SCHOOL OF LABORATORY MEDICINE AND MEDICAL SCIENCE

INVESTIGATING THE PREVALENCE OF VAGINITIS PATHOGENS IN HIV INFECTED & UNINFECTED PREGNANT WOMEN IN DURBAN, SOUTH AFRICA

BY

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September 2020

Investigating the Prevalence of Vaginitis Pathogens in HIV Infected & Uninfected Pregnant Women In Durban, South Africa

By

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Submitted in partial fulfillment of the requirements for the degree of Masters of Medicine Medical Microbiology in the School of Laboratory Medicine & Medical Science, University of KwaZulu-Natal

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South Africa

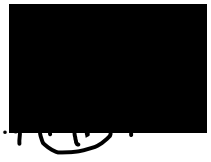
September 2020

Declaration of Authorship

1. I, Dr Ntokozo Mzimela declare as follows:
   The work described in this dissertation has not been submitted to UKZN or any other institution for the purposes of an academic qualification, whether by myself or any other party.

2. That my contribution to the project is as follows:
   I, Dr Ntokozo Mzimela did the full work on this project with assistance from the supervisors; Dr Khine Swe Swe Han and Prof Nathlee Abbai.

3. That the contributions of others to the project are as follows: Both my supervisors assisted with correction and guidance of developing research proposal and acquiring ethics approval from BREC. They also assisted with data collection and analysis. Data analysis was assisted by a Statistician, Partson Tinwaro.

SIGNATURE…  …… DATE: 05 Nov 2020

ACKNOWLEDGEMENTS

I acknowledge the following:

1. God, I put you first in all that I do. It is by your might and power that I have made it this far.
2. My family who are my constant pillar of strength and courage. I thank you.
3. My friends, Esmeraalda, Xoli, Sphe, Kelly who always listen to some struggles and successes.
4. To the special person in my life, thank you for being so supportive and your daily encouraging words.
5. The Embassy Church and My spiritual Father, Pastor Vernon Jacobs for prayers, love and support.
6. My supervisor, Dr Khine Swe Swe Han, for her professional guidance throughout this project. Your supervision and mentorship is invaluable.
7. My co-supervisor, Prof Nathlee, for her assistance and guidance throughout this project. I do appreciate the prompt responses and going an extra mile to make sure I complete this dissertation on time.
8. The statistician, Partson Tinwaro for the analysis of the data.
9. Mrs Fazana Dessai from the School of Clinical Medicine Research Laboratory, University of KwaZulu-Natal for the testing of the samples that were used in this study. This has contributed to the generation of accurate data used in this study.
10. Thanks to colleagues KEH, Inkosi Albert Luthuli Central Hospital Microbiology
11. Thank you KEH Antenatal Nurses for conducting HIV testing and Farzana Dessai for assisting with data collection

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List of Abbreviations

AIDS	Acquired Immunodeficiency Syndrome
ANC	Antenatal Care
BREC	Biomedical Research Ethics Committee
BV	Bacterial Vaginosis
HIV	Human Immunodeficiency Virus
PMTCT	Prevention of Mother to Child Transmission
STIs	Sexually Transmitted Infections
Spp	Species
T. vaginalis	*Trichomonas vaginalis*
USA	United States of America
WHO	World Health Organization

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ABSTRACT

BACKGROUND: Vaginitis in pregnancy significantly contributes to obstetric complications such as preterm labor. Human Immunodeficiency Virus (HIV) infection is thought to increase risk of acquiring vaginitis in pregnancy and vice versa. Currently, there are limited data on the prevalence of vaginitis pathogens in HIV infected and uninfected pregnant women from Durban. This study compared the prevalence of bacterial vaginosis (BV), *Candida* spp. and *Trichomonas vaginalis (T. vaginalis)* across HIV infected and uninfected pregnant women, thereby contributing to the body of knowledge in this area. In addition, this study identified risk factors associated with HIV infection, BV, *Candida* species (spp). and *T. vaginalis* in the studied population.

METHODS: This study was a sub-study of a larger study which enrolled 273 pregnant women from King Edward VIII hospital in Durban. For the larger study, data obtained from eligible women included; sociodemographic, sexual behavior and clinical data. All participants were tested for HIV as part of standard of care at the clinic and two self-collected swabs were obtained from each woman. Permission to obtain data on their HIV status was requested from each women. One vaginal swab was used to test for the presence of vaginal pathogens and the second swab was stored for future use. The BD Max vaginal assay was used to detect BV, *T. vaginalis* and *Candida* spp. from a single swab. The prevalence estimates for HIV, BV, *T. vaginalis* and *Candida* spp. were calculated using percentage frequencies. Univariate and multiple logistic regressions were used to assess risk factors for HIV status and vaginitis pathogens. All analysis were conducted using RStudio.

RESULTS: Of the n=273 enrolled in the larger study, n=128 women provided permission to access data on their HIV statuses. Therefore, for the current study a sample size of n=128 was available for analysis. Of the n=128 women in this study, 52/128 tested positive for HIV resulting in a HIV prevalence of 40.6%. The most prevalent vaginal infection diagnosed in the study population was *Candida* spp. (73/ 128, 57%), followed by BV (61/ 128, 47.7%) and *T. vaginalis* (14/128, 10.9%). BV positivity increased the likelihood for HIV by >2-fold (OR: 2.91, 95% CI: 1.08 – 8.63, p= 0.042), being *Candida* positive increased risk for HIV by 4-fold (OR: 4.04, 95% CI: 1.52 – 11.68, p= 0.007) and testing positive for *T. vaginalis* increased risk for HIV by 10-fold (OR: 10.15, 95%

CI: 2.38 – 55.81, p= 0.018). Having 2-4 lifetime sex partners increased the likelihood of being HIV infected by 9-fold (OR: 9.78, 95% CI: 2.69 – 47.07, p= 0.001) and having >4 lifetime sex partners increased the likelihood of being HIV infected by 33-fold (OR: 33.88, 95% CI: 5.62 – 274.00, p< 0.001). In the adjusted analysis, not knowing if their partner had other partners, increased the risk of being BV positive by 4-fold (OR: 4.05, 95% CI: 1.58 -11.16, p=0.005) and alcohol consumption increased the risk of being BV positive by 4-fold (OR: 4.44, 95% CI: 1.54 -14.96, p=0.009). Having a current abnormal vaginal discharge and HIV infection were significantly associated with *Candida* infection (OR: 3.63, 95% CI: 1.45- 9.90, p=0.008), and (OR: 5.19, 95% CI: 1.98 – 15.21, p=0.001), respectively. Similarly, to *Candida* infection, having a current abnormal vaginal discharge increased the risk of *T. vaginalis* infection by 5-fold (OR: 5.37, 95% CI: 1.39 – 24.59, p=0.019) and HIV infection increased the risk of *T. vaginalis* infection by 23-fold (OR: 23.25, 95% CI: 4.52 – 174.17, p=0.001).

CONCLUSION: In this study, behavioral factors played a significant role in the risk for contracting infections. It is imperative that women must first perceive themselves to be at risk for contracting infections before they can be motivated to reduce that risk. This can be accomplished by conducting future studies which administer a risk assessment tool to the women so that they can be made aware of actual risk versus perceived risk. Older age group (25-34 years) of pregnant women has also been identified to be at higher risk of HIV transmission, therefore this group should be mainly targeted for risk assessment and prompt HIV testing during pregnancy. Lastly, routine screening of BV, Candida & T. vaginalis is recommended during pregnancy, as many women in this particular study population were found to be co-infected with all three pathogens. The screening is highly recommended largely, due to the fact that vaginitis in pregnancy has been identified as a risk factor for HIV infection.

CHAPTER 1

1. INTRODUCTION

The vaginal microbiome, also known as vaginal flora consists of a collection of different organisms that inhabit the vaginal wall (1). The vaginal microbiome helps to maintain the vagina in a healthy state and protects the host from diseases such as sexually transmitted infections, bacterial vaginosis, urinary tract infections and poor outcomes in pregnancy such as preterm birth (1-4). Organisms that inhabit the vaginal wall comprise mostly of *Lactobacillus* species (spp.) that produce lactic acid, creating an acidic vaginal environment (5).

The acidic vaginal environment is key to healthy homeostasis of the vaginal tract, enhancing the role of the vaginal microbiome to act as a natural barrier (4). *Lactobacillus* spp. offers a protective role using two mechanisms; by binding to specific sites on the vaginal epithelial wall and secondly producing compounds that inhibit pathogen growth (4). However, dysbiosis in vaginal flora (microbiota imbalance) can occur as a result of multiple factors such as hormonal changes, age, ethnicity, tobacco use, stress and sexual activity (4). Literature reveals that the diversity of the microbiota has been associated with greater risk of human immunodeficiency virus (HIV) transmission(6). It has been reported that lactic acid acts as a potent microbicide that is able to destroy the HIV (6). Even though there is conflicted opinion in terms of the association between vaginal microbiota composition and HIV status in literature however some studies have revealed that there is a role of vaginal microbiota composition in HIV transmission (7). In South African women particularly, the risk of HIV acquisition is largely related to the composition of the vaginal microbiome (8).

Vaginitis can be defined as the interference of the Lactobacilli predominant vaginal microbiome and the risk of acquiring vaginitis in pregnancy is higher due to hormonal changes (1). Pregnancy disrupts the estrogen and progesterone levels inducing some physiological changes such as change in pH levels (9). The change in pH level enable growth of anaerobic bacteria and other pathogenic microorganisms inside the vaginal tract resulting in dysbiosis of the vaginal microbiome (9, 10).
Vaginitis associated sexually transmitted infection (STI) that have been identified to be common in pregnancy include the following: Bacterial vaginosis *(*BV), *Candid*a spp. and *Trichomonas vaginalis* (11, 12).

Currently, there are limited data on the prevalence of vaginitis pathogens in HIV infected and uninfected pregnant women from Durban. This study will compare the prevalence of BV, *Candida* spp. and *T. vaginalis* across HIV infected and uninfected pregnant women, thereby contributing to the body of knowledge in this area. In addition, this study will identify risk factors associated with HIV infection, BV, *Candida* spp. and *T. vaginalis* in the studied population.

CHAPTER 2

2. LITERATURE REVIEW

2.1 HIV Infections in Pregnant Women

Globally, reports reveal that 5.2 million women of reproductive age are infected with HIV and of those infected, 1.1 million women are from South Africa (13). According to World Health Organization (WHO), there were 248 000 cases of pregnant women who were living with HIV that received antiretroviral treatment in 2018. The magnitude of HIV infection in pregnant women escalated in the early 90s prior to the introduction of the prevention of mother to child transmission (PMTCT) program. Since inception of the PMTCT program in 2002, there has been up to 80% coverage of pregnant women who are enrolled in HIV prevention programs at early gestation (13).

Despite a notable decline in the mortality of HIV positive pregnant women following the introduction of PMTCT, the risk of HIV related mortality in pregnant women is 3.7-21.6 times higher when compared to HIV negative pregnant women (14). Therefore, it is hypothesized that pregnancy fast-tracks progression of HIV to AIDS, resulting in increased susceptibility to complications of HIV infection in pregnant women (13).

Literature states that South African women of reproductive age remain at high risk for acquiring HIV infection even though rigorous intentional efforts to reduce risk of HIV transmission are in place (15, 16). Figure 1 shows the global HIV prevalence rates over a 10-year period. According to the statistics, South Africa has the highest HIV infections rates amongst pregnant women when compared to other countries in the developing world.

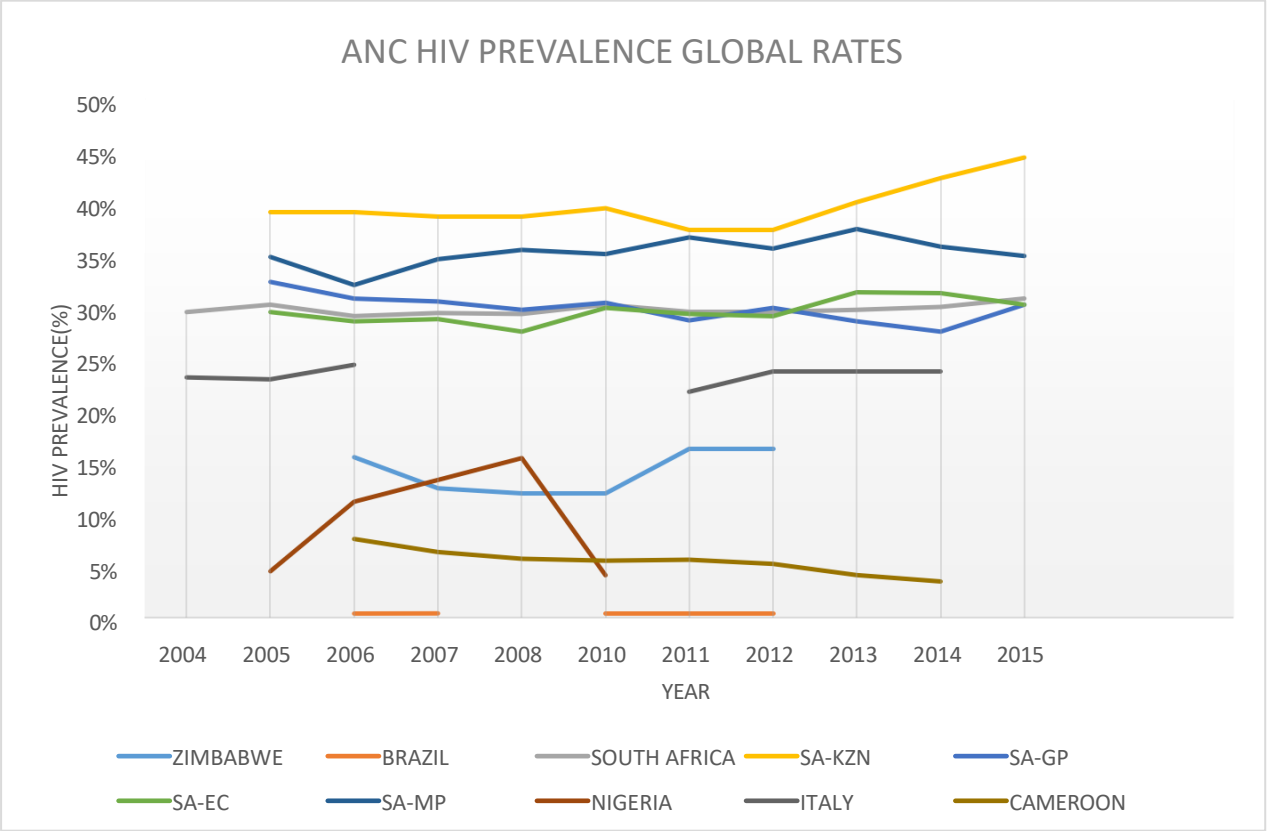

Figure 1: Graphical representation of the HIV prevalence rates of Brazil, Italy, Nigeria, Cameroon, Zimbabwe and South Africa for a 10-year period. [Abbreviations: SA-EC (South Africa-Eastern Cape), SA-GP (South Africa-Gauteng Province), SA-EC(South Africa-Eastern Cape), SA-MP(South Africa-Mpumalanga Province)]

Notably, South Africa has the highest HIV rates in pregnancy when compared to other countries such as Brazil, Italy, Nigeria, Cameroon and Zimbabwe (17-29).

In South Africa, KwaZulu-Natal is the province with the highest HIV prevalence in antenatal women (44%) with increase in HIV prevalence rates greater than 5% observed over the period

2011-2015(29, 30). Other South African provinces with high HIV prevalence rates during pregnancy include; Mpumalanga (37%), Gauteng (32%) and the Eastern Cape (31%) (28, 29). According to Figure 1, Brazil has the lowest HIV prevalence rate in pregnancy (0.42%) (20, 31). Studies conducted in Brazil have revealed that the low HIV prevalence rate in pregnancy is attributed to comprehensive pre-natal and birth care in their health care settings (20). The population in Brazil similarly to South Africa has free access to HIV testing (19).

2.2 Risk Factors for HIV in Pregnancy

According to a past study, the likelihood of becoming HIV infected increases during pregnancy due to hormonal changes (32). In a population of Brazilian women, the high risk for HIV acquisition was associated with region of habitation, older age (>35 years of age), ethnicity, being single and co-infection with syphilis (19). Similarly, a study conducted on pregnant women from Cameroon identified being single and older age to be associated with HIV risk. In that study, single women were two times more likely to be infected with HIV when compared to married or cohabiting women. In addition, women aged 25 years and older were also two times more likely to be infected when compared to women younger than 25 years of age (25). In a study conducted in Nigeria, having multiple partners, history of sexually transmitted infections (STIs), having candidiasis, having BV and cigarette smoking were significantly associated with the risk of acquiring HIV infection during pregnancy (33). Bacterial vaginosis and *Candida* species were associated with a three times higher risk of acquiring HIV during pregnancy (32).

A study conducted in KwaZulu-Natal has also shown that HIV incidence significantly increases in pregnancy when compared to non-pregnant woman (34). Associated risk factors for infection during pregnancy included; age, number of pregnancies and level of education. Women less than 25 years had twice the risk of acquiring HIV infection during pregnancy. This study also concluded that the risk was increased with increased number of pregnancies and low level of education (34). Significant evidence that STIs increase the likelihood of acquiring HIV was observed; however, this has not been studied extensively in the context of pregnant and postpartum women (32).

2.3 Composition of the vaginal microbiome during pregnancy

The vaginal microbiome comprises of a bacterial community, predominated by *Lactobacillus* spp.

that significantly affects the health of a woman (35). Other organisms that form part of the vaginal microbiome in a healthy non-pregnant woman in lesser quantity include: *Ureaplasma*, *Gardnerella, Staphylococcus, Enterococcus, Bacteroides*, *Bifidobacterium* and *Candida* (36). Various factors contribute to the differences in the composition of the vaginal microbiome in a healthy non-pregnant woman and these include; ethnicity, hygiene and sexual health (4, 35). The vaginal microbiome helps to maintain the vagina in a healthy state and protects the host from diseases such as STIs, BV, urinary tract infections and poor outcomes in pregnancy such as preterm birth (1-4). According to the literature, the vaginal microbiome in a women who is pregnant is not clearly understood (37). However, during pregnancy the levels of *L. crispatus* (healthy vaginal bacteria) is reduced and the levels of L. crispatus will differ by ethnicity(1, 38). The shift in structure and composition of the vaginal microbiome during pregnancy contributes to negative pregnancy outcomes such as miscarriage and preterm labor (2).

Studies conducted in healthy pregnant woman from the United Kingdom revealed that the vaginal microbiome in this population is predominantly *Lactobacillus* spp. (6). However, in a sub-Saharan African Black pregnant population, there was a notable difference in the vaginal bacterial composition with a lower abundance of the *Lactobacillus* spp. (6). The structure and composition of the vaginal microbiome differs by population group, however, a significant shift in the vaginal microbiota structure has been identified during the post-partum period (39).

The increased risk of HIV acquisition during pregnancy has been linked to changes in the structure and composition of the vaginal microbiome (11, 37). In addition, the presence of HIV infection has also changed the clinical course of vaginal tract infections in pregnancy resulting in complexity in terms of diagnosis and treatment (37). In a study comparing HIV infected and uninfected pregnant women the prevalence of vaginal tract infections was remarkably higher in the HIV infected pregnant women when compared to the uninfected women (40). Studies conducted estimated that shifts in the vaginal microbiome of HIV infected pregnant women affects 89% of women in Africa, which accounts for 20% of the general population (41, 42). Risk factors that further contribute to the shifts in the vaginal microbiome of HIV infected pregnant women when compared to uninfected women include; exposure to antiretroviral drugs contributing to more diversity of the vaginal microbiome (31, 43).

2.4 Vaginitis in Pregnancy

The most frequent pathogens that cause vaginitis in pregnancy include BV, *Candida* spp. and *T. vaginalis* (11, 12). BV vaginitis is characterized by a fishy foul smelling vaginal discharge whereas *Candida* vaginitis is characterized by pruritis, dysuria and a white thick discharge described as cottage cheese (44). *T. vaginalis* vaginitis is associated with pruritis accompanied by green-yellow frothy discharge (44). Various studies conducted in South Africa have revealed that vaginitis pathogen recovery is greater in HIV infected pregnant women when compared to HIV uninfected pregnant women (41). Of concern, the recovery of these pathogens is associated with adverse obstetrical events and complications such as miscarriage, chorioamnionitis or preterm labor (1-4).

Pregnancy has been identified as risk factor for *Candida* colonization, hence pregnant women are more prone to be diagnosed with *Candida* vaginitis(45). With regards to BV, there is conflicting data with certain studies revealing higher prevalence in non-pregnant women(46). Some studies have shown that prevalence of BV decreases with increasing gestational with predominant occurrence in first & second trimester(45)

2.4.1 *Trichomonas vaginalis* infections during pregnancy

T. vaginalis is a highly prevalent STI, estimated to infect up to 25 million pregnant women globally(47). The pathogen is often detected in women who also exhibit symptoms of BV such as foul-smelling vaginal discharge (24, 44).

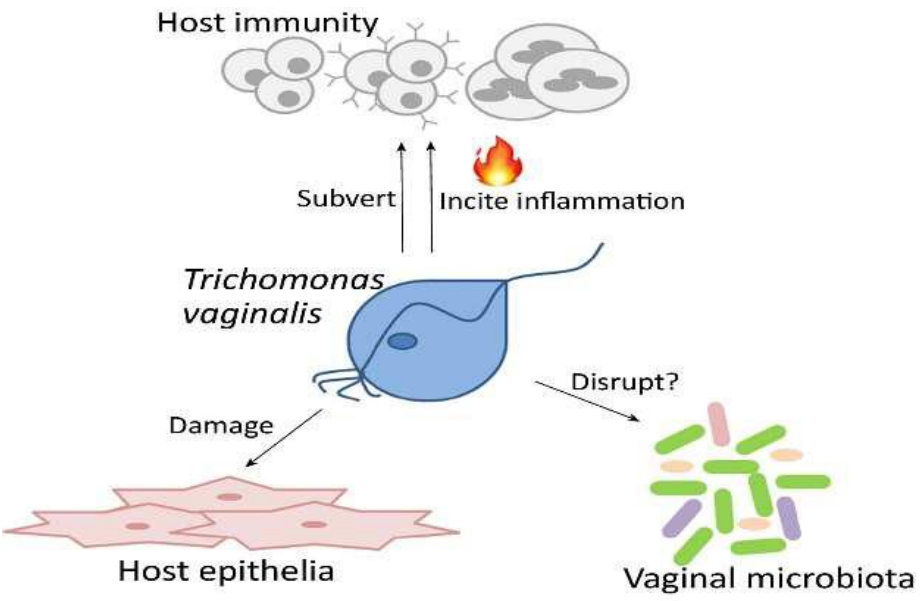

Figure 2: Diagram depicting the pathogenesis of *T. vaginalis* (48).
(*Biological factors that predispose host to acquiring T. vaginalis infection include damage to host epithelia and disruption of vaginal microbiota. Entry of T.vaginalis to host cell will incite inflammation and/or subvert host immunity)*

Various biological factors predispose pregnant women to *T. vaginalis* infection such as disruption of the vaginal microbiota and damage to the host vaginal epithelial cells (49). These factors enhance the adherence of *T. vaginalis* to vaginal epithelial cells disrupting host immunity and causing vaginitis (49) (Figure 2). *T. vaginalis* infection is significantly predominant in pregnant women due to hormonal fluctuations, discontinuing contraceptive use and change in vaginal pH ultimately disrupting the vaginal microbiome (37). The increase in vaginal pH (5.5-5.8) results in physiological modifications encouraging colonization of the vaginal tract by *T. vaginalis* and overgrowth of pathogenic organisms (37). *T. vaginalis* is also associated with adverse reproductive health outcomes including preterm birth, low birth weight, and high risk of HIV acquisition (50, 51). The relationship between HIV and *T. vaginalis* is bi-directional as *T. vaginalis* may increase susceptibility to acquire HIV infection (49). In addition, HIV infection also increases susceptibility to acquire *T. vaginalis* infection (38).

The prevalence of this infection differs according to geographical distribution, samples collected, laboratory diagnostic methods employed and HIV status (52). In Brazil, the prevalence rate of *T. vaginalis* in pregnant women ranges from 5.9-7.7% with a much higher rate in HIV positive pregnant women (10.1%) (26). In South Africa, studies have revealed prevalence rates that range from 15.3-20.2% in HIV infected pregnant women (27, 28). In KwaZulu-Natal, South Africa, the prevalence of *T. vaginalis* ranges from 10-13% in pregnant women (53, 54).

The risk factors that increase susceptibility to *T. vaginalis* in pregnant women have been previously described (26, 29). In a cross sectional survey conducted in Papua New Guinea among 400 pregnant women, the following risk factors were identified; partner at perceived risk of infection, lack of formal education, maternal extramarital intercourse, urban residence and smoking and condom use (29). Wangnapi et al (29) mentions that there is higher prevalence of TV infection with statistical significant findings in a group of women that perceived their partner to be at high risk of infection. In terms of condom use, 79% of women had never used condoms and the inconsistent use was perceived to put a pregnant at higher risk of TV infection, however the findings were statistically insignificant (29). Mabaso et al (53) also reported high prevalence of TV infection in pregnancy in the group that reported inconsistent use of condoms however this finding was also statistically insignificant. A study in Brazil by Gatti *et al* (48), shared the following associated risk factors; illicit drug use, history of STIs and less visits for antenatal care. Other factors such as age, schooling, stable partner, gestational age and presence of vaginal discharge were found to be statistically insignificant (26). A study in King Edward VIII Hospital in KwaZulu-Natal, South Africa reported a prevalence rate of 12.9% for *T. vaginalis* in pregnant women and the following risk factors were identified: being 21-25 years of age, being unemployment, being unmarried, having a regular sex partner and inconsistent condom use (53).

2.4.2 Bacterial vaginosis during pregnancy

BV is the most predominant of vaginal infections in women of reproductive age and it has been associated with obstetric complications such as preterm labor and chorioamnionitis (50, 53). Studies have also observed an association of this infection with other STIs such as *T. vaginalis*. Literature states that BV infection increases susceptibility to acquire *T. vaginalis* infection by two

folds (50). BV infection changes course during pregnancy with predominant infection arising mainly in the first trimester (55).

The prevalence of BV differs according to geographical region, race and ethnic group (56). A study conducted in the United States of America (USA) revealed that the prevalence of BV was 47% in the HIV infected pregnant women in comparison to 44% in the HIV uninfected women (41). A study in Cameroon observed a prevalence of 26.2% for BV infection in pregnant women. In pregnant women from Kenya, the prevalence of BV was reported to be from 6.4 to 38% (57). In South Africa, high prevalence rates for BV (17.6%- 49%) have been reported for antenatal women (54, 58) (43).

In a Danish population of pregnant women, significant risk factors for BV were identified, the factors were; daily coitus, single status, smoking, previous genital tract infection such as *Chlamydia trachomatis* and alcohol consumption (59). However, a study conducted in a cohort of 309 pregnant women from Cameroon (56) identified different risk factors for infection which included; vaginal douching, age (younger age), parity, area of residence(high in rural areas), previous genital infection, previous antibiotic use and second trimester in pregnancy (40, 56). Currently, there is a lack of data on the risk factors for BV in South African pregnant women according to HIV status(40).

2.4.3 Candida infections during pregnancy

Candida spp. constitute part of the normal vaginal flora in healthy women of reproductive age. This organism is an opportunistic pathogen which has been described in literature to be pathogenic in its hyphal form (60). Lactobacilli that predominantly form part of the vaginal microbiota play a role in preventing biofilm production by *Candida* spp. therefore if Lactobacilli are reduced, this results in *Candida* vaginitis (60).

Prevalence of *Candida* vaginitis is significantly reported to be higher in pregnancy and *Candida albicans* seems to be the most common pathogen in most countries (44, 61). A high prevalence of *Candida* (30.7%) has been reported for Jamaican pregnant women with *C. albicans* being the most predominant species (62). Risk factors for this cohort included the following; age less than 21 years

old, belief that sexual partner has other sexual partners and adverse outcomes in their past pregnancy (62). A study conducted in an Ethiopian antenatal population reported a prevalence of 25% with the predominant spp. being *C. albicans* (56.25%) followed by *C. krusei* (21.9%). The Ethiopian study identified the following risk factors for infection; HIV status, frequent use of contraception, number of pregnancies, prolonged use of antibiotics and gestational age (63). However, in the antenatal population in South Africa, the last reported range was 9–59% in 2005. No further studies have described the prevalence of *Candida* vaginitis in pregnancy (54, 63). In addition, there is lack of data on the risk factors for this infection in South African antenatal women according to HIV status.

2.5 Rationale for study

Various studies conducted in South Africa have revealed that pathogen recovery is greater in HIV infected pregnant women when compared to HIV uninfected pregnant women (41). Of concern, the recovery of these pathogens is associated with adverse obstetrical events and complications such as miscarriage, chorioamnionitis or preterm labor (1-4). Currently, there are limited data on the prevalence of vaginitis pathogens in HIV infected and uninfected pregnant women from Durban. This study will compare the prevalence of BV, *Candida* spp. and *T. vaginalis* across HIV infected and uninfected pregnant women, thereby contributing to the body of knowledge in this area. In addition, this study will identify risk factors associated with HIV infection, BV, *Candida* spp. and *T. vaginalis* in the studied population.

2.6 Research Questions

1. What is the prevalence and risk factors for HIV infection in antenatal women from Durban?
2. Which vaginitis pathogens are strongly associated with HIV infection?
3. What are the risk factors that are associated with the individual pathogens in pregnancy?

2.7 Aim

The aim of this study was to investigate the most prevalent vaginitis pathogens in HIV infected and uninfected pregnant women from Durban, South Africa.

2.8 Objectives

1. To determine the prevalence of HIV infection in pregnancy and to identify risk factors associated with HIV infection in pregnancy.
2. To identify the most prevalent vaginitis pathogens associated with HIV infection in the study population
3. To identify risk factors for the individual vaginitis pathogens in the HIV infected pregnant population

CHAPTER 3

3. METHODOLOGY

3.1. Ethical approval

Ethical and human participant research approvals were obtained from the Biomedical Research Ethics committee (BREC/0001494/2020) of the University of KwaZulu-Natal. In addition, this was a sub-study of a larger study that had already obtained gatekeeper permission, Department of Health approval and BREC approval (BE214/17).

3.2 Study design and setting

This study was a sub-study of a larger study which investigated the laboratory based diagnosis of vaginal infections in pregnant women from Durban. The larger study included n=273 women, 18 years and older and willing to provide written informed consent. The study population was recruited from the King Edward VIII hospital in Durban, KwaZulu-Natal between October 2017 and April 2018. Symptomatic participants (before treatment) were characterized as those who complained of an abnormal vaginal discharge/dysuria or vulval itching/burning. Asymptomatic participants were characterized as those who did not complain of any of the above symptoms. All symptomatic women were treated as per the syndromic management guidelines. The guidelines advocate the use of a 2g single dose of metronidazole and clotrimazole vaginal pessary (single dose) or clotrimazole vaginal cream (12 hourly for seven days). Participants who were asymptomatic and who tested positive for infection were not eligible for treatment as per the current syndromic management guidelines.

A questionnaire was administered to collect data on the women's demographics, sexual behaviour and clinical information. Clinical information included: current abnormal vaginal discharge, past pregnancy history, sexual practises, condom use, use of alcohol, smoking history, past abnormal vaginal discharge and previous treatment of sexually transmitted infection. All interviews were conducted in private, and all study-related information had been stored securely. All records and specimens had been identified by study identification numbers only to maintain participant confidentiality. Only participants who had given written informed consent were included in the study. During the study visit, women were asked to provide two self-collected vaginal swab

samples. Permission to obtain data on HIV status was requested from the women. Of the n=273 women, n=128 women provided permission to obtain data on their HIV status. The sample size for the sub-study was n=128.

3.3 Sample collection

Two self-collected vaginal swabs were obtained from each participant for testing and storage. Instructions for the collection of the self-collected swabs were given to the study participants (Figure 3). The swabs were transported within two hours of collection to the School of Clinical Medicine Laboratory, College of Health Sciences, Medical School campus, University of KwaZulu-Natal. At the laboratory, one vaginal swab was placed in the BD Max™ Vaginal assay swab diluent tube. Samples for the BD Max™ Vaginal assay were stored at 2-8 degrees Celsius and batch tested within five days. The second vaginal swab was stored at -20 degrees Celsius for future testing.

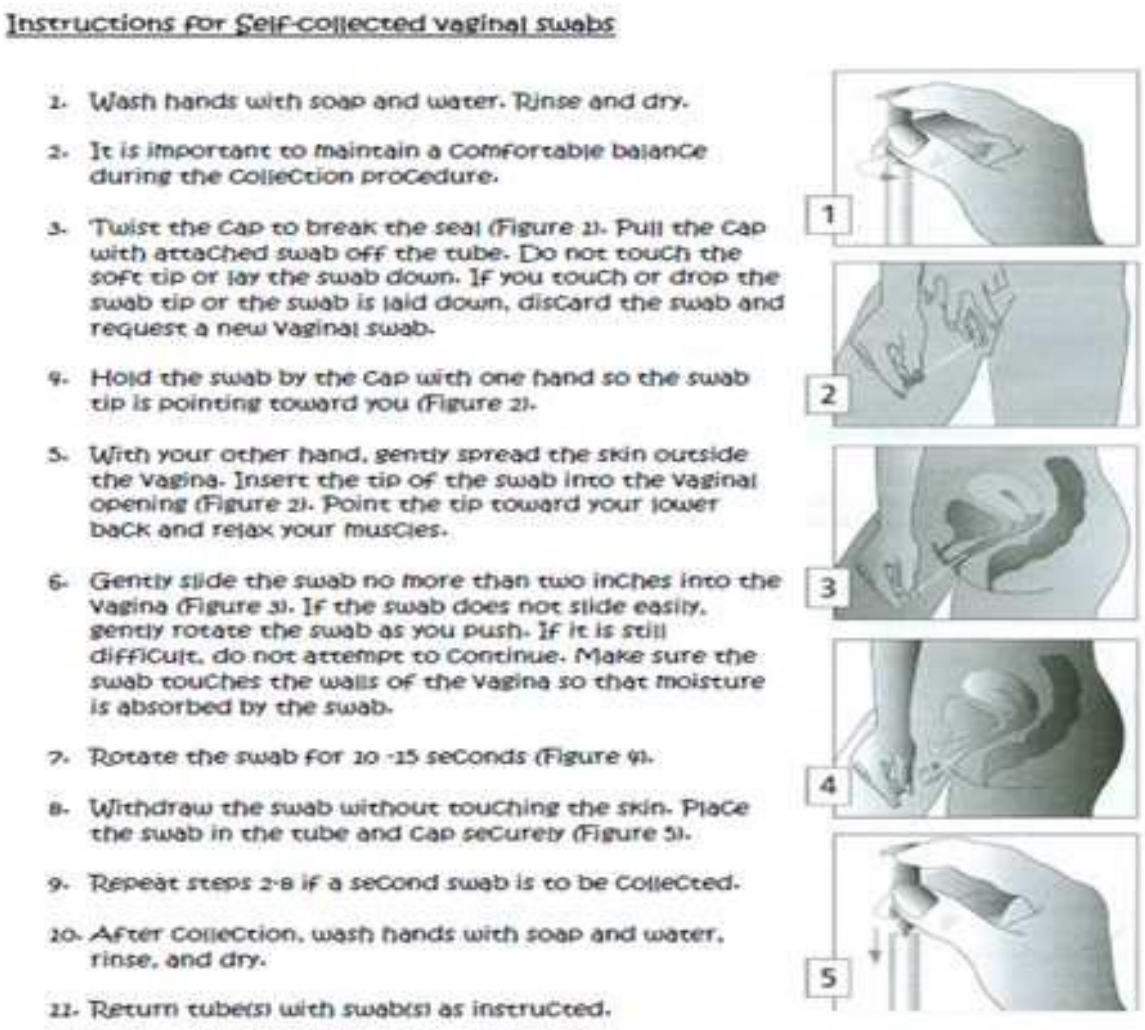

Figure 3: Instructions provided to the study women for the self-collection of vaginal swabs. Source: https://www.hey.nhs.uk/pathology/departmentofinfection/virology/vaginal-swabs

3.4. Laboratory procedures

3.4.1 Testing for HIV

Testing for HIV was part of the standard of care at the antenatal clinic. The testing was performed using the HIV 1/2/O Tri-line Human Immunodeficiency Virus Rapid Test Device test strip (ABON). The testing was performed on fingerpick whole blood. The HIV 1/2/O Tri-line Human

Immunodeficiency Virus Rapid Test Device is an *in vitro* diagnostic rapid immune-chromatographic assay for the qualitative detection of antibodies to HIV-1, including subtype O, and HIV-2 in venous and capillary whole blood, serum and plasma specimens. The test was conducted according to the manufacturer's instructions.

3.4.2 Detection of vaginal pathogens

For this study, the prevalence of BV, *T. vaginalis* and *Candida* spp. was investigated. These pathogens were detected using the BD Max™ Vaginal assay(BD Max). The testing was performed as per the manufacturer's instructions and included the following steps. Firstly, the swabs were put in sample buffer tubes and vortexed for 1 minute, and the swab was squeezed and discarded. The sample buffer tubes were uncapped and re-capped with a blue septum cap. Sample tubes were placed on the rack together with unitised reagent strips, extraction vials and master mix vials. Lastly, the samples were logged onto the instrument, and the rack was loaded into the BD Max vaginal assay instrument with the PCR cartridge and the run initiated. An average run took 2.5 hrs.

The BD Max™ results were interpreted using the guidelines below:

Assay Result Reported (Vaginitis Master Mix)	Interpretation of Results
TV POS	*Trichomonas vaginalis* DNA Detected
TV NEG	No *Trichomonas vaginalis* detected
Cgroup POS	*Candida* group DNA Detected (*Candida albicans* and/or *Candida tropicalis* and/or *Candida parapsilosis* and/or *Candida dubliniensis*)
Cgroup NEG	No *Candida* group detected (*Candida albicans* and/or *Candida tropicalis* and/or *Candida parapsilosis* and/or *Candida dubliniensis*)
Cgla POS	*Candida glabrata* DNA Detected
Cgla NEG	No *Candida glabrata* detected
Ckru POS	*Candida krusei* DNA Detected
Ckru NEG	No *Candida krusei* detected
UNR	Unresolved – inhibitory sample or reagent failure; No target detected and no amplification of Sample Processing Control
IND	Indeterminate result due to BD MAX System failure (with Warning or Error Codes[3])
INC	Incomplete Run (with Warning or Error Codes[3])
Assay Result Reported (Vaginosis Master Mix)	**Interpretation of Results**
BV POS	Vaginosis Panel DNA detected. Detection of marker combinations related to bacterial vaginosis: *Gardnerella vaginalis* and/or *L. crispatus* and/or *L. jensenii* and/or *Atopobium vaginae* and/or BVAB-2 and/or *Megasphaera-1*
BV NEG	Detection of marker combinations related to normal vaginal flora
UNR	Unresolved – inhibitory sample or reagent failure; No target detected and no amplification of Sample Processing Control
IND	Indeterminate result due to BD MAX System failure (with Warning or Error Codes[3])
INC	Incomplete Run (with Warning or Error Codes[3])

Figure 4: Interpretation of the BD Max™ assay (BD Max™ vaginal Panel package insert)

3.5. Data analysis

Descriptive and inferential statistical analysis methods were used and all the analysis were conducted in R Statistical computing software, version 3.6.3. Age was the only numerical variable and summarized using the minimum, maximum, mean, quartiles, standard deviation and the coefficient of variation. The mean or median comparisons were done on two groups only and called for either t-test or Wilcoxon test. Majority of the variables were categorical with binary responses and were summarized in the form of counts and percentage frequencies. The associations between the categorical variables were tested using either the Chi-Square or Fisher's exact test depending

on the distribution of counts within the cross tabulations. Univariate and multiple logistic regressions were used in assessing the risk factors for HIV status or infection, BV, Candida and TV. The statistical model building was used to determine the likelihood of HIV infection, BV positive, Candida positive and TV positive with respect to the socio-demographic, behavioral and clinical. Further stepwise regression was used to identify the most important risk factors as a way of variable reduction. In this case an iterative procedure was used where the least significant factors were removed first. Both the dependent variables and the socio-demographic, behavioral and clinical were set to have a reference group. All tests were conducted at 5% significance level.

CHAPTER 4

4. RESULTS

4.1 Prevalence estimates

Of the n=128 women in this study, 52/128 tested positive for HIV resulting in a HIV prevalence of 40.6%. The most prevalent vaginal infection diagnosed in the study population was *Candida* spp with prevalence of 57%, followed by BV with prevalence of 47% and *T. vaginalis* with prevalence of 10.9%. Within the HIV infected women (n=52), the prevalence of *Candida* spp. was 36/52 (69.2%) and within the HIV uninfected group (n=76), the prevalence of *Candida* spp. was 37/76 (48.7%). The prevalence of BV was 50% in the HIV infected group (26/52) and 46.1% in the HIV uninfected group (35/76). Similarly, a higher prevalence of *T. vaginalis* was observed in the HIV infected women (11/52, 21.2%) when compared to the HIV uninfected women (3/76, 3.9%) (Table 1).

4. 2 Characteristics of the study population according to HIV status

The characteristics of the study population according to HIV status is described in Table 1.

4.2.1 Socio-demographic factors

According to the analysis, age was significantly associated with HIV status ($p < 0.001$), the median age of the women in the HIV infected group was 29 years old whereas the median age of the women in the HIV uninfected group was 25 years old (Table 1). Marital status was regarded as a non-significant factor in this study population.

4.2.2 Behavioural factors

A higher proportion of women who had > 4 lifetime sex partners were HIV infected (23.1%) when compared to the HIV uninfected women (6.6%), ($p<0.001$). A higher proportion of the HIV infected women reported using condoms at their last sex act (42.3%) when compared to the HIV uninfected women (26.3%), ($p=0.05$). The insignificant behavioural factors associated with HIV status included; having a regular sex partner, co-habiting with their partner, partner having other partners, condom use, smoking, consuming alcohol, and engaging in intravaginal practices

(p>0.05) (Table 1).

4.2.3 Clinical factors

A higher proportion of the women who tested positive for *Candida* spp. (69.2%) and *T. vaginalis* (21.2%) were HIV infected when compared to the HIV uninfected women. There was a significant association between *Candida* infection and HIV infection (p=0.021). Similarly, there was a significant association between *T. vaginalis* infection and HIV infection (p=0.002). In addition, a higher proportion of the HIV infected women reported having a past abnormal vaginal discharge (50%) when compared to the HIV uninfected women (31.6%), (p=0.041) and the majority of the HIV infected women were treated for STIs in the past (51.9%) when compared to the HIV uninfected women (26.3%), (p=0.003). Both these associations were significant (p<0.05)
(Table 1).

Insignificant clinical factors in association with HIV status included; BV status, current abnormal vaginal discharge, trimester of pregnancy, having a past preterm baby, having a past miscarriage and having a past abortion (p>0.05).

Table 1: Characteristics of the study population stratified by HIV status

HIV status	Uninfected (N=76)	Infected (N=52)	p-value	Overall (N=128)
Bacterial vaginosis			0.661	
Negative	41 (53.9%)	26 (50.0%)		67 (52.3%)
Positive	35 (46.1%)	26 (50.0%)		61 (47.7%)
***Candida* spp.**			0.021	
Negative	39 (51.3%)	16 (30.8%)		55 (43.0%)
Positive	37 (48.7%)	36 (69.2%)		73 (57.0%)
T. vaginalis			0.002	
Negative	73 (96.1%)	41 (78.8%)		114 (89.1%)
Positive	3 (3.9%)	11 (21.2%)		14 (10.9%)
Age			<0.001	
Mean±SD (CV %)	26.4±5.99(22.6)	29.6±5.27(17.8)		27.7±5.89(21.3)
Median(Q1-Q3)	25.0(22.0-29.0)	29.5(25.8-34.0)		27.0(24.0-32.0)
Min-Max	18.0-43.0	18.0-42.0		18.0-43.0
Current abnormal vaginal discharge			0.517	
No	51 (67.1%)	32 (61.5%)		83 (64.8%)
Yes	25 (32.9%)	20 (38.5%)		45 (35.2%)
Married			0.231	
No	63 (82.9%)	47 (90.4%)		110 (85.9%)
Yes	13 (17.1%)	5 (9.6%)		18 (14.1%)
Has a regular sex partner			0.197	
No	12 (15.8%)	13 (25.0%)		25 (19.5%)
Yes	64 (84.2%)	39 (75.0%)		103 (80.5%)
Co-habitating with partner			0.963	
No	45 (59.2%)	31 (59.6%)		76 (59.4%)
Yes	31 (40.8%)	21 (40.4%)		52 (40.6%)
Lifetime sex partners			<0.001	
1	33 (43.4%)	4 (7.7%)		37 (28.9%)
2-4	38 (50.0%)	36 (69.2%)		74 (57.8%)
>4	5 (6.6%)	12 (23.1%)		17 (13.3%)
Partner has other partners			0.149	
No	20 (26.3%)	12 (23.1%)		32 (25.0%)
Yes	16 (21.1%)	19 (36.5%)		35 (27.3%)
Don't know	40 (52.6%)	21 (40.4%)		61 (47.7%)
Condom use (generally)			0.337	
No	25 (32.9%)	13 (25.0%)		38 (29.7%)
Yes	51 (67.1%)	39 (75.0%)		90 (70.3%)
Condom used during last sex act			0.058	
No	56 (73.7%)	30 (57.7%)		86 (67.2%)
Yes	20 (26.3%)	22 (42.3%)		42 (32.8%)
Smokes			0.700	
No	71 (93.4%)	50 (96.2%)		121 (94.5%)
Yes	5 (6.6%)	2 (3.8%)		7 (5.5%)
Consumes alcohol			0.219	
No	61 (80.3%)	46 (88.5%)		107 (83.6%)
Yes	15 (19.7%)	6 (11.5%)		21 (16.4%)
Engages in intravaginal			0.445	

practices				
No	67 (88.2%)	48 (92.3%)		115 (89.8%)
Yes	9 (11.8%)	4 (7.7%)		13 (10.2%)
Pregnancy trimester			0.216	
1st	5 (6.6%)	8 (15.4%)		13 (10.2%)
2nd	20 (26.3%)	15 (28.8%)		35 (27.3%)
3rd	51 (67.1%)	29 (55.8%)		80 (62.5%)
Past Preterm baby			0.413	
No	60 (78.9%)	39 (75.0%)		99 (77.3%)
Yes	14 (18.4%)	13 (25.0%)		27 (21.1%)
Missing	2 (2.6%)	0 (0%)		2 (1.6%)
Past miscarriage			0.406	
No	59 (77.6%)	37 (71.2%)		96 (75.0%)
Yes	17 (22.4%)	15 (28.8%)		32 (25.0%)
Past spontaneous abortion			0.668	
No	69 (90.8%)	46 (88.5%)		115 (89.8%)
Yes	7 (9.2%)	6 (11.5%)		13 (10.2%)
Past abnormal vaginal discharge			0.041	
No	51 (67.1%)	26 (50.0%)		77 (60.2%)
Yes	24 (31.6%)	26 (50.0%)		50 (39.1%)
Missing	1 (1.3%)	0 (0%)		1 (0.8%)
Previous treatment for sexually transmitted infections (STIs)			0.003	
No	56 (73.7%)	25 (48.1%)		81 (63.3%)
Yes	20 (26.3%)	27 (51.9%)		47 (36.7%)

The p-values are based on non-missing cases only (tableStack).

4. 3 Risk factors for HIV infection

In the unadjusted analysis, the significant factors association with HIV included; testing positive for *Candida*, *T. vaginalis*, lifetime sex partners, past abnormal vaginal discharge and previous treatment for STIs (Table 2). Being *Candida* positive increased the likelihood of testing positive for HIV by 2-fold (Odds Ratio [OR], 2.31, 95% Confidence Interval [CI]: 1.11 - 4.96, p=0.028). The likelihood of HIV was 6-fold greater in women who were diagnosed with *T. vaginalis* (OR: 6.26, 95% CI: 1.83 - 28.87, p=0.007). Having 2-4 lifetime sex partners increased the women's risk of contracting HIV by 8-fold (OR: 8.00, 95% CI: 2.82 – 28.93, p<0.001), however, having >4 lifetime sex partners increased the likelihood of contracting HIV by 19-fold (OR: 19.20, 95% CI: 4.81 – 95.30, p<0.001). Significant clinical factors included; past abnormal vaginal discharge and previous treatment for STIs. Having a past discharge increased the likelihood of HIV by 2-fold (OR: 2.17, 95% CI: 1.05 – 4.57, p=0.038) and previous treatment for STIs increased the likelihood by 2-fold (OR: 2.86, 95% CI: 1.36 – 6.13, p=0.006) The following factors were identified as non-

significant: BV positive, having current discharge, marital status, co-habiting with partner, past obstetric history, having regular partner, partner has other partners, don't know if partner has other partner, smoking, alcohol consumption or engaging in intravaginal practises.(Table 2).

In the adjusted analysis, being BV positive was significantly associated with the likelihood of contracting HIV (OR: 3.88, 95% CI: 1.21 – 14.45, p=0.030). Similarly, being *Candida* and *T. vaginalis* increased the risk of being HIV infected by 5-fold and 11-fold, respectively (OR: 5.84, 95% CI: 1.86 – 21.42, p= 0.004; OR: 11.82, 95% CI: 2.46 – 75.86, p=0.004). Finally, having 2-4 and >4 lifetime sex partners significantly increased the risk for HIV. Having 2-4 lifetime sex partners increased the likelihood of being HIV infected by 12-fold (OR: 12.68, 95% CI: 2.78 – 87.11, p=0.003) and having >4 lifetime sex partners increased the likelihood of being HIV infected by 50-fold (OR: 50.98, 95% CI: 6.29 – 626.99, p=0.001). Similarly, the following factors were identified as non-significant: BV positive, having current discharge, marital status, co-habiting with partner, past obstetric history, having regular partner, partner has other partners, don't know if partner has other partner, smoking, alcohol consumption or engaging in intravaginal practises. (Table 2).

After performing a further stepwise adjustment in which confounders were removed, testing positive for BV, *Candida* and *T. vaginalis* and lifetime sex partners were still significantly associated with increased risk for HIV (Table 2). BV positivity increased the likelihood for HIV by >2-fold (OR: 2.91, 95% CI: 1.08 – 8.63, p=0.042), being *Candida* positive increased risk for HIV by 4-fold (OR: 4.04, 95% CI: 1.52 – 11.68, p=0.007) and testing positive for *T. vaginalis* increased risk for HIV by 10-fold (OR: 10.15, 95% CI: 2.38 – 55.81, p=0.018). Having 2-4 lifetime sex partners increased the likelihood of being HIV infected by 9-fold (OR: 9.78, 95% CI: 2.69 – 47.07, p=0.001) and having >4 lifetime sex partners increased the likelihood of being HIV infected by 33-fold (OR: 33.88, 95% CI: 5.62 – 274.00, p<0.001) (Table 2).

Table 2: Risk factors for HIV infection

Factors	Unadjusted Odds Ratio [OR] (Confidence Interval [CI], p-value)	Adjusted Odds Ratio [OR] (Confidence Interval [CI], p-value)	Odds Ratio [OR] (Confidence Interval [CI], p-value) Back Step
BV positive vs negative	1.21 (0.59-2.48, p=0.597)	3.88 (1.21-14.56, p=0.030)	2.91 (1.08-8.63, p=0.042)
***Candida* spp. Positive vs Negative**	2.31 (1.11-4.96, p=0.028)	5.84 (1.86-21.42, p=0.004)	4.04 (1.52-11.68, p=0.007)
***T. vaginalis* positive vs negative**	6.26 (1.83-28.87, p=0.007)	11.82 (2.46-75.86, p=0.004)	10.15 (2.38-55.81, p=0.003)
Having current discharge: Yes vs No	1.20 (0.57-2.51, p=0.629)	0.44 (0.12-1.49, p=0.196)	--
Married: Yes vs No	0.54 (0.16-1.57, p=0.278)	0.45 (0.06-3.02, p=0.420)	--
Has regular sex partner: Yes vs No	0.59 (0.24-1.43, p=0.241)	0.53 (0.11-2.25, p=0.395)	--
Co-habitating with partner: Yes vs No	0.97 (0.47-2.00, p=0.936	0.60 (0.15-2.31, p=0.466)	--
Lifetime sex partners: 2-4 vs 1	8.00 (2.82-28.93, p<0.001)	12.68 (2.78-87.11, p=0.003)	9.78 (2.69-47.07, p=0.001)
Lifetime sex partners: >4 vs 1	19.20 (4.81-95.30, p<0.001)	50.98 (6.29-626.99, p=0.001)	33.88 (5.62-274.00, p<0.001)
Partner has other partners: Yes vs No	1.88 (0.71-5.12, p=0.208)	1.54 (0.33-7.44, p=0.582)	--
Don't know if partner has other partners: don't know vs No	0.88 (0.36-2.18, p=0.771)	0.80 (0.19-3.30, p=0.753)	--
Uses condoms: Yes vs No	1.29 (0.59-2.94, p=0.529)	0.55 (0.15-1.94, p=0.363)	--
Used condom at last sex: Yes vs No	2.08 (0.98-4.49, p=0.058)	2.68 (0.77-10.18, p=0.129)	--
Smokes: Yes vs No	0.69 (0.09-3.68, p=0.675)	0.39 (0.03-4.91, p=0.469)	--
Alcohol consumption: Yes vs No	0.50 (0.17-1.35, p=0.190)	0.17 (0.02-1.02, p=0.068)	0.19 (0.04-0.72, p=0.021)
Engages in intravaginal practices: Yes vs No	0.59 (0.15-1.94, p=0.407)	0.59 (0.07-4.20, p=0.600)	--
Pregnancy trimester: 2nd vs 1st	0.52 (0.13-1.90, p=0.329)	0.44 (0.06-2.72, p=0.391)	--
Pregnancy trimester: 3rd vs 1st	0.36 (0.10-1.19, p=0.100)	0.16 (0.02-0.82, p=0.037)	--
Had a previous preterm baby: Yes vs No	1.40 (0.59-3.32, p=0.437)	1.93 (0.51-8.18, p=0.346)	--
Had a past miscarriage: Yes Vs No	1.34 (0.59-3.01, p=0.483)	0.84 (0.21-3.28, p=0.797)	--
Had a past spontaneous abortion Yes vs No	1.23 (0.37-3.94, p=0.725)	0.73 (0.13-4.37, p=0.722)	--
Had a past discharge: Yes vs No	2.17 (1.05-4.57, p=0.038)	0.92 (0.16-4.78, p=0.917)	--
Had previous treatment for STIs: Yes vs No	2.86 (1.36-6.13, p=0.006)	2.24 (0.43-13.32, p=0.349)	2.36 (0.89-6.50, p=0.087)

4. 4 Characteristics of the study population according to BV status

The characteristics of the study population according to BV status is described in Table 3.

4.4.1 Socio-demographic factors

There was no significant association between any of the socio-demographic variables and BV status. Despite the lack of significance, a lower proportion of married women were BV positive (13.1%) when compared to women who were BV negative (14.9%) (p=0.769). The median age of the women in the BV positive group was 26 years old and the median age of the women who were BV negative was 27 years old, p=0.553.

4.4.2 Behavioural factors

Partner having other partners and alcohol consumption were significantly associated with BV status. A higher proportion of women who were unaware if their partner had other partners were BV positive (63.9%) when compared to women who were BV negative (32.8%), p=0.002. The remaining behavioural factors such as; having a regular sex partner, co-habiting with their sex partner, lifetime number of sex partners, condom use, smoking and intravaginal practices were not significantly associated with BV status (p>0.05).

4.4.3 Clinical factors

None of the clinical factors were significantly associated with BV status, p>0.05. Despite the lack of significance, a higher percentage of women who reported current abnormal vaginal discharge (36.1%) were BV positive when compared to 34.3% of the women who were BV negative. A higher percentage of women who were HIV infected (42.6%) were also BV positive when compared to 38.8% who were BV negative.

Table 3: Characteristics of the study population according to BV status

Bacterial vaginosis	Negative (N=67)	Positive (N=61)	p-value	Overall (N=128)
***Candida* spp.**			0.522	
Negative	27 (40.3%)	28 (45.9%)		55 (43.0%)
Positive	40 (59.7%)	33 (54.1%)		73 (57.0%)
T. vaginalis			0.703	
Negative	59 (88.1%)	55 (90.2%)		114 (89.1%)
Positive	8 (11.9%)	6 (9.8%)		14 (10.9%)
Age			0.553	
Mean±SD (CV %)	28.0±5.92(21.1)	27.4±5.89(21.5)		27.7±5.89(21.3)
Median(Q1-Q3)	27.0(24.0-32.0)	26.0(23.0-31.0)		27.0(24.0-32.0)
Min-Max	19.0-43.0	18.0-43.0		18.0-43.0
Current abnormal vaginal discharge			0.837	
No	44 (65.7%)	39 (63.9%)		83 (64.8%)
Yes	23 (34.3%)	22 (36.1%)		45 (35.2%)
Married			0.769	
No	57 (85.1%)	53 (86.9%)		110 (85.9%)
Yes	10 (14.9%)	8 (13.1%)		18 (14.1%)
Has a regular sex partner			0.683	
No	14 (20.9%)	11 (18.0%)		25 (19.5%)
Yes	53 (79.1%)	50 (82.0%)		103 (80.5%)
Co-habitating with partner			0.661	
No	41 (61.2%)	35 (57.4%)		76 (59.4%)
Yes	26 (38.8%)	26 (42.6%)		52 (40.6%)
Lifetime sex partners			0.613	
1	21 (31.3%)	16 (26.2%)		37 (28.9%)
2-4	36 (53.7%)	38 (62.3%)		74 (57.8%)
>4	10 (14.9%)	7 (11.5%)		17 (13.3%)
Partner has other partners			0.002	
No	23 (34.3%)	9 (14.8%)		32 (25.0%)
Yes	22 (32.8%)	13 (21.3%)		35 (27.3%)
Don't know	22 (32.8%)	39 (63.9%)		61 (47.7%)
Condom use (generally)			0.464	
No	18 (26.9%)	20 (32.8%)		38 (29.7%)
Yes	49 (73.1%)	41 (67.2%)		90 (70.3%)
Condom used during last sex			0.711	
No	46 (68.7%)	40 (65.6%)		86 (67.2%)
Yes	21 (31.3%)	21 (34.4%)		42 (32.8%)
Smokes			0.257	
No	65 (97.0%)	56 (91.8%)		121 (94.5%)
Yes	2 (3.0%)	5 (8.2%)		7 (5.5%)
Alcohol consumption			0.004	
No	62 (92.5%)	45 (73.8%)		107 (83.6%)
Yes	5 (7.5%)	16 (26.2%)		21 (16.4%)
Engages in intravaginal practices			0.637	
No	61 (91.0%)	54 (88.5%)		115 (89.8%)
Yes	6 (9.0%)	7 (11.5%)		13 (10.2%)
Pregnancy trimester			0.872	

1st	7 (10.4%)	6 (9.8%)		13 (10.2%)
2nd	17 (25.4%)	18 (29.5%)		35 (27.3%)
3rd	43 (64.2%)	37 (60.7%)		80 (62.5%)
Past Preterm baby			0.214	
No	49 (73.1%)	50 (82.0%)		99 (77.3%)
Yes	17 (25.4%)	10 (16.4%)		27 (21.1%)
Missing	1 (1.5%)	1 (1.6%)		2 (1.6%)
Past miscarriage			0.609	
No	49 (73.1%)	47 (77.0%)		96 (75.0%)
Yes	18 (26.9%)	14 (23.0%)		32 (25.0%)
Past spontaneous abortion			0.637	
No	61 (91.0%)	54 (88.5%)		115 (89.8%)
Yes	6 (9.0%)	7 (11.5%)		13 (10.2%)
Past abnormal vaginal discharge			0.555	
No	39 (58.2%)	38 (62.3%)		77 (60.2%)
Yes	28 (41.8%)	22 (36.1%)		50 (39.1%)
Missing	0 (0%)	1 (1.6%)		1 (0.8%)
Previous treatment for STIs			0.379	
No	40 (59.7%)	41 (67.2%)		81 (63.3%)
Yes	27 (40.3%)	20 (32.8%)		47 (36.7%)
HIV status			0.661	
Uninfected	41 (61.2%)	35 (57.4%)		76 (59.4%)
Infected	26 (38.8%)	26 (42.6%)		52 (40.6%)

The p-values are based on non-missing cases only (tableStack).

4.5 Risk factors for BV infection

In the unadjusted analysis, not knowing if their partner had other partners and alcohol consumption increased the risk of being BV positive (Table 4). Not knowing if their partner had other partners, increased the risk of being BV positive by 4-fold (OR: 4.11, 95% CI: 1.65 -10.93, p=0.003). Similarly, alcohol consumption also increased the risk of being BV positive by 4-fold (OR: 4.54, 95% CI: 1.64 -14.74, p=0.006). These factors were also significant in the adjusted analysis. In the adjusted analysis, not knowing if their partner had other partners, increased the risk of being BV positive by 5-fold (OR: 5.91, 95% CI: 1.91 -20.52, p=0.003) and alcohol consumption increased the risk of being BV positive by 8-fold (OR: 8.27, 95% CI: 1.99 -42.71, p=0.006). After performing further adjustments in which confounders were removed, not knowing if their partner had other partners and alcohol consumption were still significantly associated with risk for BV. Not knowing if their partner had other partners, increased the risk of being BV positive by 4-fold (OR: 4.05, 95% CI: 1.58 -11.16, p=0.005) and alcohol consumption increased the risk of being BV positive by 4- fold (OR: 4.44, 95% CI: 1.54 -14.96, p=0.009) (Table 4).

Table 4: Risk factors for BV

Factors	Unadjusted Odds Ratio [OR] (Confidence Interval [CI], p-value)	Adjusted Odds Ratio [OR] (Confidence Interval [CI], p-value)	Odds Ratio [OR] (Confidence Interval [CI], p-value) Back Step
Candida positive vs negative	0.77 (0.38-1.57, p=0.47)	0.70 (0.27-1.79, p=0.461)	
***T. vaginalis* positive vs negative**	0.82 (0.26-2.51, p=0.730)	0.67 (0.14-2.93, p=0.594)	
Having current discharge: Yes vs No	1.11 (0.53-2.32, p=0.777)	1.23 (0.47-3.23, p=0.673)	--
Age: Median vs Mode	0.99 (0.93-1.05, p=0.805)	1.00 (0.91-1.10, p=0.990)	--
Married: Yes vs No	0.99 (0.35-2.79, p=0.990)	0.93 (0.21-4.26, p=0.927)	--
Has regular sex partner: Yes vs No	1.17 (0.49-2.89, p=0.720)	0.78 (0.25-2.42, p=0.660)	--
Co-habitating with partner: Yes vs No	1.29 (0.63-2.65, p=0.482)	2.26 (0.77-6.98, p=0.144)	--
Lifetime sex partners: 2-4 vs 1	1.25 (0.56-2.82, p=0.586)	0.66 (0.22-1.92, p=0.445)	
Lifetime sex partners: >4 vs 1	0.88 (0.26-2.80, p=0.823)	0.24 (0.04-1.32, p=0.113)	
Partner has other partners: Yes vs No	1.44 (0.52-4.16, p=0.486)	1.89 (0.54-6.90, p=0.326)	1.45 (0.50-4.33, p=0.498)
Don't know if partner has other partners: don't know vs No	4.11 (1.65-10.93, p=0.003)	5.91 (1.91-20.52, p=0.003)	4.05 (1.58-11.16, p=0.005)
Uses condoms: Yes vs No	0.79 (0.36-1.73, p=0.555)	1.24 (0.43-3.63, p=0.694)	--
Used condom at last sex: Yes vs No	1.10 (0.52-2.33, p=0.805)	0.76 (0.26-2.09, p=0.591)	--
Smokes: Yes vs No	2.33 (0.44-17.25, p=0.340)	2.26 (0.21-28.78, p=0.502)	--
Alcohol consumption: Yes vs No	4.54 (1.64-14.74, p=0.006)	8.27 (1.99-42.71, p=0.006)	4.44 (1.54-14.96, p=0.009
Engages in intravaginal practices: Yes vs No	1.35 (0.42-4.42, p=0.613)	0.85 (0.18-3.73, p=0.828)	--
Pregnancy trimester: 2nd vs 1st	1.10 (0.30-4.09, p=0.887)	1.66 (0.32-8.93, p=0.546)	--
Pregnancy trimester: 3rd vs 1st	1.03 (0.31-3.45, p=0.964)	1.50 (0.32-7.41, p=0.605)	--
Had a previous preterm baby: Yes vs No	0.59 (0.24-1.39, p=0.235)	0.40 (0.12-1.20, p=0.111)	--
Had a past miscarriage: Yes Vs No	0.83 (0.37-1.86, p=0.651)	0.84 (0.28-2.57, p=0.762)	--
Had a past spontaneous abortion Yes vs No	1.35 (0.42-4.42, p=0.613)	1.68 (0.37-7.80, p=0.499	--
Had a past discharge: Yes vs No	0.75 (0.36-1.54, p=0.435)	0.57 (0.14-2.21, p=0.416)	--
Had previous treatment for STIs: Yes vs No	0.74 (0.35-1.53, p=0.420)	1.84 (0.47-7.66, p=0.384	
HIV positive vs negative	1.21 (0.59-2.48, p=0.597	2.77 (0.98-8.46, p=0.062)	

4.6 Characteristics of the study population according to *Candida* status

The characteristics of the study population according to *Candida* status is described in Table 5.

4.6.1 Socio-demographic factors

There was no significant association between any of the socio-demographic variables and *Candida* status. The median age of the women in the *Candida* positive group was 26 years old and the median age of the women who were *Candida* negative was 28 years old, p=0.122 (Table 5).

4.6.2 Behavioural factors

Condom use (generally) and condom use at last sex act was significantly associated with *Candida* status. A higher proportion of women who reported using condoms generally were *Candida* positive (78.1%) when compared to women who were *Candida* negative (60%), p=0.027. In addition, a higher proportion of women who reported using condoms at their last sex act were *Candida* positive (39.7%) when compared to women who were *Candida* negative (23.6%), p=0.055 (Table 5). The remaining behavioural factors such as; having a regular sex partner, co-habiting with sex partner, partner having other partners, lifetime number of sex partners, smoking, alcohol consumption, and intravaginal practices were not significantly associated with *Candida* status (p>0.05) (Table 5).

4.6.3 Clinical factors

Of the clinical factors, current abnormal vaginal discharge, past abortion and HIV status were significantly associated with *Candida* status, p<0.05. With respect to discharge, 43.8% of the women who reported abnormal discharge at study enrolment tested positive for *Candida* when compared to 23.6% who tested negative, p=0.018. A lower proportion of women who had experienced a past abortion (5.5%) tested positive for *Candida* spp. when compared to women who tested negative (16.4%), p=0.044. With respect to HIV status, 49.3% of the women who were HIV infected were also *Candida* positive, p=0.021 (Table 5). The remaining clinical factors such as trimester of pregnancy, past miscarriage, past preterm baby, past abnormal vaginal discharge and previous treatment for STIs were not significantly associated with *Candida* infection, p>0.05 (Table 5).

Table 5: Characteristics of the study population according to *Candida* status

***Candida* spp.**	**Negative (N=55)**	**Positive (N=73)**	**p-value**	**Overall (N=128)**
BV			0.522	
Negative	27 (49.1%)	40 (54.8%)		67 (52.3%)
Positive	28 (50.9%)	33 (45.2%)		61 (47.7%)
T. vaginalis			0.088	
Negative	46 (83.6%)	68 (93.2%)		114 (89.1%)
Positive	9 (16.4%)	5 (6.8%)		14 (10.9%)
Age			0.122	
Mean±SD (CV %)	28.6±6.02(21.1)	27.1±5.75(21.2)		27.7±5.89(21.3)
Median(Q1-Q3)	28.0(24.0-32.5)	26.0(22.0-30.0)		27.0(24.0-32.0)
Min-Max	18.0-42.0	18.0-43.0		18.0-43.0
Current abnormal vaginal discharge			0.018	
No	42 (76.4%)	41 (56.2%)		83 (64.8%)
Yes	13 (23.6%)	32 (43.8%)		45 (35.2%)
Married			0.516	
No	46 (83.6%)	64 (87.7%)		110 (85.9%)
Yes	9 (16.4%)	9 (12.3%)		18 (14.1%)
Has regular sex partner			0.908	
No	11 (20.0%)	14 (19.2%)		25 (19.5%)
Yes	44 (80.0%)	59 (80.8%)		103 (80.5%)
Co-habitating with partner			0.334	
No	30 (54.5%)	46 (63.0%)		76 (59.4%)
Yes	25 (45.5%)	27 (37.0%)		52 (40.6%)
Lifetime sex partners			0.469	
1	19 (34.5%)	18 (24.7%)		37 (28.9%)
2-4	29 (52.7%)	45 (61.6%)		74 (57.8%)
>4	7 (12.7%)	10 (13.7%)		17 (13.3%)
Partner has other partners			0.414	
No	16 (29.1%)	16 (21.9%)		32 (25.0%)
Yes	12 (21.8%)	23 (31.5%)		35 (27.3%)
Don't know	27 (49.1%)	34 (46.6%)		61 (47.7%)
Condom use			0.027	
No	22 (40.0%)	16 (21.9%)		38 (29.7%)
Yes	33 (60.0%)	57 (78.1%)		90 (70.3%)
Condom used during last sex			0.055	
No	42 (76.4%)	44 (60.3%)		86 (67.2%)
Yes	13 (23.6%)	29 (39.7%)		42 (32.8%)
Smokes			0.138	

No	50 (90.9%)	71 (97.3%)		121 (94.5%)
Yes	5 (9.1%)	2 (2.7%)		7 (5.5%)
Alcohol consumption			0.991	
No	46 (83.6%)	61 (83.6%)		107 (83.6%)
Yes	9 (16.4%)	12 (16.4%)		21 (16.4%)
Intravaginal practices			0.349	
No	51 (92.7%)	64 (87.7%)		115 (89.8%)
Yes	4 (7.3%)	9 (12.3%)		13 (10.2%)
Pregnancy trimester			0.879	
1st	6 (10.9%)	7 (9.6%)		13 (10.2%)
2nd	16 (29.1%)	19 (26.0%)		35 (27.3%)
3rd	33 (60.0%)	47 (64.4%)		80 (62.5%)
Preterm baby			0.802	
No	43 (78.2%)	56 (76.7%)		99 (77.3%)
Yes	11 (20.0%)	16 (21.9%)		27 (21.1%)
Missing	1 (1.8%)	1 (1.4%)		2 (1.6%)
Past miscarriage			0.471	
No	43 (78.2%)	53 (72.6%)		96 (75.0%)
Yes	12 (21.8%)	20 (27.4%)		32 (25.0%)
Past spontaneous abortion			0.044	
No	46 (83.6%)	69 (94.5%)		115 (89.8%)
Yes	9 (16.4%)	4 (5.5%)		13 (10.2%)
Past abnormal vaginal discharge			0.231	
No	36 (65.5%)	41 (56.2%)		77 (60.2%)
Yes	18 (32.7%)	32 (43.8%)		50 (39.1%)
Missing	1 (1.8%)	0 (0%)		1 (0.8%)
Previous treatment for STIs			0.416	
No	37 (67.3%)	44 (60.3%)		81 (63.3%)
Yes	18 (32.7%)	29 (39.7%)		47 (36.7%)
HIV status			0.021	
Uninfected	39 (70.9%)	37 (50.7%)		76 (59.4%)
Infected	16 (29.1%)	36 (49.3%)		52 (40.6%)

4.7 Risk factors for *Candida* infection

In the unadjusted analysis having a current abnormal vaginal discharge, using condoms (generally and at last sex act) and HIV infection were significantly associated with the risk for *Candida* infection. Having a current abnormal vaginal discharge increased the risk of *Candida* infection by 2-fold (OR: 2.46, 95% CI: 1.15- 5.50, p=0.024). Condom use (generally and at last sex act) both increased the risk of *Candida* infection by 2-fold (OR: 2.30, p=0.040). In addition, being HIV positive also increased the risk of *Candida* infection by 2-fold (OR: 2.31, 95% CI: 1.11 – 4.96, p=0.028) (Table 6).

In the adjusted analysis, having a current abnormal vaginal discharge, had a spontaneous abortion and HIV infection were significantly associated with the risk for *Candida* infection. In this analysis, women who had a current abnormal vaginal discharge had a 4-fold risk of testing positive for *Candida* infection (OR: 4.10, 95% CI: 1.46 – 12.73, p=0.010). In addition, being HIV infected increased the risk of testing positive for *Candida* infection by 6-fold (OR: 6.73, 95% CI: 2.08 – 25.45, p=0.003) (Table 6). After performing further adjustments, current abnormal vaginal discharge was still significantly associated with *Candida* infection (OR: 3.63, 95% CI: 1.45- 9.90, p=0.008) (Table 6).

Table 6: Risk factors for *Candida* infection

Factors	Unadjusted Odds Ratio [OR] (Confidence Interval [CI], p-value)	Adjusted Odds Ratio [OR] (Confidence Interval [CI], p-value)	Odds Ratio [OR] (Confidence Interval [CI], p-value) Back Step
BV positive vs negative	0.77 (0.38-1.57, p=0.472)	0.73 (0.27-1.91, p=0.521)	---
***T. vaginalis* positive vs negative**	0.36 (0.11-1.13, p=0.088)	0.07 (0.01-0.34, p=0.002)	0.08 (0.02-0.33, p=0.001)
Having current discharge: Yes vs No	2.46 (1.15-5.50, p=0.024)	4.10 (1.46-12.73, p=0.010)	3.63 (1.45-9.90, p=0.008)
Married: Yes vs No	0.80 (0.29-2.29, p=0.676)	0.78 (0.15-4.07, p=0.770)	--
Has regular sex partner: Yes vs No	1.09 (0.44-2.62, p=0.856)	2.29 (0.69-7.92, p=0.179)	--
Co-habitating with partner: Yes vs No	0.73 (0.35-1.49, p=0.382)	0.92 (0.28-3.05, p=0.893)	--
Lifetime sex partners: 2-4 vs 1	1.57 (0.70-3.54, p=0.272)	1.39 (0.45-4.40, p=0.567)	
Lifetime sex partners: >4 vs 1	1.43 (0.45-4.73, p=0.549)	1.51 (0.22-10.64, p=0.671)	
Partner has other partners: Yes vs No	1.80 (0.67-4.93, p=0.247)	0.95 (0.25-3.53, p=0.937)	
Don't know if partner has other partners: don't know vs No	1.19 (0.50-2.86, p=0.696)	0.57 (0.16-1.91, p=0.367)	
Uses condoms: Yes vs No	2.30 (1.05-5.17, p=0.040)	1.62 (0.56-4.72, p=0.372)	2.10 (0.85-5.28, p=0.109)
Used condom at last sex: Yes vs No	2.30 (1.06-5.26, p=0.040)	2.05 (0.69-6.44, p=0.204)	--
Smokes: Yes vs No	0.35 (0.05-1.87, p=0.236)	0.29 (0.02-3.30, p=0.321)	--
Alcohol consumption: Yes vs No	0.98 (0.38-2.59, p=0.963)	1.08 (0.23-5.40, p=0.922)	
Engages in intravaginal practices: Yes vs No	1.75 (0.54-6.77, p=0.375)	1.76 (0.38-9.21, p=0.479)	--
Pregnancy trimester: 2nd vs 1st	1.03 (0.28-3.76, p=0.966)	1.89 (0.31-11.48, p=0.483)	--

Pregnancy trimester: 3rd vs 1st	1.26 (0.37-4.13, p=0.702)	3.08 (0.53-18.66, p=0.210)	--
Had a previous preterm baby: Yes vs No	1.09 (0.46-2.65, p=0.844)	1.03 (0.29-3.78, p=0.963)	--
Had a past miscarriage: Yes Vs No	1.31 (0.58-3.06, p=0.516)	2.25 (0.64-8.72, p=0.218)	--
Had a past spontaneous abortion Yes vs No	0.29 (0.07-0.94, p=0.048)	0.15 (0.03-0.74, p=0.023)	--
Had a past discharge: Yes vs No	1.47 (0.71-3.10, p=0.304)	1.63 (0.38-7.09, p=0.509)	--
Had previous treatment for STIs: Yes vs No	1.31 (0.63-2.77, p=0.472)	0.43 (0.10-1.82, p=0.256)	
HIV positive vs negative	2.31 (1.11-4.96, p=0.028)	6.73 (2.08-25.45, p=0.003)	

4.8 Characteristics of the study population according to *T. vaginalis* status

The characteristics of the study population according to *T. vaginalis* status is described in Table 7.

4.8.1 Socio-demographic factors

There was no significant association between any of the socio-demographic variables and *T. vaginalis* status. The median age of the women in the *T. vaginalis* positive group was 27 years old and the median age of the women who were *T. vaginalis* negative was 26.5 years old, p=0.494 (Table 7).

4.8.2 Behavioural factors

Similarly, there were no behavioural factors that were significantly associated with *T. vaginalis* status. However, a higher proportion of women who tested positive for *T. vaginalis* reported having a regular sex partner (35.7%) when compared to the women who tested negative (17.5%), p=0.147. A higher proportion of the women who had reported 2-4 lifetime sex partners (71.4%) were *T. vaginalis* positive when compared to the women who were negative (56.1%), p=0.470. In addition, a higher proportion of women who reported that their partner had other partners (42.9%) tested positive for *T. vaginalis* when compared to women who remained negative (25.4%), p=0.379. The majority of the positive women reported not using condoms at their last sex act (71.4%) when compared to the women who were negative (66.7%), p=1.000 (Table 7).

4.8.3 Clinical factors

Of the clinical factors, only HIV status was significantly associated with *T. vaginalis* status, p=0.002. A higher proportion of HIV infected women (78.6%) also tested positive for *T. vaginalis* (Table 7). The remaining clinical factors such as current and past abnormal vaginal discharge, trimester of pregnancy, past miscarriage, past preterm baby and previous treatment for STIs were not significantly associated with *T. vaginalis* infection status, p>0.05 (Table 7).

Table 7: Characteristics of the study population according to *T. vaginalis* status

T. vaginalis	**Negative (N=114)**	**Positive (N=14)**	**p-value**	**Overall (N=128)**
BV			0.703	
Negative	59 (51.8%)	8 (57.1%)		67 (52.3%)
Positive	55 (48.2%)	6 (42.9%)		61 (47.7%)
***Candida* spp.**			0.088	
Negative	46 (40.4%)	9 (64.3%)		55 (43.0%)
Positive	68 (59.6%)	5 (35.7%)		73 (57.0%)
Current abnormal vaginal discharge			0.081	
No	77 (67.5%)	6 (42.9%)		83 (64.8%)
Yes	37 (32.5%)	8 (57.1%)		45 (35.2%)
Age			0.494	
Mean±SD (CV %)	27.7±6.02(21.8)	28.3±4.86(17.2)		27.7±5.89(21.3)
Median(Q1-Q3)	26.5(23.3-32.0)	27.0(25.3-32.8)		27.0(24.0-32.0)
Min-Max	18.0-43.0	20.0-35.0		18.0-43.0
Married			0.691	
No	97 (85.1%)	13 (92.9%)		110 (85.9%)
Yes	17 (14.9%)	1 (7.1%)		18 (14.1%)
Has a regular sex partner			0.147	
No	20 (17.5%)	5 (35.7%)		25 (19.5%)
Yes	94 (82.5%)	9 (64.3%)		103 (80.5%)
Co-habitating with partner			0.449	
No	69 (60.5%)	7 (50.0%)		76 (59.4%)
Yes	45 (39.5%)	7 (50.0%)		52 (40.6%)
Lifetime sex partners			0.470	
1	35 (30.7%)	2 (14.3%)		37 (28.9%)
2-4	64 (56.1%)	10 (71.4%)		74 (57.8%)
>4	15 (13.2%)	2 (14.3%)		17 (13.3%)
Partner has other partners			0.379	
No	29 (25.4%)	3 (21.4%)		32 (25.0%)
Yes	29 (25.4%)	6 (42.9%)		35 (27.3%)
Don’t know	56 (49.1%)	5 (35.7%)		61 (47.7%)
Condom use			0.228	
No	36 (31.6%)	2 (14.3%)		38 (29.7%)
Yes	78 (68.4%)	12 (85.7%)		90 (70.3%)
Condom used during last sex			1.000	
No	76 (66.7%)	10 (71.4%)		86 (67.2%)
Yes	38 (33.3%)	4 (28.6%)		42 (32.8%)
Smokes			0.565	

No	108 (94.7%)	13 (92.9%)		121 (94.5%)
Yes	6 (5.3%)	1 (7.1%)		7 (5.5%)
Alcohol consumption			0.464	
No	94 (82.5%)	13 (92.9%)		107 (83.6%)
Yes	20 (17.5%)	1 (7.1%)		21 (16.4%)
Intravaginal practices			1.000	
No	102 (89.5%)	13 (92.9%)		115 (89.8%)
Yes	12 (10.5%)	1 (7.1%)		13 (10.2%)
Pregnancy trimester			0.829	
1st	12 (10.5%)	1 (7.1%)		13 (10.2%)
2nd	30 (26.3%)	5 (35.7%)		35 (27.3%)
3rd	72 (63.2%)	8 (57.1%)		80 (62.5%)
Preterm baby			0.177	
No	90 (78.9%)	9 (64.3%)		99 (77.3%)
Yes	22 (19.3%)	5 (35.7%)		27 (21.1%)
Missing	2 (1.8%)	0 (0%)		2 (1.6%)
Past miscarriage			0.748	
No	86 (75.4%)	10 (71.4%)		96 (75.0%)
Yes	28 (24.6%)	4 (28.6%)		32 (25.0%)
Past spontaneous abortion			1.000	
No	102 (89.5%)	13 (92.9%)		115 (89.8%)
Yes	12 (10.5%)	1 (7.1%)		13 (10.2%)
Past discharge			0.149	
No	71 (62.3%)	6 (42.9%)		77 (60.2%)
Yes	42 (36.8%)	8 (57.1%)		50 (39.1%)
Missing	1 (0.9%)	0 (0%)		1 (0.8%)
Previous treatment for STIs			0.275	
No	74 (64.9%)	7 (50.0%)		81 (63.3%)
Yes	40 (35.1%)	7 (50.0%)		47 (36.7%)
HIV status			0.002	
Uninfected	73 (64.0%)	3 (21.4%)		76 (59.4%)
Infected	41 (36.0%)	11 (78.6%)		52 (40.6%)

The p-values are based on non-missing cases only (tableStack).

4.9 Risk factors for *T. vaginalis* infection

In the unadjusted analysis, only HIV infection was significantly associated with the risk for *T. vaginalis* infection. Women who were HIV infected had a 6-fold likelihood of testing positive for *T. vaginalis* (OR: 6.26, 95% CI: 1.83 – 28.87, p=0.007) (Table 8). This association was still significant in the adjusted analysis. In the adjusted analysis, being HIV infected increased the risk for *T. vaginalis* by 22-fold (OR: 22.34, 95% CI: 2.88 – 303.73, p=0.007). In the adjusted analysis, current abnormal vaginal discharge was also significantly associated with *T. vaginalis* infection, women who had reported having a current abnormal vaginal discharge had an 8-fold likelihood of testing positive for *T. vaginalis* (OR: 8.89, 95% CI: 1.23 – 104.51, p=0.047) (Table 8). After performing further adjustments being HIV infected and a current abnormal vaginal discharge were still associated with the risk for infection. Having a current abnormal vaginal discharge increased the risk of infection by 5-fold (OR: 5.37, 95% CI: 1.39 – 24.59, p=0.019) and HIV infection increased the risk of infection by 23-fold (OR: 23.25, 95% CI: 4.52 – 174.17, p=0.001) (Table 8).

Table 8: Risk factors for *T. vaginalis* infection

Factors	Unadjusted Odds Ratio [OR] (Confidence Interval [CI], p-value)	Adjusted Odds Ratio [OR] (Confidence Interval [CI], p-value)	Odds Ratio [OR] (Confidence Interval [CI], p-value) Back Step
BV positive vs negative	0.82 (0.26-2.51, p=0.730)	1.07 (0.15-8.05, p=0.945)	---
***Candida* spp. positive vs negative**	0.36 (0.11-1.13, p=0.088)	0.04 (0.00-0.29, p=0.006)	0.07 (0.01-0.35, p=0.002)
Having current discharge: Yes vs No	2.67 (0.87-8.64, p=0.089)	8.89 (1.23-104.51, p=0.047)	5.37 (1.39-24.59, p=0.019)
Married: Yes vs No	0.46 (0.02-2.55, p=0.465)	0.59 (0.01-12.13, p=0.743)	--
Has regular sex partner: Yes vs No	0.40 (0.12-1.40, p=0.128)	0.51 (0.07-3.67, p=0.481)	--
Co-habitating with partner: Yes vs No	1.52 (0.49-4.74, p=0.460)	4.33 (0.64-35.81, p=0.140)	2.84 (0.73-12.30, p=0.140)
Lifetime sex partners: 2-4 vs 1	2.74 (0.67-18.51, p=0.209)	1.96 (0.20-27.02, p=0.576)	
Lifetime sex partners: >4 vs 1	2.27 (0.25-20.39, p=0.434)	1.62 (0.04-79.94, p=0.797)	
Partner has other partners: Yes vs No	1.93 (0.46-9.86, p=0.383)	0.62 (0.04-7.89, p=0.707)	
Don't know if partner has other partners: don't know vs No	0.86 (0.20-4.46, p=0.849)	0.29 (0.02-4.14, p=0.366)	
Uses condoms: Yes vs No	2.54 (0.65-16.89, p=0.239)	2.27 (0.25-27.86, p=0.480)	
Used condom at last sex: Yes vs No	0.80 (0.21-2.57, p=0.721)	0.63 (0.09-4.02, p=0.627)	--
Smokes: Yes vs No	1.63 (0.08-11.20, p=0.666)	14.40 (0.17-2400.95, p=0.224)	--
Alcohol consumption: Yes vs No	0.35 (0.02-1.92, p=0.325)	0.21 (0.00-6.65, p=0.453)	
Engages in intravaginal practices: Yes vs No	0.63 (0.03-3.65, p=0.674)	0.09 (0.00-2.06, p=0.197)	--
Pregnancy trimester: 2nd vs 1st	2.14 (0.30-43.38, p=0.507)	3.92 (0.23-194.96, p=0.394)	--
Pregnancy trimester: 3rd vs 1st	1.35 (0.22-26.24, p=0.785)	3.53 (0.26-157.36, p=0.406)	--
Had a previous preterm baby: Yes vs No	2.25 (0.64-7.21, p=0.182)	2.24 (0.32-17.26, p=0.408)	--
Had a past miscarriage: Yes Vs No	1.19 (0.31-3.86, p=0.787)	1.34 (0.15-11.17, p=0.784)	--
Had a past spontaneous abortion Yes vs No	0.63 (0.03-3.65, p=0.674)	0.20 (0.00-3.69, p=0.335)	--
Had a past discharge: Yes vs No	2.28 (0.74-7.35, p=0.152)	3.41 (0.32-53.78, p=0.332)	--
Had previous treatment for STIs: Yes vs No	1.77 (0.57-5.54, p=0.314)	0.58 (0.04-6.22, p=0.657)	
HIV positive vs negative	6.26 (1.83-28.87, p=0.007)	22.34 (2.88-303.73, p=0.007)	23.25 (4.52-174.17, p=0.001)

CHAPTER 5

5. 1 DISCUSSION

In this study, we describe the prevalence of HIV infection in pregnant women and risk factors related to HIV acquisition in pregnancy. We further describe prevalence of vaginal pathogens in HIV infected and uninfected pregnant women and the risk factors thereof.

The HIV prevalence rate in South Africa (29.1-30.8%) is the highest worldwide when compared to Brazil (0.38-0.42%), Italy (21-24.4%), Cameroon (4-10%), Zimbabwe (12-16%) and Nigeria (4-15%)(17-30). In this study, the HIV prevalence in this antenatal group of participants was 40.6% and this prevalence rate was similar to rates observed in rural KwaZulu-Natal during the period 2009-2015 (37.0-44.4%) (64). KwaZulu-Natal still has a notably high HIV prevalence rate when compared to the rest of South Africa since 1990 (30). With these findings, we therefore conclude that KwaZulu-Natal still has a high burden of HIV infection during pregnancy and most likely more adverse obstetric complications such as preterm labor, the latter needing more investigations. The high HIV prevalence reported for the studied population could be due to a combination of demographic, behavioral and clinical factors. In this study, age (25-34 years old), having a greater number of lifetime sexual partners, having a prevalent *T. vaginalis* and *Candida* infection and being treated for STIs in the past were significantly associated with HIV infection. A study conducted in a population of pregnant women from Cameroon showed a significant association between increasing age and being unmarried with the risk for HIV infection (25). A more recent study conducted by Hamda *et al* (65) in pregnant women from Botswana also reported an association between older age and HIV infection. Based on these findings, there is a need for prevention strategies which are targeted towards pregnant women who are older. In the current study, an increasing number of lifetime sexual partners was significantly associated with HIV infection. Similarly, studies conducted by Lukhele *et al* (66) and Rosenberg *et al* (67) in pregnant women from Swaziland and Malawi, respectively also showed an association between increasing number of lifetime sexual partners and HIV infection. Findings from the study conducted by Price *et al* (68) observed a high prevalence of *T. vaginalis* in a population of HIV infected pregnant women

seeking antenatal care at public health centers in South Africa. Similarly, in the current study, a significant association was observed for *T. vaginalis* and HIV infection. A recent study conducted by Varnasiri *et al (69)* reported a high prevalence of vulvovaginal *Candida* in a population of HIV infected women. However, there is a dearth of studies which have explored the prevalence of *Candida* infection in pregnant women stratified by HIV status, thus the findings of this study fills this gap in the literature.

In this study, after performing a further stepwise adjustment, testing positive for BV, *Candida* and *T. vaginalis*, lifetime number of sex partners and alcohol consumption were significantly associated with increased risk for HIV. Taha *et al* (70) reported that sexually transmitted infections such as *T. vaginalis* and genital tract infections such as BV increases the risk of acquiring HIV infection in pregnancy. Based on these findings it is recommended that routine screening for BV, *Candida* and *T. vaginalis* infections among pregnant women should be encouraged in order to reduce the risk of HIV acquisition in our setting. Our study reported a significant association between alcohol consumption and HIV risk. Identifying the factors associated with alcohol use among HIV infected pregnant women residing in high HIV prevalence settings such as South Africa may be critical to improving adherence and HIV treatment outcomes (71).

In this study, the most prevalent vaginal infection identified in the HIV infected and uninfected women were *Candida* spp. (69. 2% vs 48.7%), followed by BV (50% vs 46.1%) and *T. vaginalis* (21.2% vs 3.9%), respectively. A higher prevalence of these infections were observed for the HIV infected women when compared to the HIV uninfected women in this study cohort. Previous studies have shown that vaginitis pathogens recovery is higher in HIV infected pregnant women and results in greater peripartum complications (41, 72). A study which compared the prevalence of vaginitis pathogens in women from the USA and Africa revealed that the most common pathogens were *C. albicans* and *T. vaginalis* with a higher prevalence observed in HIV infected pregnant women (41). A previous study conducted in South Africa also revealed that the most common vaginitis pathogens in pregnancy include *T. vaginalis* (52%), BV (21%) and *Candida* spp. (10%) (44). In addition, a study conducted in a primary health clinic in KwaZulu-Natal detected *T. vaginalis* (15,3%) as the most prevalent vaginitis pathogen in pregnancy and the prevalence of

T. vaginalis was 1.8 times higher in the HIV infected pregnant women when compared to the uninfected women (73).

Candida spp. was shown to be the most prevalent vaginitis pathogen in the study population (57%). HIV infected women had the highest rate of *Candida* vaginitis (69.2%) when compared to the uninfected women (48.7%). The prevalence rate of *Candida* infection reported in this study was notably higher when compared to other countries such as Jamaica (30.7%) and Ethiopia (25%) (62, 63). However, the prevalence estimates reported in this study are similar to findings from other South African studies (37, 41). However, there is a lack of prevalence data for *Candida* spp. in HIV infected pregnant woman from KwaZulu-Natal. This study fills in this gap in the literature.

The risk factors identified in this study, which contributed to the acquisition of *Candida* infection, included; having a current abnormal vaginal discharge, condom use and HIV status. These findings are in keeping with an Ethiopian study, however in our findings, no statistical significance was observed with trimester of pregnancy, number of pregnancies and previous treatment for STIs (63). We observed that 43.8% of pregnant women who presented with abnormal vaginal discharge at enrolment tested positive for *Candida*. Studies conducted on pregnant women from Italy and Jamaica have also shown a significant association between abnormal vaginal discharge and *Candida* infection (44, 61).

Literature has reported that condom use is associated with increased risk of acquiring vaginal candidiasis (67). This has been understood to occur as a consequence of the spermicidal preparation used in condoms which disrupt the vaginal microbiota balance and additionally promotes adherence of *Candida* spp. to vaginal epithelial cells resulting in a shift of the Lactobacilli dominant vaginal microbiota (74). In this study, being HIV infected increased the risk of *Candida* vaginitis. These findings are confirmed by a study conducted in Brazil that also described higher rates of Candidiasis in HIV infected pregnant women when compared to HIV uninfected pregnant women (75).

In this study, an overall prevalence of 10.9% (14/128) was reported for *T. vaginalis* in the studied population. However, a higher prevalence of infection was found in HIV infected women (21.2%)

when compared to HIV uninfected women (3.9%). A study conducted by Mudau *et al* (76) in South African HIV infected pregnant women also reported high rates of *T. vaginalis* infection (23.9%). Another South African study also reported high rates for *T. vaginalis* infection (20%) in HIV infected pregnant women (68). Of the study women who were infected with *T. vaginalis*; 42.9% of the women had BV and 35.7% had *Candida* infections. After a survey of the literature, there was a lack of published data on the prevalence of *T. vaginalis*, BV and *Candida* co-infections in pregnant women. This study adds to the body of evidence on vaginal co-infections during pregnancy.

Only HIV status has been identified as risk factor for acquisition of *T. vaginalis* were identified in this study population. In a recent study conducted by Kim *et al* (77), younger age was significantly associated with the risk for *T. vaginalis* infection. A study conducted in South African pregnant women found a significant association between prevalent HIV infection and the risk for acquiring STIs including *T. vaginalis* (78). Even though other risk factors were not statistically significant it was noted that the majority of the women reported having a regular sex partner, having 2-4 lifetime sex partners and lack of condom use. This correlated with findings from a Papua New Guinea study which also identified behavioral risk factors such as multiple partners to be associated with high infection rates (79). Women with *T. vaginalis* infections should be counselled on the use of condoms and the risk of new infections as a result of behavioral practices.

The prevalence of BV in the study cohort was 47.7% and this rate was higher than prevalence rates reported for Gauteng (17.4%), Botswana (38%), India (20.5%), Sweden (9.3%) and Zimbabwe (32.5%) (54, 57, 58). Notably a higher proportion of HIV infected pregnant women (42.6%) were diagnosed with BV when compared to HIV uninfected pregnant women (38.8%). However, in this study there was no significant association between BV prevalence and HIV status. This confirms the findings by Kamga *et al* (56) and Shayo *et al* (80) stating that there is no strong relationship between BV and HIV infection (56). However, there are conflicting studies that highlight a strong relationship between HIV and BV infection as reported by Mbu *et al* (81) who observed a higher prevalence of BV in HIV infected pregnant women when compare to HIV uninfected pregnant women (81). In terms of risk factors, none of the socio-demographic and clinical factors were significantly associated with BV acquisition in pregnancy. The significant risk factors included;

partner having other partners and alcohol consumption. Both these variables showed a 4-fold increase in the risk of BV acquisition. A study conducted in a Danish population also observed a strong association between alcohol use and BV infection in pregnancy (59). Therefore, in this study cohort, it is suggested that behavioral factors play a major role in increasing risk of acquiring BV infection during pregnancy. In this study, women who had BV also had been co-infected with *Candida* spp. (54.1%) and *T. vaginalis* (9.8%). However, a lower proportion of participants were HIV positive. Sobet *et al* (56) also reported a BV co-infection with *T. vaginalis* and *Candida* spp. Based on these findings, women with BV need to be counselled on modifying their behavior and reducing their risk of acquiring other vaginal infections.

5.2 LIMITATIONS

The study had the following limitations; firstly, sampling was performed at a single antenatal clinic, therefore the data is not reflective of all pregnant women from KwaZulu-Natal. Despite this limitation, we were able to describe factors which were significantly associated with HIV infection and vaginitis pathogens. Secondly, due to budgetary constraints, we were unable to investigate other pathogens that may be associated with vaginal infections such as genital mycoplasmas. However, this can be a future research endeavor.

5.3. CONCLUSION

In sub-Saharan Africa, the risk for HIV infection is greatest among women of reproductive age and a high incidence of infection is reported during pregnancy(34). In this study, a prevalence rate of 40.6% for HIV was reported in a population of pregnant women. The continuous rate of new HIV transmission cases within young women of reproductive age is however still concerning despite the widespread access to information and testing at health facilities. Behavioral factors noted in this study which contributed to the high risk of HIV acquisition included; inconsistent condom use and multiple partners. We suggest including men to play major role in helping to improve behavioral changes especially when it comes to consistent condom use.

In this study, prevalent *Candida, T. vaginalis* and BV infections were also shown to be risk factors for HIV infection. Many of the study women were also co-infected with all three pathogens. We also recommend routine screening of the most common vaginitis pathogens during each clinic visit and prompt treatment to decrease risk of HIV transmission and therefore negate obstetric complications such as preterm labor aggravated by these vaginitis pathogens. For all three pathogens, the most common behavioral factor shown to be associated with infection was condom use. Changing behavior in regards to condom use is a complex task for women. Women must first perceive themselves to be at risk for contracting infections before they can be motivated to reduce that risk. Therefore, women will need to be made aware of their actual risk of contracting these infections. This can be accomplished by conducting future studies which administer a risk assessment tool to the women so that they can distinguish actual risk from perceived risk.

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APPENDIX 1

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

09 July 2020

Dr Ntokozo Mzimela (200267163)
School of Lab Med & Medical Sc
Medical School

Dear Dr Mzimela,

Protocol reference number: BREC/00001494/2020
Project title: Assessing the diversity of the vaginal microbiome in HIV infected and uninfected pregnant women
Degree Purposes: MMed

EXPEDITED APPLICATION: APPROVAL LETTER

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application.

The conditions have been met and the study is given full ethics approval and may begin as from 09 July 2020. Please ensure that outstanding site permissions are obtained and forwarded to BREC for approval before commencing research at a site.

This approval is subject to national and UKZN lockdown regulations dated 5th June 2020 which is available on the BREC website (http://research.ukzn.ac.za/Libraries/BREC/Proposed_UKZN_BREC_revision_to_research_constraints_anticipating_change_to_Level_3_lockdown.sflb.ashx).

This approval is valid for one year from 09 July 2020. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be noted by a full Committee at its next meeting taking place on 11 August 2020.

Yours sincerely

[redacted]

Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

Biomedical Research Ethics Committee
Chair: Professor D R Wassenaar
UKZN Research Ethics Office Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
Email: BREC@ukzn.ac.za
Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx

APPENDIX 2

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

22 July 2020

Dr Ntokozo Mzimela (200267163)
School of Laboratory Medicine & Medical Science
Medical School

Dear Dr Mzimela,

Protocol reference number: BREC/00001494/2020
Project title: Assessing the diversity of the vaginal microbiome in HIV infected and uninfected pregnant women
Degree Purposes: MMed
NEW TITLE: ***To investigate the differences in the vaginal microbiome during pregnancy in HIV infected and uninfected pregnant women***

We wish to advise you that your correspondence received on 14 July 2020 submitting an application for amendments to change the title to the new title above has been **noted and approved** by a subcommittee of the Biomedical Research Ethics Committee.

The committee will be notified of the above at its next meeting taking place on 11 August 2020.

Yours sincerely

[redacted]

Ms A Marimuthu
(for) Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

Biomedical Research Ethics Committee
Chair: Professor D R Wassenaar
UKZN Research Ethics Office Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
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APPENDIX 3

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

04 September 2017

Dr N Abbai
Discipline of Chemical Pathology
School of Clinical Medicine
abbain@ukzn.ac.za

Dear Dr Abbai

Protocol: Laboratory-based detection of microflora associated with vaginitis in pregnant women presenting at a KwaZulu-Natal hospital. Degree: Non-degree
BREC reference number: BE214/17

EXPEDITED APPROVAL

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application received on 22 May 2017.

The study was provisionally approved pending appropriate responses to queries raised. Your response received on 23 August 2017 to BREC letter dated 28 June 2017 have been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given **full ethics approval** and may begin as from 04 September 2017.

This approval is valid for one year from **04 September 2017**. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be RATIFIED by a full Committee at its next meeting taking place on 10 October 2017.

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

[redacted]

Professor J Tsoka-Gwegweni
Chair: Biomedical Research Ethics Committee

cc postgraduate administrator: [redacted]

Biomedical Research Ethics Committee
Professor J Tsoka-Gwegweni (Chair)
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