KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

KUMASI.

COLLEGE OF HEALTH SCIENCE

SCHOOL OF MEDICAL SCIENCE

DEPARTMENT OF CLINICAL MICROBIOLOGY

PREVALENCE OF BACTERIAL VAGINOSIS, TRICHOMONIASIS AND VULVOVAGINAL CANDIDIASIS IN PREGNANT WOMEN ATTENDING ANTENATAL CLINIC AT THE KINTAMPO MUNICIPAL HOSPITAL, KINTAMPO

BY

DENNIS GYASI KONADU,

JULY 2015

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DECLARATION

The experimental work described in this thesis was carried out at the Department of Clinical Microbiology, KNUST. Any assistance obtained has been duly acknowledged. This work has not been submitted for any other degree.

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ABSTRACT

Introduction: Vaginal infections are usually caused by *Candida albicans, Trichomonas vaginalis* and organisms responsible for bacterial vaginosis including *Gardneralla vaginalis, Bacteriodes spp,* and *Mobilincus.* These infections in pregnancy are associated with considerable discomfort and adverse pregnancy outcome including preterm delivery, low birth weight, miscarriage.

Objectives: The study determined the prevalence of bacterial vaginosis, trichomoniasis and vulvovaginal candidiasis in pregnant women attending antenatal clinic at the Kintampo Municipal Hospital.

Methods: A prospective study of 589 consecutive vaginal swabs of pregnant women was taken after administration of a semi-structured questionnaire. The samples were analysed using wet mount, culture and Gram stain for vaginal infection. Univariate and multivariate analysis were used to investigate association of vaginal symptoms and risk factors to vaginal infections.

Results: The overall prevalence of vaginal infections was 56.4%. The individual prevalence of bacterial vaginosis, trichomoniasis and vulvovaginal candidiasis was 30.6%, 1.4% and 36.5% respectively. In multivariate analysis, vaginal symptom pruritus was significantly associated with vulvovaginal candidiasis. Considering number of pregnancies, less than three pregnancies was an independent risk factor for bacterial vaginosis. Being in the third trimester of pregnancy was however found to be protective to bacterial vaginosis.

Conclusion: The prevalence of vaginal infections was high among pregnant women in Kintampo. The data confirms pruritus as a key symptom of vulvovaginal candidiasis. Adequate investigations and early treatment of vaginal infections will reduce the disease burden and avoid complications associated with it.

DEDICATION

I dedicate this work to my parents K. K. Akyeampong and Sussana Mansah, my siblings Dorcas Konadu and Kwame Opoku Konadu.

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I thank the Almighty God for making it possible for me to go through this course successfully. My heartfelt gratitude goes to Dr. Alexander Owusu Ofori (my project supervisor) for his unflinching support and guidance. His patience, kindness and encouragements helped me immensely in carrying out this work.

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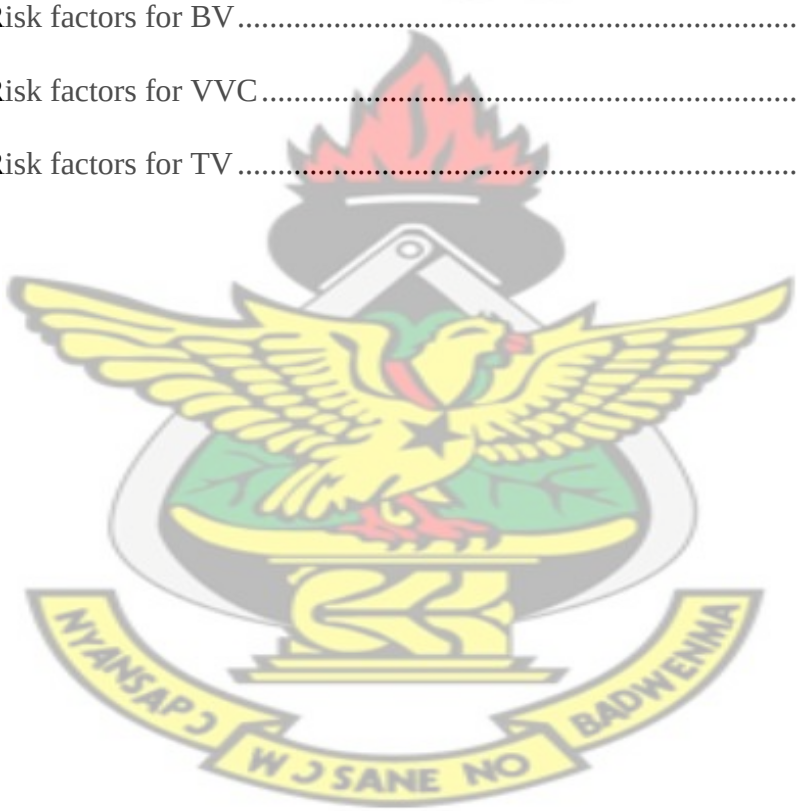

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LIST OF ABBREVIATIONS

ANC	Antenatal Clinic
BV	Bacterial vaginosis
CDC	Centre for disease Control and Prevention
HIV	Human Immune Deficiency Virus
HPF	High-Power Field
HSV-2	Herpes Simplex Virus Type-2
KHRC	Kintampo Health Research Centre
KHRC - IEC	Kintampo Health Research Centre -Institutional Ethics Committee
KHRC – SRC	Kintampo Health Research Centre - Scientific Review Committee
KMH	Kintampo Municipal Hospital
OR	Odd Ratio
PCR	Polymerase Chain Reaction
PID	Pelvic Inflammatory Disease
PROM	Premature Rupture Of Membrane
ReTI	Reproductive Tract Infection
STD	Sexually Transmitted Disease
STI	Sexually Transmitted Infection
TV	Trichomoniasis
VVC	Vulvovaginal Candidiasis

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Reproductive tract infection (ReTI) including human immune deficiency virus (HIV) is an important public health problem especially in developing countries (WHO, 2005). These infections are sometimes symptomatic or asymptomatic (Marrazzo, 2011). Common symptoms seen are vaginal discharge, itching, irritation, unpleasant odor and discomfort. Causative organisms for vaginal infections are usually *Candida albicans, Trichomonas vaginalis* and organisms responsible for bacterial vaginosis including *Gardnerella vaginalis, Bacteriodes spp,* and *Mobilincus* (Pepin *et al.,* 2011; Workowski *et al.,* 2010).

Bacterial vaginosis (BV) is the most common cause of abnormal vaginal discharge in women of child bearing age (Cherpes *et al.,* 2003; Donders, 2010; Sobel, 2000). Studies have shown BV to be more common among blacks especially in sub-saharan Africa than whites (Allsworth & Peipert, 2007) with prevalence between 6 to 54% (Jespers *et al.,* 2014; Kirakoya-Samadoulougou *et al.,* 2008; Pepin *et al.,* 2011). BV is as a result of alterations of the normal flora in the vagina mainly dominated by lactobacillus species to an overgrowth of anaerobic species (Hillier, 1993; Sobel, 2005). Epidemiological studies have shown that women with disrupted vagina flora are more likely to acquire infections resulting from herpes simplex virus type-2 (HSV-2) (Cherpes *et al.,* 2003; Kirakoya-Samadoulougou *et al.,* 2011), *Trichomonas vaginalis* (Martin *et al.,* 1999), *Neisseria gonorrhoeae* and HIV (Atashili *et al.,* 2008). This fact shows that hydrogen peroxide producing lactobacilli act as important defense mechanism against pathogenic organism causing infections. The main effect of BV on pregnancy is preterm delivery (Hillier *et al.,* 1995; Klebanoff *et al.,* 2005). However, pelvic inflammatory disease (PID),

spontaneous abortion, (Leitich *et al.,* 2003), low birth weight, infertility, premature rupture of membrane (PROM), miscarriage (Leitich & Kiss, 2007) have also been linked to BV. Treatment of BV is by antibiotics such as metronidazole and clindamycin but treatment failures in about 30% of women have been recorded (Marrazzo, 2011).

Vulvovaginal candidiasis (VVC) is a female genital tract infection caused by the fungus candida species (Akah *et al.,* 2010; Sobel, 2007). In about 20-50 % of healthy women, the presence of candida species in their lower genital tract is asymptomatic (Akah *et al.,* 2010; Alli *et al.,* 2011; Donbraye-Emmanuel *et al.,* 2010; McClelland *et al.,* 2009). Candida species commonly known to cause candidiasis include C. *albicans*, C. *glabrata* and C. *tropicalis.* (Cronje *et al.,* 1994; Garcia Heredia *et al.,* 2006). Candida infections are very common in pregnant women as a result of the increased levels of estrogens and corticoids reducing the vaginal defense mechanisms (Sobel, 1997). An estimated 75% of women experience at least one episode of vulvovaginal candidiasis during their lifetime, with 40% to 50% having two or more episodes (Sobel, 2007). Increased rate of vaginal colonization by candida have been attributed to a number of factors, these include pregnancy, prolonged use of broad spectrum antibiotics and poor personal hygiene. (Akah *et al.*, 2010; Alli *et al.,* 2011)

Trichomoniasis (TV) is an infection caused by a flagellated parasite known as *Trichomonas vaginalis*. The infection is transmitted mainly through sexual intercourse (Heterosexual or homosexual) (Hobbs *et al.,* 2006) and also vertically through vaginal delivery. There has been varying prevalence of Trichomonas infections across African countries including Ghana. According to studies in Ghana, prevalence of Trichomoniasis is between 2 to 6% (Adu-Sarkodie *et al.,* 2004; Agyarko-Poku, 2011; Apea-Kubi *et al.,* 2006). The parasite is usually found in the vagina, cervix, urethra, bladder, prostrate,

periurethral gland and kidney (Workowski *et al.,* 2010). Approximately 25% of women with *Trichomonas vaginalis* infection are asymptomatic (Sena *et al.,* 2007). Symptomatic patients experience common signs and symptoms such as vulvovaginal erythema, dysuria, pruritus, irritation, edema, frothy yellow-gray or green vaginal discharge and an elevated pH (>6). Metronidazole has been the drug of choice for the treatment of *Trichomonas vaginalis* infection but there have been emerging cases of resistances in 2.5 to 9.6% of patients (Perez *et al.,* 2001; Schmid *et al.,* 2001; Schwebke & Barrientes, 2006).

1.2 Problem Statement

In developing countries including Ghana, surveillance of ReTI is unreliable and in some places not existence; hence prevalence data are not available. Few studies have been carried out in Africa on the prevalence of Bacterial Vaginosis, Vulvovaginal candidiasis and Trichomoniasis.

In Ghana, available data on prevalence only exist in special group of women with incomplete abortion in Accra and attendees to sexually transmitted disease (STD) clinic in Kumasi but not in pregnant women (Agyarko-Poku, 2011; Lassey *et al.*, 2004).

Moreover, vaginal infections have been associated with serious pregnancy outcomes and complication such as preterm delivery, low birth weight, miscarriage etc.

Furthermore, BV has been associated with increased susceptibility to acquisition of sexually transmitted infections including HIV (Atashili *et al.,* 2008) and Herpes simplex virus 2 (HSV-2)(Kirakoya-Samadoulougou *et al.,* 2011; Kirakoya-Samadoulougou *et al.,* 2008; Nagot *et al.,* 2007).

1.3 Justification

Pregnant women are an appropriate group for studying ReTI especially STI. This is due to the risk of the infection on the unborn baby and the mother. Several studies had associated the effect of vaginal infection in pregnancy to preterm delivery, low birth rate and neonatal death (Gray *et al.*, 2001; Hillier *et al.*, 1995). Moreover, knowing women sexual behaviours and practices which disrupt the normal flora of the vagina and predisposing them to STI is essential. In Kintampo and surrounding areas, the prevalence of BV, VVC and TV is unknown both in pregnant and non-pregnant women.

This cross-sectional study was conducted to determine the prevalence of vaginal infection and its associated risk factors among pregnant women. The results of this study will be important in planning intervention studies aimed at reducing vaginal infections and improving birth outcomes in Ghana and Sub-Saharan Africa.

1.4 Study Objectives

1.4.1Aim

To determine the prevalence of Bacterial Vaginosis, Trichomoniais and Vulvovaginal candidiasis in pregnant women attending antenatal clinic at the Kintampo Municipal Hospital (KMH).

1.4.2 Specific Objectives

1. To determine the prevalence of BV, TV and VVC.
2. To determine the association between vaginal infection (BV, TV and VVC) and clinical symptoms/signs presented.
3. To determine the risk factors for vaginal infection (BV, TV and VVC).

CHAPTER TWO

LITERATURE REVIEW

2.1 Bacterial Vaginosis

BV is characterized by an imbalance of the vaginal flora with the replacement of the usual hydrogen peroxide producing lactobacillus species with anaerobic microorganisms. These anaerobic microorganisms consist of *Gardneralla vaginalis, Prevotella, Peptostreptococcus, Bacteriodes spp, Mycoplasma* etc (Livengood III, 2009; Turovskiy *et al.,* 2011). BV causes little or no inflammation but a change in the vaginal flora, hence the name vaginosis rather than vaginitis (Turovskiy *et al.,* 2011).

2.1.1 Prevalence of Bacterial Vaginosis

BV prevalence varies with several factors such as geographical location (Tolosa *et al.,* 2006), race/ethnicity and socioeconomic factors (Koumans *et al.,* 2007). In a multicenter study conducted in 8 institutions around the world on 1461 asymptomatic pregnant women, the prevalence rate was 12.3% (Tolosa *et al.,* 2006). In this study, the developed countries such as USA and Ireland had the lowest prevalence of BV (5.8 and 5.9%) as compared to the developing countries such as Thailand and Zimbabwe where prevalence was (11.5 and 24.4%). This gives an indication of how geographical location and socioeconomic factors play a role in BV prevalence.

BV prevalence in developed countries is generally low but there have been few studies which have recorded prevalence of above 20% (Koumans *et al.,* 2007; Yen *et al.,* 2003). This is especially in North American where studies included Black Americans in USA and Aboriginals in Canada. Goldenberg *et al.,* in 1996 compared BV in whites and blacks women had 9% and 23% respectively (Goldenberg *et al.,* 1996). This high rate in blacks backs evidence of race and ethnicity as being a major factor in BV prevalence.

In a combined data from the 2001-2002 and 2003-2004 National Health and Nutrition Examination Surveys in 3700 American women reviewed, BV rate was 29% (Allsworth & Peipert, 2007).

BV prevalence in Africa has been very high as reported in a multicenter study carried out in West Africa women with a rate of 54% (Pépin *et al.*, 2011) and in the Gambia (Prevalence of 47%) (Demba *et al.*, 2005). However, a large survey in Burkina Faso reported a very low BV prevalence of 6.4% in pregnant women (Kirakoya-Samadoulougou *et al.*, 2008).

In Ghana, there have been few BV prevalence studies in sex workers (37%), women with incomplete abortion (47%) and students with vaginitis (28%)(Aubyn & Tagoe, 2013; Lassey *et al.*, 2004).

2.1.2 Pathogenesis of BV

The pathogenesis of BV condition is unclear. It is known to involve the substitution of the predominant beneficial microorganism (*Lactobacillus spp*) with other anaerobic microorganism (*G. Vaginalis*) and a corresponding increase in vaginal pH (>4.5). The major reason for the uncertainty is due to the lack of reflective animal models for the role of *G. vaginalis* (Johnson *et al.*, 1984). Gardner and Duke in 1955, were the first scientist to link *G. vaginalis* to BV after they reported isolating the organism in the lower genital tract of 92% of BV-affected females as compared to 0% in the non-infected (Gardner & Dukes, 1955). The two demonstrated that bacterial vaginosis was transmissible sexually.

Recent studies using polymerase chain reaction (PCR) have demonstrated that BV is not only caused by G. vaginalis alone but other anaerobic microorganism such as *Prevotella, Peptostreptococcus, Bacteriodes spp, Mycoplasma* (Fredricks *et al.*, 2005).

The pathophysiology of BV is an overgrowth of anaerobic microorganisms (Livengood III, 2009). This is then accompanied by the production of proteolytic enzymes that act on vaginal peptides to release several biologic products such as polyamines, which is enhanced in an alkaline environment to give out foul-smelling trimethylamine (Brand & Galask, 1986). The polyamines act to facilitate the transudation of vaginal fluid and exfoliation of epithelial cells, creating a copious discharge. *Gardnerella vaginalis* present in high numbers adhere to exfoliated epithelial cells in the presence of a high pH to form what is known as clue cells (Turovskiy *et al.*, 2011).

2.1.3 Diagnosis of BV

Several clinical and microscopic scoring systems for the diagnosis of BV have been validated. The most widely used criterion is the Amsel and Nugent criteria.

2.1.3.1 Amsel Criteria

Clinical diagnosis can be made by means of Amsel criteria; this method looks out for three out of the four of the following diagnosis (Amsel *et al.*, 1983)

1. Homogenous non-inflammatory discharge adherent to vaginal walls
2. Clue cells on microscopy of a smear (Usually > 20% of epithelial cells)
3. Vaginal fluid pH >4.5
4. Fishy odour when mixing vaginal fluid with 10.0% potassium hydroxide (KOH) (Positive whiff test).

2.1.3.2 Nugent criteria

This criterion is a scoring method for evaluating the vaginal flora using a 0 to 10 point scale. Nugent score is calculated by assessing for the presence of large gram-positive

rods (*Lactobacillus* morphotypes; scored as 0 to 4), small gram-variable rods (*G. vaginalis, Bacteriodes spp* morphotypes; scored as 0 to 4), and curved gram-variable rods (*Mobiluncus* spp. morphotypes; scored as 0 to 2). A score of 0-3 is normal vaginal flora, 4-6 is intermediate, and 7-10 is positive for bacterial vaginosis (Nugent *et al.,* 1991).

2.1.4 Adverse effects of BV

There have been many adverse health outcomes in connection to bacterial vaginosis, the most being related to women's reproductive health morbidity

Complications of certain gynaecological procedure and adverse pregnancy outcomes have been reported to be due to bacterial vaginosis. These include preterm delivery (Allsworth & Peipert, 2007; Hay *et al.,* 1994; Klebanoff *et al.,* 2005; Madhivanan *et al.,* 2008; Purwar *et al.,* 2001), pelvic inflammatory disease (PID) (Allsworth & Peipert, 2007; Koumans *et al.,* 2007), postoperative infections (Allsworth & Peipert, 2007), low birth weight (Hillier *et al.,* 1995; Madhivanan *et al.,* 2008), premature rapture of membrane (PROM)(Purwar *et al.,* 2001), late miscarriage/spontaneous abortion (Allsworth & Peipert, 2007; Hay *et al.,* 1994), and postpartum endometritis (Allsworth & Peipert, 2007).

Even though, studies have linked gynaecological problems to BV, a study by Larson *et al.,* found no association between BV and previous miscarriage, history of preterm delivery, infertility and extra-uterine pregnancies (Larsson *et al.,* 2007).

Several cross-sectional studies have shown possible links of BV to sexually transmitted diseases (STDs)(Atashili *et al.,* 2008; Bukusi *et al.,* 2006; Kirakoya-Samadoulougou *et al.,* 2011; Madhivanan *et al.,* 2008; Myer *et al.,* 2005), including HIV (Atashili *et al.,*

2008). This is very disturbing since BV prevalence is very high. There is a higher possibility for greater transmission of HIV infections. Recommendations are that, there is the need for further studies to understand the role of BV in HIV infection (Atashili *et al.*, 2008) and ways of preventing BV to reduce HIV spread (Koumans *et al.*, 2001; Myer *et al.*, 2005).

2.1.5 Risk factors for BV

Because the cause of BV is unknown, several factors have been suggested to be the major cause of the condition. These have been classified into socio-demographic and behavioral characteristics:

2.1.5.1 Socio-demographics

1. Age: Bukusi *et al.*, in a study in Kenya showed that BV prevalence decrease with increasing age of the women (Bukusi *et al.*, 2006). Similar finding in an intervention study in pregnant using clindamycin found a higher incidence of BV with younger age group (Larsson *et al.*, 2007).

2. Race/Ethnicity: Several studies of BV show a significant correlation to race (Goldenberg *et al.*, 1996; Koumans *et al.*, 2007). Some studies have shown more than 2 fold increases in prevalence of BV in the black race compared to whites (Cherpes *et al.*, 2008).

3. Educational status: Studies have associated BV with lower educational levels (Ashraf Ganjoei, 2005; Koumans *et al.*, 2007).

2.1.5.2 Behavioral Characteristics

1. Smoking: Findings from studies around the world show a strong association between BV and smoking (Georgijevic *et al.,* 2000). Smoking was found to be an independent predictor of BV in women (Cherpes *et al.,* 2008). Larsson et al studies found BV to be more than twice in smokers compared with non-smokers (Larsson *et al.,* 2007) but there were doubt if these could be used as a screening tool for BV. Smoking substances such as Cigarette in pregnancy has been found to be associated with an increase in anti-inflammatory cervical cytokines (Simhan *et al.,* 2005). These anti-inflammatory cervical cytokines are believed to create immune hypo-responsiveness environment which may increase risk for pregnancy complications such as preterm delivery.

2. Feminine Hygiene practices (Douching): Certain feminine practices such as vaginal douching have been strongly associated with BV (Holzman *et al.,* 2001; Ness *et al.,* 2002). In pregnant women, a study found significant correlation of BV with douching three or more times before or during pregnancy (Trabert & Misra, 2007).

   A woman's timimg and/or frequency of douching is highly predictive of BV (Schwebke & Weiss, 2001). However, the reason for douching by many women is because of abnormal vaginal symptoms (Ness *et al.,* 2002). It is possible BV is not the consequence of douching but rather the reason why women douche.

   There are however a few studies that did not find any relationship between BV and douching (Bukusi *et al.,* 2006; Demba *et al.,* 2005).

3. Contraceptive use: Studies on contraceptive use (hormonal and barrier) have revealed a decreased incidence of BV (Calzolari *et al.,* 2000; Riggs *et al.,* 2007). On the other hand intrauterine contraceptive devices have been shown to increase risk for acquiring BV (Calzolari *et al.,* 2000; Georgijevic *et al.,* 2000).

   Cohort studies using very large sample sizes gives evidence of the protective effects of hormonal and barrier contraceptive use to BV. In one of such studies (Riggs *et al.,* 2007), the users of oral contraceptive had reduced risk of getting BV (OR=0.76; 95%CI=0.63-0.90).

   A cross-sectional study in the United Kingdom showed taking oral or injectable hormonal contraceptive had a protective effect against BV (Brabin *et al.,* 2005). Other cross-sectional studies in the United States of America (USA) (Yen *et al.,* 2003), Australia (Bradshaw *et al.,* 2006) also had similar finding.

   Despite the benefits of hormonal and barrier conceptive use against BV, a study by Calzolari *et al.,* reported increased in BV among IUD user (Calzolari *et al.,* 2000).

4. History of abortion: Bacterial vaginosis also significantly increased the risk of spontaneous abortion (odds ratio, 9.91; 95% CI, 1.99-49.34)(Leitich *et al.,* 2003).

2.1.5 Treatment

Metronidazole and Clindamycin have been the conventional drug for treating BV (Livengood *et al.,* 1994; Sobel *et al.,* 2001), It has been the recommended drug for BV treatment by the Centre for disease Control and Prevention (CDC). These drugs are only effective in about 60% to 80% in BV cases (Eriksson *et al.,* 2005; Livengood *et al.,* 1994; Paavonen *et al.,* 2000). A study by Paavonen *et al.,* in 2000 had metronidazole

cure rate of 80% and that of clindamycin to be 68.1% in BV infected women (Paavonen *et al.*, 2000). The use of probiotics as a means to improve the vaginal flora and prevent BV has shown mixed results. Whiles some studies have shown beneficial effect (Anukam *et al.*, 2006), some other did not (Eriksson *et al.*, 2005).

2.2 Trichomoniasis

Trichomonas Vaginalis is motile, about 9 by 7µm in size, ovoid, flagellated and an anaerobic protozoan. The organism has a cytoplasm, large nucleus and a highly developed golgi complex (Hashimoto *et al.*, 1964). The organism has five flagella, four in the anterior area and the fifth forms part of the undulating membrane (Ovcinnikov *et al.*, 1975). The quivering motility characteristic of the parasite is due to flagella and the undulating membrane (Honigberg & King, 1964). TV is composed of tubulins and actin fibers which is the cytoskeleton. Its nucleus is at the anterior portion and surrounded by a porous nuclear envelope (Hashimoto *et al.*, 1964). The axostyle is a slender hyaline, rod-like structure which starts at the nucleus and bisects the organism longitudinally. It protrudes through the end of the protozoan, terminating in a sharp point (Honigberg & King, 1964). It uses the axostyle to anchor the whole body to the vaginal walls. Under light microscopy, granules can be seen in the TV. There are two sets of these granules: paracostal and paraxostylar. Glycogen granules are also present in *T. vaginalis* and can be observed by transmission electron microscopy. It contains lysosome-like structures such as phagosomes and demonstrates hydrolase activity (Ovcinnikov *et al.*, 1975).

2.2.1 Prevalence of Trichomoniasis

Data on the incidence and prevalence of trichomoniasis is limited worldwide (WHO, 2004). Few studies on the prevalence of trichomoniasis shows varying findings

depending on the geographical locations, methods used in sample analysis and the selected group of people used for the studies.

In the United States, a large population based survey spanning a period of 5 years recorded a prevalence of 3.1% in women aged 14-49 (Sutton *et al.*, 2007). In this study, 13.3% of the infection was in Black women.

Another recent study published in 2012 using a very accurate tool Aptima Combo 2 assay (Gen-Probe), found trichomoniasis prevalence rate of 8.7% in women aged 18 – 89 (Ginocchio *et al.*, 2012).

An Asian country study (Iran) on trichomoniasis prevalence using culture and wet mount as their diagnostic tools found a lower prevalence of 2% in both male and females aged 16 – 60 (Arbabi *et al.*, 2014). Studies in Africa on trichomoniasis prevalence amongst women shows rate from 9.9% to 41.4% (Crucitti *et al.*, 2011; Francis *et al.*, 2014) using various diagnostic methods.

In Ghana, cross sectional surveys amongst female sex works in Ghana's two biggest cities Accra and Kumasi found very high prevalence of 31.4% (Deceuninck *et al.*, 2000). A similar survey recently conducted in a STI clinic in Kumasi had a lower rate of 5% using wet mount (Agyarko-Poku, 2011). In pregnant women attending ANC in Kumasi, the prevalence of TV was 5.4% using latex agglutination method with wet mount and culture as the gold standard (Adu-Sarkodie *et al.*, 2004).

2.2.2 Pathogenesis of TV

Trichomoniasis in humans is a prevalent STI which has the ability to cause significant morbidity (Valadkhani, 2004). The squamous epithelium of the vagina is the main site of infection in humans. However, the exact pathogenesis of TV is not clearly understood

due to lack of good animal model (Petrin *et al.,* 1998). Studies have demonstrated a number of factors for pathogenesis of TV.

One of such is the invasion of TV in the host epithelium by adhesion and adherence. It uses the flagellum and the side opposite the undulating membrane to adhere to the epithelium (Alderete & Garza, 1988).

TV also makes use of cysteine proteinase which is lysosomal. These cysteine proteins could possibly be a factor in erythrocyte hemolysis. Immunoglobulin's (IgA and IgG) found in the vagina are degraded by cysteine proteinase (Neale & Alderete, 1990).

Another pathogenesis factor is the use of cell detaching factor by TV. This factor has been shown to cause cytopathic effect in cell culture of TV (Pindak *et al.,* 1986).

2.2.3 Adverse effects of Trichomoniasis

Several studies have proven an infection with TV could lead to many complications in pregnancy. Two very large population based studies showed that TV had a significant association with low birth weight, premature rapture of membrane (PROM) and preterm delivery (Cotch *et al.,* 1997; Sutton *et al.,* 1999). In a similar study, there were significant correlation between TV and PROM. Prevalence of PROM was 27.5% in women with TV compared with 12.8% in those without (Minkoff *et al.,* 1984). TV was independently associated with prematurity and low birth weight in another study in pregnant adolescents (Hardy *et al.,* 1984).

Trichomoniasis has been attributed to increased risk of HIV infection. The incidence of trichomoniasis was significantly associated with HIV seroconversion (OR (Odd Ratio): 1.9) in a cohort of women in Zaire (Laga *et al.,* 1993). Similarly, a study by McClelland *et al.,* in 2007, found a 1.52 fold increased risk of trichomoniasis association to HIV 1 acquisition (McClelland *et al.,* 2007).

2.2.4 Diagnosis

2.2.4.1 Wet preparation

The easiest and less expensive means of diagnosing TV is by the wet mount preparation. This is done by placing a saline preparation of the vaginal fluid on a microscope slide and observing for motile trichomonads (Domeika *et al.*, 2010). This must be done within 10 to 20 minutes of the vaginal fluid collection or the trichomonads will lose its viability. The test's sensitivity is limited, ranging from 60 to 70% though very quick and less expensive (Hegazi *et al.*, 2009; Krieger *et al.*, 1988).

2.2.4.2 Culture

The diagnosis of Trichomoniasis using culture is known to be the Gold standard. Medium such as the Diamond medium had been widely used until a recently commercially available liquid medium in a clear pouch was introduced (Draper *et al.*, 1993). This liquid medium in a clear pouch has been shown to be as good as the previous and works for both clinician-obtained and self-obtained specimen. Results of the culture test are available in 2 to 5 days.

2.2.4.3 Antigen Detection Test

The diagnosis of Trichomoniasis can be done using a point-of-care antigen detection test. This recently introduced diagnostic test is very effective in women and has been licensed (Genzyme Corp. Cambridge, Mass). STD clinic study demonstrated, the enzyme-linked immunosorbent assay had a sensitivity and specificity of 78.5 and 98.6%, respectively compared to culture. When the rapid assay was compared with wet-preparation, the former was more sensitive (78.5 and 72.4%, respectively; $P = 0.04$) but was less specific

(98.6 and 100.00%, respectively; $P = 0.001$)(Kurth *et al.,* 2004). This rapid test is ideal for rural health care providers.

2.2.4.4 Polymerase Chain reaction (PCR)

This method for detecting TV is still being developed. Many investigators have published their findings of PCR technique for diagnosis. A report of primers (TVA5 and TVA6) by Riley in 1992 for the detection of TV was published (Riley *et al.,* 1992). Others researcher have also reported additional primers (Kengne *et al.,* 1994). PCR has a sensitivity and specificity ranging between 85 to 100% (Lawing *et al.,* 2000; Ryu *et al.,* 1999). This method is not very accurate for TV compared to fastidious organisms such as *Neisseria gonorrhoeae.* The reason for this may be because *T. vaginalis* is much less fastidious to culture than *Neisseria gonorrhoeae.* It requires only the multiplication of a single organism in culture, the same as that needed for PCR.

2.2.5 Treatment

Metronidazole is the drug of choice for the effective treatment of Trichomoniasis. A single dose of 2g taken orally has a reported cure rate of 97% (Lossick, 1980). In pregnant women, there has been controversy regarding the adverse effect of metronidazole causing preterm delivery. However, CDC have not revived their recommended the 2g single dose treatment regimen for pregnant women at any stage of the pregnancy (CDC, 2010).

Metronidazole may display some level of resistance to treatment in about 2.5 to 5% in all cases of Trichomoniasis (Schmid *et al.,* 2001). Higher doses of the drug can overcome this resistance (Lossick *et al.,* 1986). Resistant strains of TV can be solved with a 2g oral

dose of tinidazole. The drug has been shown to have equal efficacy to metronidazole (90 to 100%) (Manorama & Shenoy, 1978; Sawyer *et al.,* 1976).

2.3 Vulvovaginal Candidiasis

Vulvovaginal candidiasis is an infection caused by a number of pathogens in the Genus Candida. These species are *Candida albicans* (accounting for about 80 to 90%), *candida glabrata* (10%), *Candida tropicalis, Candida krusei* and *Candida parapsilosis* (Mohanty *et al.,* 2007; Sobel, 1997). The infection is characterized by a curd-like discharge, itching and erythema. Vaginal candida colonization is known to increase during pregnancy. The reason for this is thought to be due to increased oestrogen levels, glycogen and other substrates in the vagina (Sobel, 2007). Increased level of *Candida spp* may cause an imbalance in normal flora of the vaginal, thereby decreasing lactobacillus dominance (Cassone, 2015; Jefferson, 2012).

2.3.1 Prevalence of Candidiasis

The infection is very prevalent in women and especially pregnant women are more at risk. Even though *Candida spp* causes Vulvovaginal candidiasis, it is asymptomatic in about 20 to 30% of women and part of their normal flora. Approximately, 75% of healthy women develop VVC at least once during their reproductive age (Fidel, 2004; Sobel, 2007).

Several studies around the world have shown VVC infection range between 10.0 to 55.0% (Ahmad & Khan, 2009; Mohanty *et al.,* 2007; Okonkwo & Umeanaeto, 2010; Olowe *et al.,* 2014).

In Ghana, a study among female sex workers in 2000 reported a rate of 24.4% (Deceuninck *et al.,* 2000). Apea-Kubi *et al.,* recorded a rate of 34.2% in pregnant women antenatal and gynaecological patients in Ghana (Apea-Kubi *et al.,* 2006).

In neighbouring African countries, Burkina Faso reported a very low prevalence rate of 6.3% (Kirakoya-Samadoulougou *et al.,* 2008) whiles in Nigeria a much higher rate of 81.5 was reported in a university hospital (Okonko *et al.,* 2012).

2.3.2 Pathogenesis of Vulvovaginal candidiasis

Studies in *Candida spp* infection have not been able to establish any definite pathogenic factor for the organism (Odds, 1988; Sobel, 2007). However, the organism possesses multiple virulent factors which aid in its invasive capacity (Rodrigues *et al.,* 1999). The germ tube is a major pathogenic factor in vulvovaginal candidiasis. It helps in adherence and invasion of the vaginal mucosal surfaces (Sobel *et al.,* 1981). *Candida albicans* tends to show greater ability to adhere to vaginal epithelial cells than other *Candida spp*. such as *C. krusei* and *C.tropicalis* (Garcia-Tamayo *et al.,* 1981).

Another virulence factor is the production of enzymes. Studies have shown that the intracellular compartment of *C. albicans* contains at least three proteinases (Portillo & Gancedo, 1986). There is an irreversible denaturation of secreted proteinases at neutral pH (pH = 7). Acute vaginal inflammation is therefore the pathogenic effect of the inducible proteinases produced (Macdonald, 1984).

A very important factor for the *Candida spp* virulence is using adhesion to mucosal linings. It is thought that candida might use surface blastoconidiamanno proteins to mediate adhesion to epithelial cells (McCourtie & Douglas, 1985).

2.3.3 Diagnosis of Candidiasis

2.3.3.1 Microscopy

Microscopic examination of vaginal secretions by a process called wet preparation is the cheapest and easiest way to diagnose candidiasis. A drop of saline is added to the vaginal secretions and observed under the light microscope for yeast cells. Phase contrast and polarized light microscope makes it easier to observe details.

2.3.3.2 Culture

The use of culture is the most commonly acceptable method for diagnosing candidiasis. Sabouraud dextrose agar (usually containing cycloheximide to inhibit overgrowth of unrelated mould species) is the most appropriate media for of samples collected from the vaginal fornix (Nyirjesy *et al.,* 1995). Vaginal swab samples can be transported to the laboratory using amies transport medium before culture is done. It is incubated at 36^{o}C for 48 to 72hrs or two week when left on at room temperature.

2.3.3.3 Nucleic acid assays

Developments of PCR probes that can directly detect candida DNA in clinical samples have made diagnosis of candidiasis easier (Giraldo *et al.,* 2000). But this is only available for research purposes.

2.3.3.4 Agglutination tests

Rabbit antibodies are used in latex agglutination against candida cell wall antigens. This latex agglutination test had sensitivity ranging between 71 to 81% and specificity of 96 to 98% but its sensitivity is lower than wet and gram-stained smears (Evans *et al.,* 1986; Martinez-de-Oliveira & Fonseca, 1990).

2.3.4 Adverse effect of VVC

Aside the costs and discomfort associated with VVC, several prospective studies have reported that VVC may increase a woman's chances of getting infected with HIV-1 (Hester & Kennedy, 2003; Kilmarx *et al.*, 1998). A systematic review by Rottingen *et al.*, found VVC to be associated with a 2-fold increase in the risk for HIV-1 acquisition (Røttingen *et al.*, 2001). Due to the high prevalence of this VVC, it could contribute substantially to the population-level risk of HIV-1 (Simon *et al.*, 2006).

2.3.5 Risk factors for Candidiasis

Several authors have demonstrated that pregnancy (Apalata *et al.*, 2014; Donders *et al.*, 2011), diabetes (de Leon *et al.*, 2002) and antibiotic treatment (Eckert *et al.*, 1998) key risk factors for candidiasis. In a study by de Leon et al s, candida carriage was associated with recent antibiotic use (p = 0.03), lifetime history of chlamydia (p = 0.04), and having oral sex during the past 2 weeks (p = 0.08). A study by Eckert *et al.*, attributed risk factor for *C albicans* isolation to condom use, sex frequency greater than four times a month, recent antibiotic use, young age, past infection with *Neisseria gonorrhoeae* (Eckert *et al.*, 1998). Other risk factors known to increase the VVC are oral contraceptive use, vaginal douching, tight-fitting clothing, synthetic underwear and increase sexual activities (Bradford *et al.*, 2013; Reed *et al.*, 2003). Some studies have reported HIV infection to be a risk factor to getting VVC (Achkar & Fries, 2010; Duerr *et al.*, 2003; Sebitloane *et al.*, 2011).

2.3.6 Treatments

Vulvovaginal candidiasis is grouped into complicated and uncomplicated candidiasis (Sobel, 2007). Short oral or topical therapy such as azole, itraconazole, clotrimazole,

butoconazole, nystatin, miconazole, terconazole, ketoconazole, boric acid, tioconazole and single dose fluconazole are used for the treatment of uncomplicated candidiasis (Pappas *et al.*, 2004). For complicated candidiasis, treatment needs seven days or more antimycotic therapy either topical therapy or as two 150mg doses of fluconazole. Non-albicans species are best treated with topical boric acid or flucytosin (Sobel, 2007). A systematic review publication recommended imidazole for treatment in symptomatic pregnant women suffering from candidiasis for a longer duration of about seven day (Young & Jewell, 2001).

2.4 Mixed infections/co-infections

Mixed infection as defined by Sobel et al is the concurrent presence of two or more potential pathogens in the lower genital tract, irrespective of the clinical significance of the individual pathogens (Sobel *et al.*, 2013). Mixed infections are usually the coexistence of two or more of endogenous infections (BV and VVC) and sexually transmitted infections (*C. trachomatis* and *N. gonorrhoeae*,, *T. vaginalis* and HSV). The coexistence of vaginal infections (BV, VVC and TV) is less documented but few studies have demonstrated that co-infections in this organism are common (Gatski, Martin, Clark, *et al.*, 2011; Gatski, Martin, Levison, *et al.*, 2011; Moodley *et al.*, 2002; Rivers *et al.*, 2011).

Rivers et al. study in an STD clinic of symptomatic women reported 72.3 % and 15.7 % of BV and VVC respectively with an overall prevalence of mixed infection (BV/VVC) of only 4.4 % (Rivers *et al.*, 2011). In another study of co-infection of BV and TV, the prevalence rates were 51.4% for BV, 28.0% for TV, and 17.5% for TV/BV co-infection (Gatski, Martin, Clark, *et al.*, 2011).

CHAPTER THREE

METHODS

3.1 Study Area

The study area is Kintampo North Municipality and Kintampo South District of Ghana. The study area covers 7162 km^2 with a resident population of approximately 140,000 (KHRC, 2010). It is located within the forest-savannah transitional ecological zone in central Ghana where community members are predominantly subsistent farmers and traders. The study area has two (2) government Hospitals, one (1) private hospital, four (4) Health Centres, three (2) Rural Clinics, two (2) private Maternity Homes, one (1) private clinic and (25) CHPS compound. The study hospital is located in Kintampo, the capital of the Kintampo North Municipality. Kintampo is the geographical centre of the country. It is in the Brong Ahofo region of Ghana between latitude 8˚45N and 7˚45N and longitudes 1˚20W and 2˚1W.

3.2 Study Site

The study site is the Kintampo Municipal Hospital (KMH). The KMH serves as a referral centre for all medical conditions from the surrounding hospitals, health centre, clinics and CHPS compound in the Kintampo North, South and some Districts of the Northern Region. The hospital has an Out-Patient-Department, an In-patient wards, an emergency unit, a theater, an ANC and a laboratory. The laboratory is well equipped to handle blood, urine and stool samples. It has a haematology, biochemistry and microbiology units.

3.3 Sampling and Sample size Calculation

The sample size was calculated with a prevalence of 6.4% of BV in ANC attendees in Burkina Faso (Kirakoya-Samadoulougou *et al.,* 2008). It was assumed that the prevalence of BV in a predominantly rural setting such as Kintampo was slightly higher due to female practices such as douching.

3.3.1 Using STATA Statistical software version 12 for the calculation (Stata Corp, TX USA):

Estimated sample size for one-sample comparison of proportion to hypothesized value

Test Ho: p = 0.0640, where p is the proportion in the population

Assumptions:

alpha = 0.0500 (two-sided)

power = 0.9000

alternative p = 0.1000

Estimated required sample size:

n = 577

An estimated minimum sample size of 589 pregnant women was adequate to detect an assumed prevalence of 10% in the Kintampo population at 90% power and with 95% confidence level taking into account 2% refusals.

3.4 Study design

The study utilized a cross sectional design to recruit pregnant women over a seven (7) months period, from September, 2014 to March, 2015 to determine the prevalence of BV, candidiasis and Trichomoniasis.

3.5 Study Population

The study consented and recruited 589 pregnant women. No age limits were set and pregnant women of all gestational ages who visited the antenatal clinic (ANC) of the Kintampo Municipal Hospital for routine care or for treatments of any ailments were eligible for enrolment into the study.

3.6 Data Collection

The data for the survey was collected using a pre-tested study questionnaire that included demographic, gynaecological, medical and obstetric characteristics. Some records on the pregnant women and laboratory test were reviewed from the participant's maternal health record book and transcribed onto the questionnaire (Appendix 2). The questionnaire was checked for consistency and correct transcription errors before the participant proceeded for sample collection.

3.7 Ethical consideration

Ethical approval was sought from the Kintampo Health Research Centre -Institutional Ethics Committee (KHRC-IEC) and the Kintampo Health Research Centre -Scientific Review Committee (KHRC - SRC). Data collection commenced after ethical approval was granted.

Confidentiality of data in this study was a priority since data was sensitive. There was no breach of confidentiality. Confidentiality was ensured as part of the acceptable standard practice in the Kintampo Health Research Centre (KHRC) using non-identifiable study identification codes. This was applied to storage of hard copies of the data collected in locked cabinets.

3.8 Treatment

Pregnant women who were found to have Bacterial Vaginosis or other vaginal infections were referred to the health facility for treatment. They were advised to bring their partner for examination and treatment to avoid possible re-infection of the participant in the event of another unprotected sex.

3.9 Specimen collection

The entire sample collection procedure was first explained to the participants. A total of two (2) vaginal swabs were collected. Participants were instructed to assume a lithotomy position on an examination couch with stirrups and knee support, under a well-lit environment. A trained midwife examined the vulva for the presence of genital warts, genital ulcer and lesions. Using a gloved hand, the labia of the vulva were then separated and a sterile swab was introduced into the vagina to the posterior fornix to take the first swab. The second swab was also taken, placed in amies transport medium and the two swabs were immediately transported to the Kintampo Health Research Clinical Laboratory for processing. The first vaginal swab was used to prepare a smear on a slide for Gram staining and also used for wet mount preparation whilst second vaginal swab was used specifically for culture.

3.10 Laboratory Processing

3.10.1 First Vaginal swab – Gram stain smear and wet mount

The swab upon receipt in the laboratory was immediately used to prepare a Gram stain vaginal smear and for wet mount preparation for microscopy.

The Gram stain smear was prepared by gently rolling the cotton bud on a clean microscopic slide and labeled appropriately. This was used for the diagnosis of BV by Nugent criteria.

The same swab was used for the wet mount by adding 15 drops of physiological saline into the cover tube of the swab stick and the swab stick was firmly caped into it. It was then shaken vigorously to get the vaginal discharge into the fluid in the saline. A drop of the vaginal fluid in saline was placed on a glass slide; cover slipped and observed under X10 and X40 objective lenses. The wet mount preparation was used to diagnose *Trichomonas vaginalis* infection. Other indicators for infection such as yeast cells, clue cells, pus cells, epithelial cells were all taken note of.

3.10.2 BV Diagnosis

BV diagnosis was done by means of microscopic examination of Gram-stained vaginal smear slides. The prepared vaginal smears were air-dried, heat-fixed and stained manually; using Gram's staining method (Appendix 3), at the Kintampo Health Research Centre Clinical Laboratory. The gram-stained slides were then examined microscopically by a qualified laboratory personnel's for BV diagnosis, using the Nugent's criteria scoring system (Nugent *et al.*, 1991) (Appendix 4). The laboratory personnel's were blinded to the clinical findings of the participants, or any other details, except their study numbers and specimen collection dates.

3.10.3 Nugent Criteria

The Nugent's method was used to evaluate the Gram-stained smears under oil immersion (X1000 magnification; high-power field (HPF)), for three groups of bacteria morphotypes. These were Lactobacilli (Gram positive rods. medium to large),

Gardnerella vaginalis/ Bacteroidesspp (small Gram-variable rods/Gram negative, coccobacilli), and *Mobiluncus spp*. (Gram-variable thin curved rods).

The numbers of bacterial morphotypes per HPF were quantitated for *Lactobacillus* and *Gardnerlla vaginalis/Bacteroides spp* from 1 to 4+ score system. This was done by assigning 0 for no morphotypes, 1+ for less than 1 bacterial morphotype, 2+ for 1 to 4 bacterial morphotypes, 3+ for 5 to 30 bacterial morphotypes, and 4+ for 30 or more bacterial morphotypes. Bacterial morphotypes quantification for Mobilincus spp was done by a 1 to 2+ scoring system; by assigning 0 for no bacterial morphotypes, 1 to 4+ for less than 1 bacterial morphotype, 2+.

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

Slides were read independently by two competent readers and results compared. Any discordant slide was re-read by the two readers and a discussion followed until agreement was reached. Ten percent of slides were re-read by an experienced external microscopist, who was blinded to the initial results.

3.10.4 Second Vaginal Swab – Culture

The second swab which was placed in the transport medium was inoculated on blood agar, chocolate agar and Sabouraud dextrose agar and incubated at a temperature range of 35 – 37^{o}C for 18 to 24hrs. Organisms that grew on the culture media were identified using their colonial morphology, Gram stain and biochemical reactions.

3.11 Inclusion Criteria

1. Pregnant women of any gestational age attending antenatal care at the Kintampo Municipal Hospital.
2. Pregnant women who gave consent and completed the informed consent form.

3.12 Exclusion Criteria

1. Pregnant women with pregnancy complications such as prolong rupture of membrane etc.
2. Pregnant women who withdrew or refused consent to part-take in the study.
3. Pregnant women who were enrolled in the current/ongoing study.

3.13 Data Management and Statistical Analysis

All questionnaires were checked for completeness and consistency at the data collection site. Laboratory results were double-checked. All questionnaires were double entered using MS-access software (Microsoft Corporation Copyright 2003) and checked for consistencies.

Data was analysed using STATA Version 12 (Stata Corp, TX USA). All categorical variables were summarized as proportions whilst continuous variables were summarized as means or median based on the distribution of the variables. Associations between categorical variables were explained using the chi-square tests or the Fisher's exact test as was appropriate. Univariate and Multivariate logistic regression models were used to identify potential risk factors of vaginal infections.

CHAPTER FOUR

RESULTS

4.1 Demographic characteristics of the study population

4.1.1 Age distribution

The age of the pregnant women ranged from 12 to 54 years. Age group with the highest number of women was 21-30 (52.0%, 306/589) whiles the least was 41 and above (2.2%, 13/589).

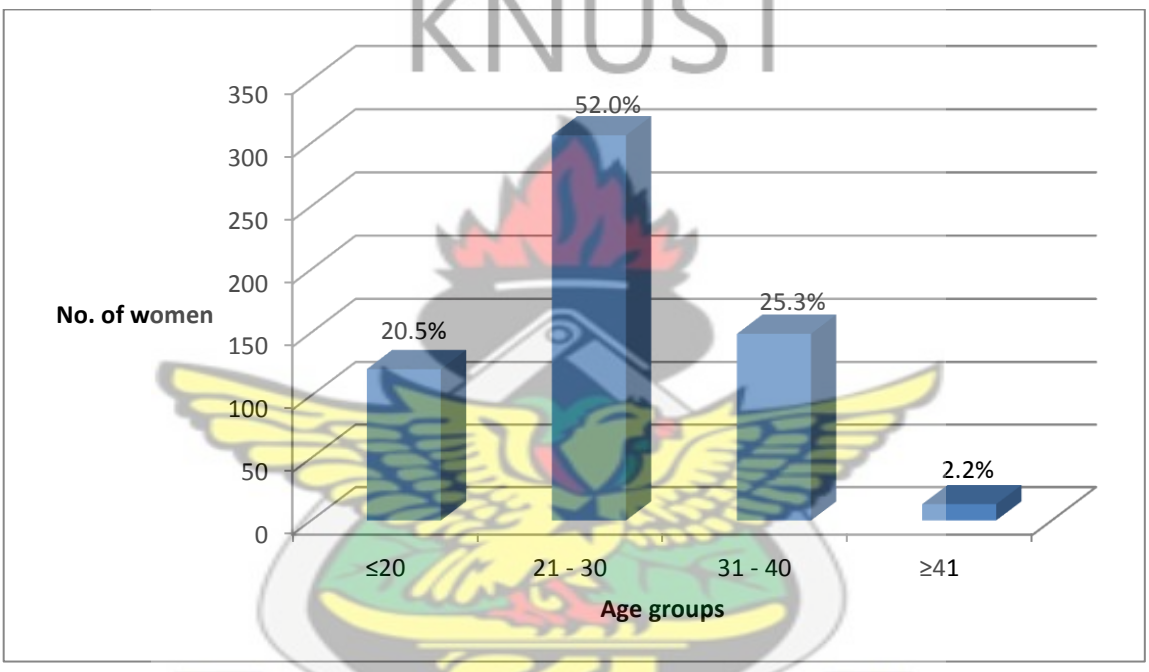

Figure 1.0 Age distribution of pregnant women

4.1.2 Marital Status

Among the 589 pregnant women interviewed, more than half 68.6% (404/589) were married. There were 16.5% (97/589) single women while 15.0% (88/589) were co-habiting (Not bound by any matrimonial contract but lives as couples).

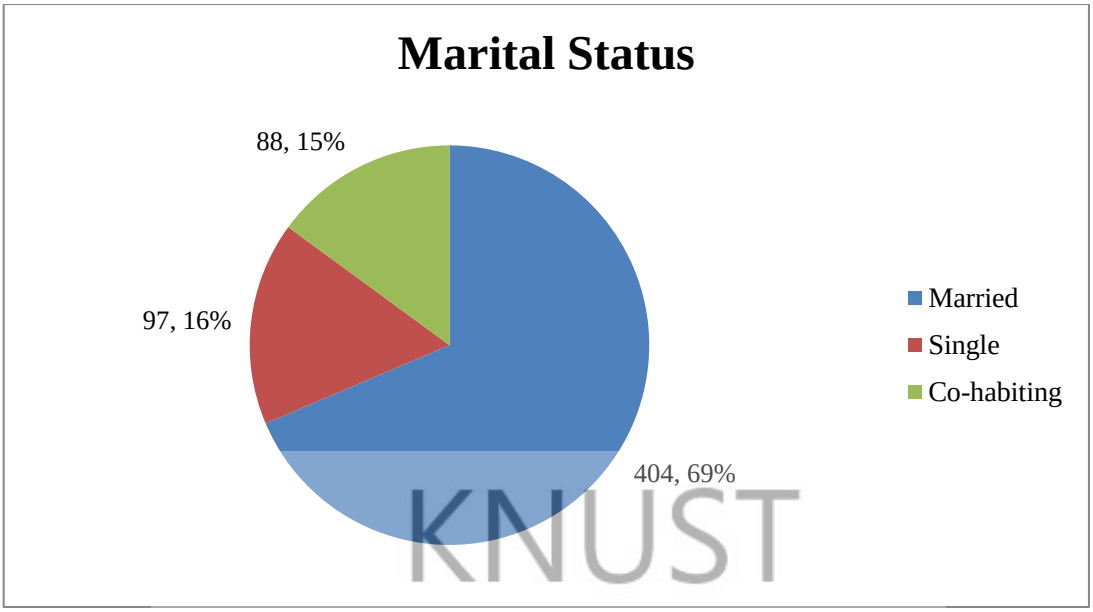

Figure 2.0 Marital Status of pregnant women

4.1.3 Employment Status

Majority of the participants 62.1% (366/589) were employed (Skilled, unskilled or professional work) whiles the minority 37.9% (233/589) were unemployed.

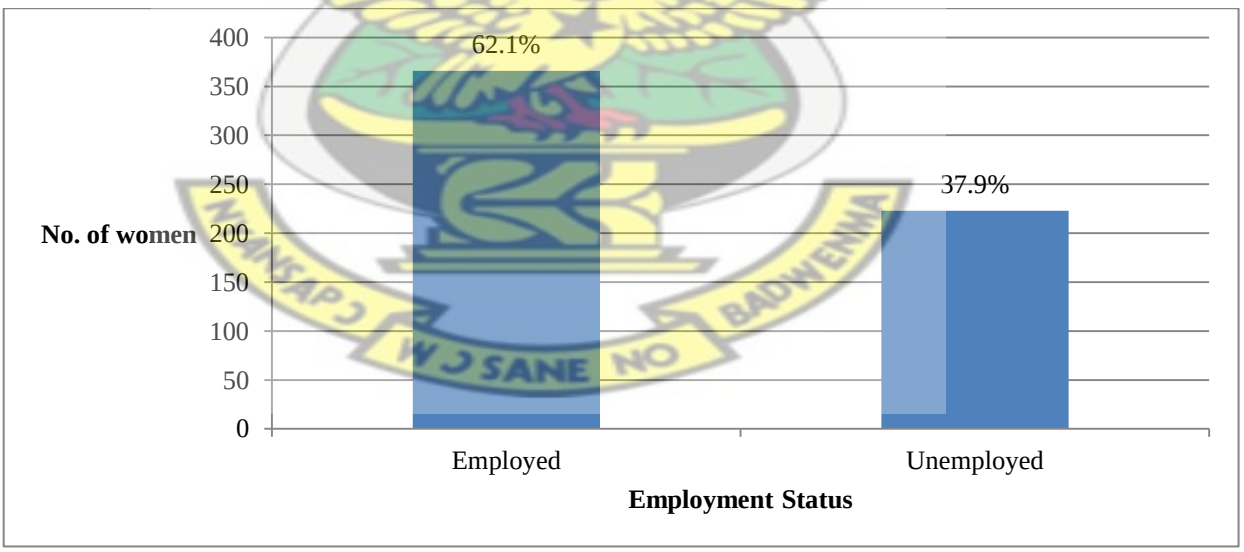

Figure 3.0 Employment status of pregnant women

4.1.4 Religious status

Majority (65.9%, 388/589) of the pregnant women were Christians followed by Muslims (31.4%, 185/589). Ten pregnant women (1.7%, 10/589) were traditional worshipers whiles only six (1.02%, 6/589) did not believe in any religious faith.

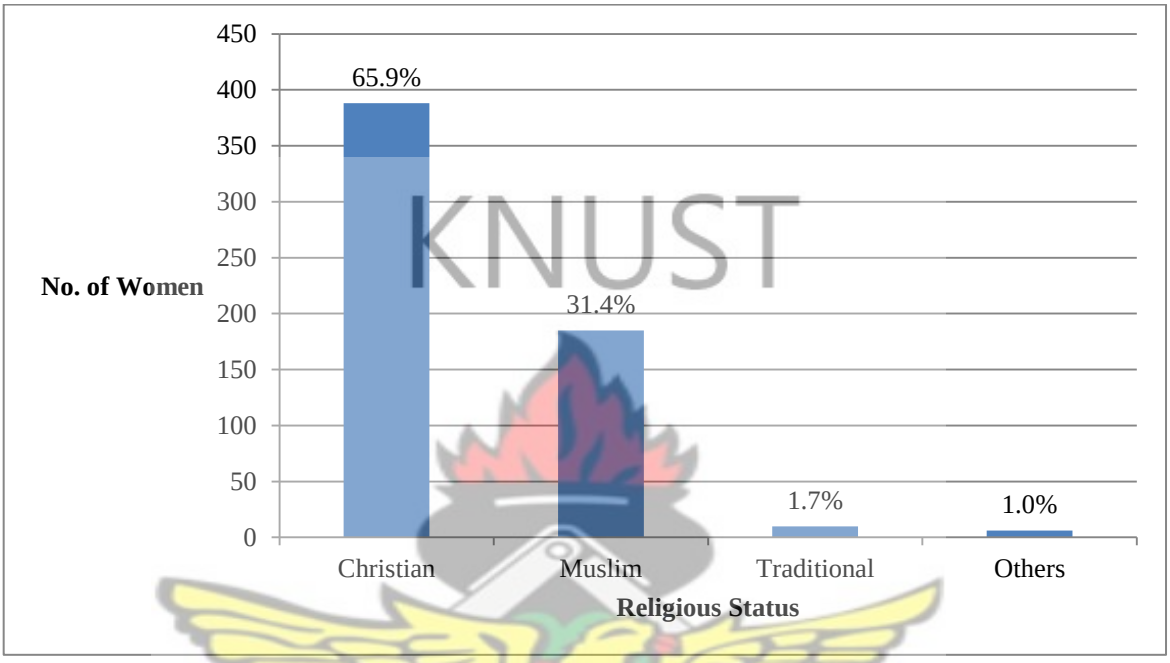

Figure 4.0 Religious status of pregnant women

4.1.5 Educational status of pregnant women

Out of 589 pregnant women enrolled, more than half (52.5%, 309/589) had completed elementary education (Defined as having completed primary or Junior Secondary school). Fifty (8.5%) reported having had secondary education (defined as having completed senior secondary/technical/Vocational/Commercial school) while about one-third (35.8%, 211/589) had no formal education.

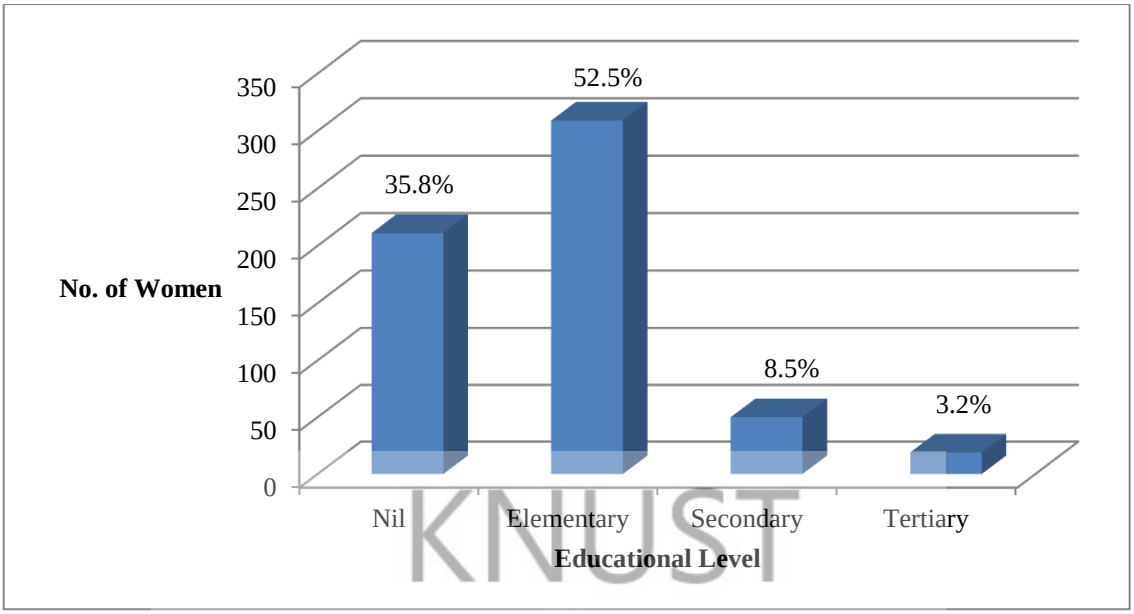

Figure 5.0 Educational status of pregnant women

4.2 Clinical Presentation

4.2.1 Symptoms of vaginal infection

More than sixty percent (61.0%, 359/589) of the pregnant women reported having no symptom/sign of vaginal infection. Two hundred and thirty (39.1%, 230/589) reported of having symptoms of vaginal infection.

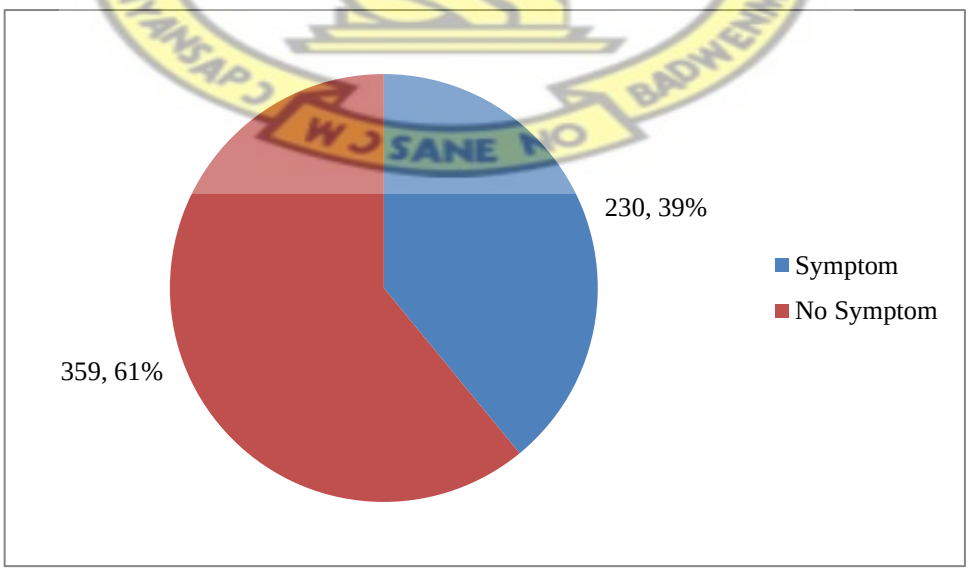

Figure 6.0 Presence of Symptom in pregnant women

4.2.2 Frequency of symptoms

The reported vaginal symptoms were vaginal discharge (36.3%, 214/589), vulva itching (24.1%, 142/589) and lower abdominal pains (23.3%, 137/589). The rest are dysuria (9.8%, 58/589), genital warts (1.7%, 10/589) and genital sores (4.2, 25/589).

Symptoms	Number of women (N=589)
	n (%)
Discharge	214 (36.3)
Itching	142 (24.1)
Lower Abdominal pains	137 (23.3)
Dysuria	58 (9.8)
Genital warts	10 (1.7)
Genital sores	25 (4.2)

Table 1.0 Frequencies of symptoms of pregnant women

4.3 Behavioural Characteristics

4.3.1 Douching practices among women

Majority of the pregnant women (62.3%, 371/589) were not practicing douching whiles minority (37.0%, 218/589) was into active douching practices.

4.3.2Douching substances

Out of the 218 pregnant women who reported douching, bathing soap (59/376) was the commonest douching substances, followed by antiseptics (Dettol and camel) (92/376). The use of lime water was the least (1/376).

Douching substance (N=218)	n (%)
Bathing soap	92 (42.20)
Antiseptics	59 (27.06)
Water	55 (25.23)
Salty Water	9 (4.13)
Herbs	2 (0.92)
Lime Water	1 (0.46)

Table 2.0 Type of douching substances

4.3.3Antimicrobial use

Twenty six (4.41%, 26/589) had taken antibiotic within three weeks prior to enrolments whiles 563 (95.59%, 563/589) had not taken any antibiotics within the past three weeks.

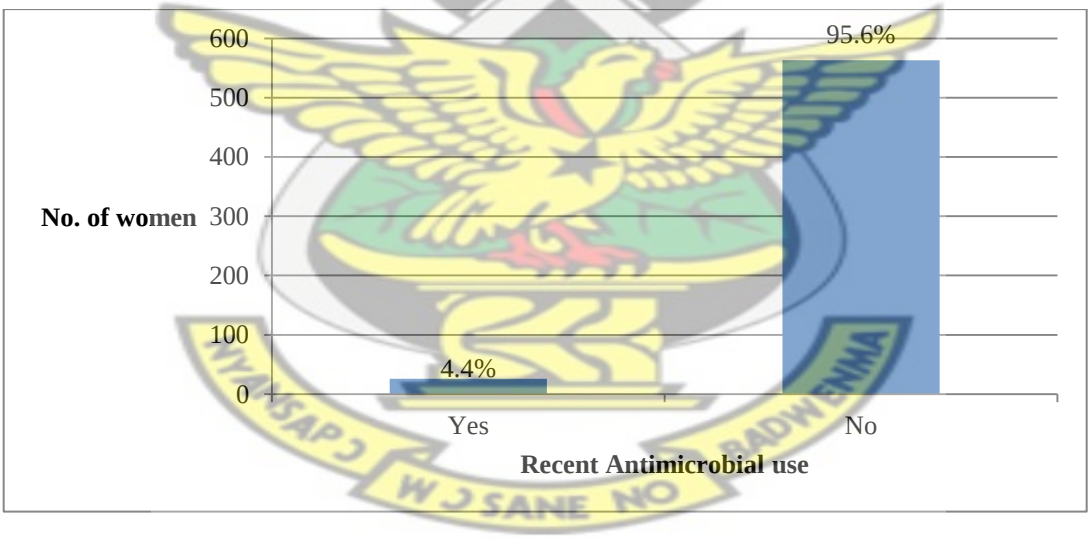

Figure 7.0 Recent antibiotic use

4.4 Obstetric Characteristics

4.4.1 Gravidae

Majority (35.0%, 206/589) of the pregnant women had had more than three pregnancies (>gravidae 3) followed by primigravidae (24.5%, 144/589) which was slightly above gravidae 2 (24.1%, 142/589). The least was gravidae 3 (16.5%, 97/589).

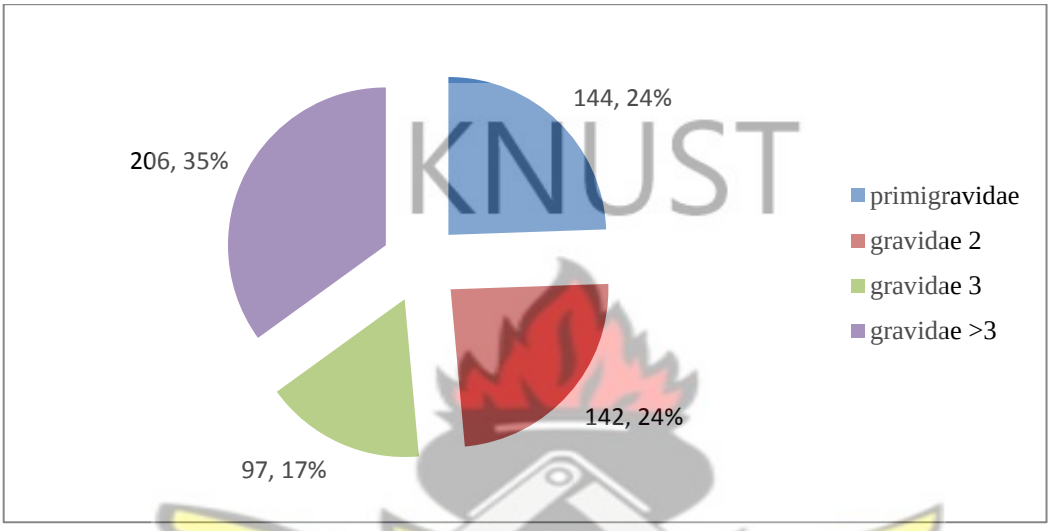

Figure 8.0 Number of pregnancies each pregnant women had ever had

4.4.2 Age of Pregnancy (Trimester)

More than half of the pregnant women in the study were in their second trimester (53.7%, 316/589) of their pregnancy. The first trimester (14.1%, 83/589) recorded the least number of pregnant women.

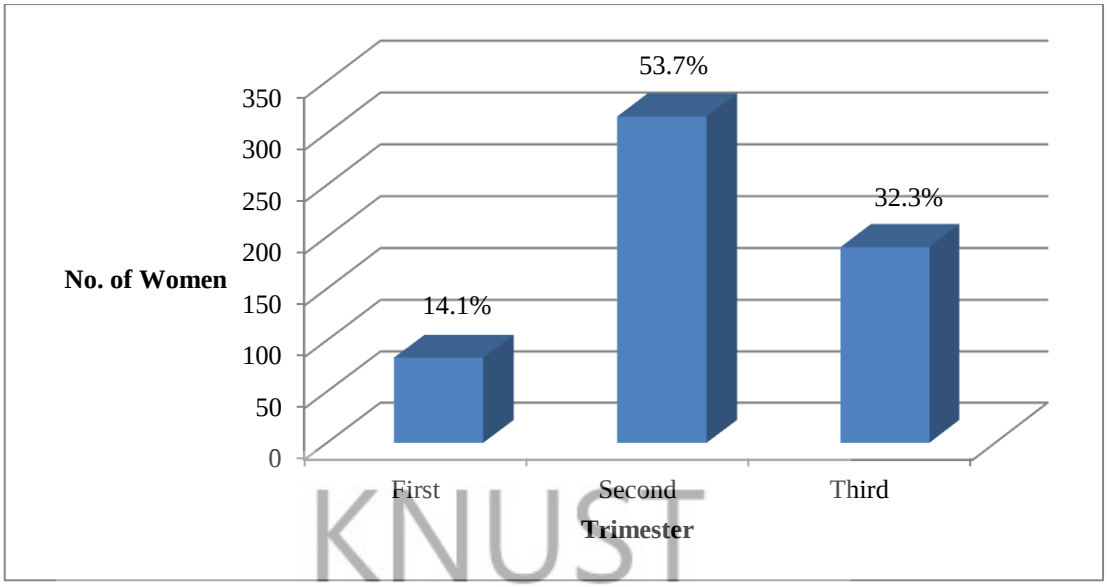

Figure 9.0 Trimester of pregnancy

4.5 Laboratory findings

4.5.1 Prevalence of vaginal infections

VVC had the highest prevalence rate among the pregnant women of 36.5%. (215/589) followed by BV (defined by Nugent score of 7 – 10) with a rate of 30.9% (182/589). TV had the least prevalence rate of 1.36% (8/589). The overall prevalence of vaginal infection was more than half of the study's sample size (56.2%, 332/589).

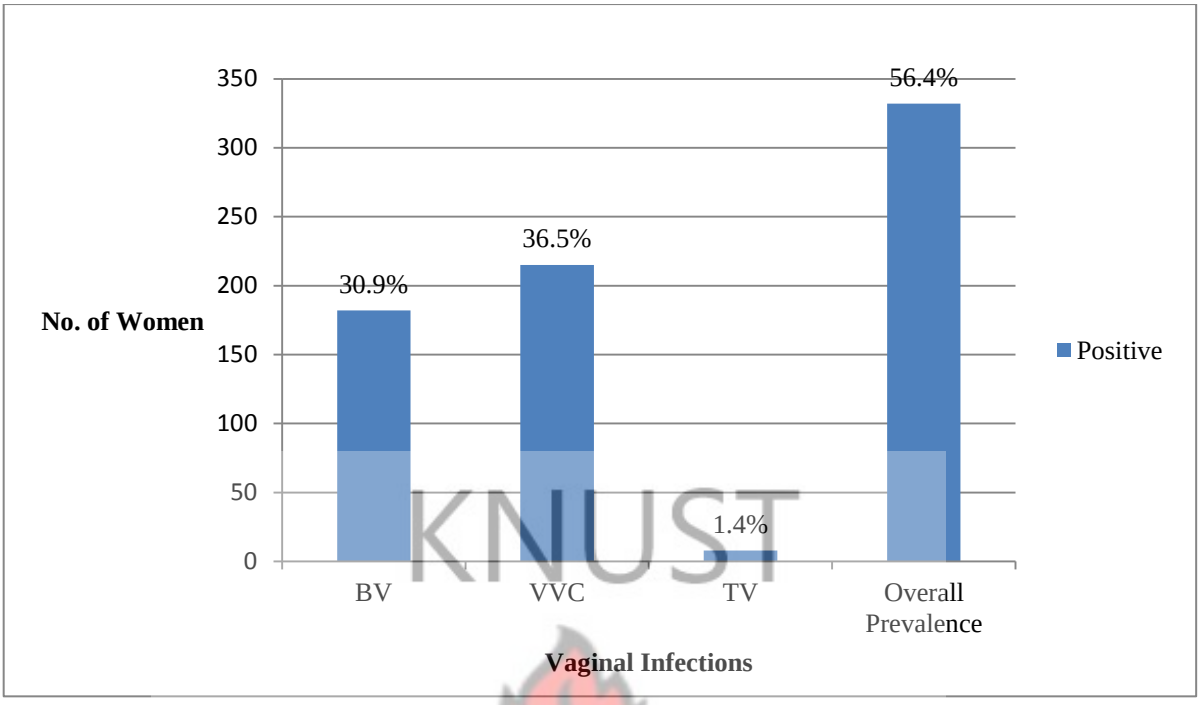

Figure 10.0 Prevalence of the vaginal infections among pregnant women

4.5.2 Distribution of vaginal infection

The highest number of vaginal infections (36.67%, 216/589) was in the age group between 21 to 30 years with VVC, TV and BV individually having over 50% infection rate. The least vaginal infection rate was 41 years and above (1.02%, 6/589).

Thirty seven percent (37.01%, 21/589) of the pregnant women did not report any symptom/ sign of vaginal infection but had one or more of laboratory confirmed vaginal infections. Whiles, 31% (187/589) pregnant women who reported symptom/sign of vaginal infection had laboratory confirmed infection.

Characteristics	No. of women	VVC	Trichomoniasis	BV Positive	Vaginal Infection
	N (%)	No. (%)	No. (%)	No. (%)	Total no. (%)
Age group					
≤20	121 (20.54)	38 (17.67)	3 (37.50)	54 (29.67)	95 (16.13)
21-30	306 (51.95)	120 (55.81)	4 (50.00)	92 (50.55)	216 (36.67)
31-40	149 (25.30)	52 (24.19)	1 (12.50)	35 (19.23)	88 (14.94)
≥41	13 (2.21)	5 (2.33)	0 (0.00)	1 (0.55)	6 (1.02)
Total	589 (100.0)	215 (100.0)	8 (100.0)	182 (100.0)	405 (100.0)
Symptoms/Signs					
No	359 (60.95)	126 (58.60)	3 (37.50)	89 (48.90)	218 (37.01)
Yes	230 (39.05)	89 (41.40)	5 (62.50)	93 (51.10)	187 (31.75)
Total	589 (100.0)	215 (100.0)	8 (100.0)	182 (100.0)	405 (100.0)

Table 3.0 Distribution of vaginal infections among population considering age and symptoms

4.5.3 Co-infection

Co-infection among the pregnant women is shown in figure 11.0. BV and VVC co-infection was the highest (11.2%, 66/589). TV/VVC co-infection (0.5%, 3/589) and the BV/TV co-infection (0.3%, 2/589) were very low. Only one pregnant woman had all three infections.

The overall co-infection rate (two or more infections) was 12.2% (72/589).

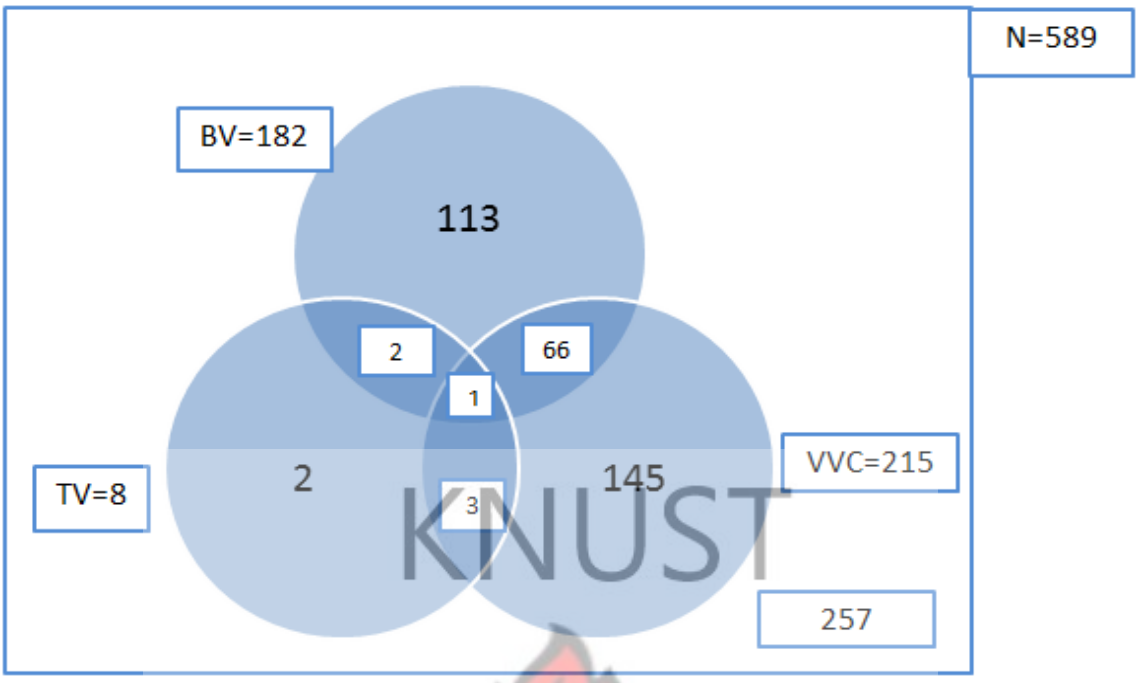

Figure 11.0 Co-infection among pregnant women

4.5.4 Association between vaginal infections and symptoms/signs presented in univariate and multivariate analysis

4.5.4.1 The association between BV and symptoms/sign

In univariate analysis of the association of BV with vaginal symptoms, vaginal discharge, lower abdominal pain, pruritus and dysuria were all independently significantly associated with the infection.

In multivariate analysis, adjusting for possible confounding relationship, none of the vaginal symptoms was significantly associated with BV.

Symptom	Total N	BV n (%)	OR (Unadjusted)	*P-value* for OR	AOR (Adjusted)	*P-value* for AOR
Discharge						
No	375	95	1.0		1.0	
Yes	214	87	2.02 (1.41-2.9)	<0.001	1.48 (0.84-2.63)	0.18
Lower abdominal pain					1.0	
No	452	125	1.0		1.0	
Yes	137	57	1.86 (1.25-2.77)	0.002	1.18 (06.8-2.02)	0.56
Pruritus						
No	447	121	1.0		1.0	
Yes	142	61	2.03 (1.37-3.00)	<0.001	1.36 (0.79-2.34)	0.27
Dysuria						
No	531	157	1.0		1.0	
Yes	58	25	1.80 (1.04-3.13)	0.039	1.09 (0.57-2.06)	0.78

Table 4.0 The association between BV and symptoms/signs

4.5.4.1 The association between VVC and symptoms/sign

Unadjusted logistic regression (bivariate) analysis of the association of VVC with vaginal symptoms revealed a possible independent association to be only pruritus (p=0.026, OR = 1.90, 95%CI 1.08-2.32) The remaining vaginal symptoms (vaginal discharge, lower abdominal pain and dysuria) showed negative association with VVC.

In the adjusted analysis, using possible confounding relationship (pruritus, vaginal discharge, lower abdominal pain and dysuria), pruritus still remained significantly associated with VVC.

Symptom	Total N	VVC n (%)	OR (Unadjusted)	*P-value* for OR	AOR (Adjusted)	*P-value* for AOR
Discharge						
No	375	134	1.0		1.0	
Yes	214	81	1.10 (0.77-1.55)	0.61	0.6 (0.35-1.14)	0.13
Lower abdominal pain						
No	452	160	1.0		1.0	
Yes	137	55	1.22 (0.83-1.81)	0.31	1.17 (0.68-2.01)	0.58
Pruritus						
No	447	153	1.0		1.0	
Yes	142	62	1.49 (1.01-2.19)	0.04	1.90 (1.08-2.32)	0.026
Dysuria						
No	531	190	1.0		1.0	
Yes	58	25	1.36 (0.79-2.35)	0.28	1.22 (0.64-0.68)	0.53

Table 5.0 The association between VVC and symptoms/sign

4.5.4.1 The association between TV and symptoms/sign

The association of TV with vaginal symptoms, in both the adjusted and unadjusted logistic regression revealed no statistically significant association.

Symptom	Total N	TV n (%)	OR (Unadjusted)	*P-value* for OR	AOR (Adjusted)	*P-value* for AOR
Discharge						
No	375	3	1.0	0.13	1.0	
Yes	214	5	2.97 (0.70-12.53)		3.38 (0.39-29.45)	0.10
Low abdominal pain						
No	452	7	1.0	0.44	1.0	
Yes	137	1	0.48 (0.05-3.83)		0.15 (0.02-1.43)	0.27
Pruritus						
No	447	4	1.0		1.0	
Yes	142	4	3.21 (0.79-13.00)	0.11	2.24 (0.28-17.80)	0.45
Dysuria						
No	531	8	1.0			
Yes	58	0	1.0			

Table 6.0 The association between TV and symptoms/sign

4.5.5 Risk Factors associated with vaginal infections in univariate and multivariate analysis

4.5.5.1 Risk factors for BV

In the univariate analysis shown in table 7.0, independent risk factor for BV were the age group 20 years and below (p=0.005, OR=1.87, 95% CI=1.21-2.89) and having 2 or less number of pregnancies (p<0.001, OR=2.37, 95% CI=1.65-3.39). However, the third trimester of pregnancy was protective of BV (p=0.05, OR=0.66, 95% CI=0.44-1.00).

Douching, recent antibiotic use and sex frequency were not risk factor for BV

In multivariate analysis, BV was significantly associated with gravidae (2 or less pregnancies) (p=0.003, OR=1.95, 95% CI=1.25-3.04). Being in the third trimester of pregnancy was protective for BV (p=0.04, OR=0.65, 95% CI=0.42-0.98). Age group, douching practice, recent antimicrobial use and sex frequency are not risk factors for BV.

	Total N	BV n (%)	OR (Unadjusted)	*P-value* for OR	AOR (Adjusted)	*P-value* for AOR
Age group						
≤20	121	54 (44.63)	1.87(1.21-2.89)	0.005	1.54(0.96-2.46)	0.071
21-30	306	92 (30.07)	1.0		1.0	
31-40	149	35 (23.46)	0.71 (0.45-1.12)	0.143	0.97(0.60-1.64)	0.991
≥41	13	1 (7.69)	0.19(0.02-1.51)	0.118	0.30(0.03-2.36)	0.249
Trimester						
First	83	31 (37.35)	1.22(0.73-2.01)	0.447	1.18(0.70-1.98)	0.540
Second	316	104 (32.91)	1.0		1.0	
Third	190	47 (24.74)	0.66 (0.44-1.00)	0.052	0.65(0.42-0.98)	0.040
Gravidae						
<3 pregnancy	286	115 (40.21)	2.37(1.65-3.39)	<0.001	1.95(1.25-3.04)	0.003
≥3 pregnancy	303	67 (22.11)	1.0		1.0	
Douche						
No	371	119 (32.80)	1.0		1.0	
Yes	218	63 (28.90)	0.86(0.60-1.24)	0.421	0.82(0.56-1.20)	0.316
Antibiotic use						
No	563	171 (30.37)	1.0		1.0	
Yes	26	11 (42.31)	1.68(0.75-3.74)	0.202	1.75(0.77-3.99)	0.184
Sex Frequency						
<2 per week	420	130 (30.95)	1.0		1.0	
≥2 per week	169	52 (30.77)	0.99 (0.67-1.46)	0.965	0.88(0.59-1.32)	0.540

Table 7.0 Risk factors for BV

4.5.5.2 Risk factors for VVC

Univariate and multivariate analysis shows that age group, trimester of pregnancy, gravidae, douching practices, recent antibiotic use and sex frequency were not risk factors for acquiring VVC.

	Total N	VVC n (%)	OR (Unadjusted)	*P-value* for OR	AOR (Adjusted)	*P-value* for AOR
Age group						
≤20	121	38 (31.4)	0.71(0.45-1.11)	0.133	0.77(0.47-1.24)	0.278
21-30	306	120 (39.2)	1.0		1.0	
31-40	149	52 (34.9)	0.83(0.55-1.25)	0.373	0.72(0.49-1.19)	0.235
≥41	13	5 (38.5)	0.97(0.31-3.03)	0.956	0.83(0.25-2.68)	0.756
Trimester						
First	83	30 (36.1)	1.06(0.64-1.75)	0.821	1.08(0.65-1.79)	0.773
Second	316	110 (34.8)	1.0		1.0	
Third	190	75 (39.5)	1.22(0.84-1.77)	0.292	1.22(0.84-1.78)	0.305
Gravidae						
<3 pregnancy	286	100 (35.0)	0.88(0.63-1.23)	0.452	0.86(0.57-1.30)	0.476
≥3 pregnancy	303	115 (38.0)	1.0		1.0	
Douche						
No	371	136 (36.7)	0.92(0.65-130)		0.94(0.66-1.34)	0.725
Yes	218	79 (36.2)	1.0	0.624	1.0	
Antibiotic use						
No	563	202(35.9)	1.79(0.81-3.93)	0.149	1.83(0.82-4.05)	0.138
Yes	26	13(50.0)	1.0		1.0	
Sex Frequency						
<2 per week	420	161 (38.3)	1.0		1.0	
≥2 per week	169	54 (32.0)	0.76(0.52-1.10)	0.146	0.76(0.52-1.11)	0.156

Table 8.0 Risk factors for VVC

4.5.5.3 Risk factors for TV

Both the unadjusted and adjusted logistic regression analysis in table 9.0 did not show any significant risk association between TV and age group, trimester, gravidae, douching practices, recent antibiotic use and sex frequency.

	Total N	TV n (%)	OR (Unadjusted)	*P-value* for OR	AOR (Adjusted)	*P-value* for AOR
Age group						
≤20	121	3 (2.5)	1.91(0.42-8.71)	0.398	1.65(0.32-8.67)	0.550
21-30	306	4 (1.3)	1.0		1.0	
31-40	149	1 (0.6)	0.51(0.57-4.60)	0.549	0.49(0.05-5.17)	0.556
≥41	13	0 (0.0)				
Trimester						
First	83	1 (1.2)	0.95(0.10-8.63)	0.965	1.12(0.12-10.48)	0.921
Second	316	4 (1.3)	1.0		1.0	
Third	190	3 (1.6)	1.25(0.28-5.65)	0.771	1.44(0.31-6.61)	0.641
Gravidae						
<3 pregnancy	286	5 (1.8)	1.77(0.42-7.51)	0.433	1.0	
≥3 pregnancy	303	3 (1.0)	1.0		1.11(0.20-6.29)	0.904
Douche						
No	371	3 (0.8)	1.0		1.0	
Yes	218	5 (2.3)	2.99(0.71-12.6)	0.137	2.97(0.69-12.80)	0.144
Antibiotic use						
No	563	8 (1.4)	1.0		1.0	
Yes	26	0 (0)				
Sex Frequency						
<2 per week	420	5 (1.2)	1.0		1.0	
≥2 per week	169	3 (1.8)	1.5(0.35-6.35)	0.58	1.42(0.33-6.14)	0.637

Table 9.0 Risk factors for TV

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

The prevalence rate of BV reported in this study was 30.9%. In comparison with other similar studies, which enrolled pregnant women and using same diagnostic method (Nugent criteria), the results from this study were considered to be higher but also lower in some others.

The 30.9% BV prevalence rate found in this study was much lower than 64.3% reported by Ajani et al in Nigeria using Nugent criteria (Ajani *et al.,* 2012). However, a relatively much lower rate of 6.4% was reported in a large population based study in neighbouring Burkina Faso in pregnant women attending ANC (Kirakoya-Samadoulougou *et al.,* 2008). The study related the low rate of BV to the low national prevalence of HVS-2, vaginal hygiene practices and sexual behaviour of the study population.

In Ghana, a BV prevalence study conducted in women with incomplete abortion in Accra reported a rate of 47% (Lassey *et al.,* 2004). The high rate might be due to the selection of women with incomplete abortion. Several studies have found a possible association between BV and abortion (Allsworth & Peipert, 2007; Hay *et al.,* 1994). (Kirakoya-Samadoulougou *et al.,* 2008). Ralph *et al.,* cohort study of women undergoing in vitro fertilization, found an association between BV and an increased risk of miscarriage in the first trimester (OR=2.67, 95% CI=1.26 to 5.63) (Ralph *et al.,* 1999).

A study in 2011 revealed a BV rate of 37% among sex workers in Kumasi (Agyarko-Poku, 2011). These non-pregnant women were involved in multiple sexual partners which some studies have reported to be a risk factor for BV (Georgijevic *et al.,* 2000; Yen *et al.,* 2003).

In a multicenter study in West Africa including Ghana, an aggregate rate of 54% was reported in women with vaginal discharge (Pépin *et al.,* 2011). Demba *et al.,* in Gambia had a rate of 47.6% in women with vaginal discharge syndrome (Demba *et al.,* 2005). This high rate is due to the study's selection of women with vaginal discharge which is usually a symptom of vaginal infection.

In comparable BV prevalence studies, the National Health and Nutrition Examination surveys in non-pregnant women (USA), reported prevalence rate of 29% which was similar to what was obtained in this study (Allsworth & Peipert, 2007). A similar study conducted in tertiary students in Ghana had a comparable rate of 28% (Aubyn & Tagoe, 2013). Bella *et al.,* in India had a rate 32.8% in non-pregnant women with as much as 31.2% being asymptomatic (Bhalla *et al.*, 2007).

Studies have suggested very high prevalence rates in the black race compared to the white (Allsworth & Peipert, 2007; Cherpes *et al.*, 2008). The outcome of this study was not different from what was reported when one considers the 30.9% prevalence of BV among the study participants. This is also consistent with a multicenter study involving 6 countries where Zimbabwe (the only African and black race country) had the highest BV prevalence rate of 24.4% (Tolosa *et al.*, 2006). This might support the belief that BV prevalence is high among the black race.

The study data suggest a high of rate of BV (50.55%, 92/182) among the age group between 21 to 31 years of the BV positive pregnant women. A prevalence study in non-pregnant women conducted in Rwanda found the highest percentage BV rate in same (21-30) age group (52.8%, 28/53) (Muvunyi & Hernandez, 2009). This age group is predominantly the reproductive age and perhaps the high rate might be due to the increased sexual activity.

Almost half (48.9%) of BV positive pregnant women reported no symptom of the infection. In Koumans et al study in the USA, 84% of women with BV did not report symptoms (Koumans *et al.,* 2007). These asymptomatic pregnant women are less likely to seek treatment and are therefore prone to the adverse effects such as miscarriage, premature rapture of membrane of the disease.

Trichomonas infection prevalence was the lowest at 1.4% compared to the other vaginal infections. The study employed the use of the wet mount diagnostic technique for *Trichomonas vaginalis* which has an estimated sensitivity of 60 to 70% when compared to culture (Hegazi *et al.,* 2009).

In Ghana, the 1.4% trichomoniasis prevalence rate reported in the present study was much lower than 5.4% in reported by Adu-Sakordie *et al.,* in pregnant women using latex agglutination kit with wet mount and culture as the gold standard (Adu-Sarkodie *et al.,* 2004). The antigen detection test used in this study is more sensitive but less specific test compared with the wet mount (Kurth *et al.,* 2004). In Adu-Sakordie et al study, the sensitivity for the antigen test kit was 98.8% sensitive when compared with an expanded Gold method based on results from culture and wet mount. This higher sensitivity might have accounted for differences in prevalence rate. In another prevalence study in Ghana among sex workers, 31.4% for TV was recorded (Deceuninck *et al.,* 2000). This high rate might be due to the risky sexual behavior of the study participants. Apea-Kubi et al in Accra reported a rate of 2.7% in antenatal and gynaecological patients in Ghana (Apea-Kubi *et al.,* 2006).

Two comparable studies carried out in Vietnam using wet mount techniques, reported a rate of 1.3% and 1% in women attending maternal and child health and family planning clinics and women of reproductive age respectively (Anh *et al.,* 2003; Lan *et al.,* 2008).

Another wet mount diagnostic technique study by Sunday-Adeoye et al in Nigeria, reported a much lower rate of 0.5% (Sunday-Adeoye *et al.,* 2009). The Nigerian Author recommended the use of more sophisticated method such as culture or PCR for diagnosis of *Trichomonas vaginalis*. Other studies by Bahram et al (2009), Olowe et al (2014) in Iran and Nigeria, had 6.6%, and 2% respectively (Bahram *et al.,* 2009; Olowe *et al.,* 2014).

However, another study in Burkina Faso using culture which is a more accurate diagnostic tool had 1.5% which was similar to the current study (Kirakoya-Samadoulougou *et al.,* 2008). The study related the low prevalence to vaginal hygiene practices and less risky sexual behaviour of study participants. Another study in Iran which compared wet mount and culture diagnostic method for *Trichomonas vaginalis* had rates of 2.1% and 1.7% respectively (Matini *et al.,* 2012). The reason for the similarities in both the wet mount and culture is unknown.

Other studies conducted in the United States of America (USA) found rates to be between 3.1% and 8.7% (Ginocchio *et al.,* 2012; Sutton *et al.,* 2007) and these were higher than the present study.

The low prevalence of TV in the present study may be related to the decent sexual behavior of the participants which is the main determinant of trichomoniasis. This is evident in the fact that only one out of the 589 participants enrolled reported having more than one sexual partner in the past 3 months. In addition, majority of the participants were married and have only one sexual partner.

VVC had the highest vaginal infection prevalence rate of 36.5% in this study. The reported rate compares well with 34.2% in Accra, 36.0% in Nigeria, 37.4% in Turkey (Apea-Kubi *et al.,* 2006; Guzel *et al.,* 2011; Olowe *et al.,* 2014).

However, in some other studies in pregnant women, the present study prevalence was slightly higher than 24.4% and 30% in Nigeria (Donbraye-Emmanuel *et al.,* 2010; Okonkwo & Umeanaeto, 2010).

In another related study in only asymptomatic pregnant women in the United Kingdom, *Candida spp* prevalence was 12.5% as compared to 36.5% in this study (Akinbiyi *et al.,* 2008). This disparity in the prevalence might be due to the fact that only asymptomatic patients were included in the study. This might have affected their prevalence and would have been much higher.

Even though, the study recorded very high prevalence 36.5% for VVC, it was lower than finding reported by Limia et al at 42.3% using Immunologic Latex Agglutination Test (Limia & Lantero, 2004). Another study in two hundred (200) women reported a much higher rate of 81.5% in patients attending a Medical Centre in Nigeria (Okonko *et al.,* 2012).

The high *Candida spp* colonization of the study participants' vagina might be because of their pregnancy status. This is due to the high concentration of oestrogen hormone during pregnancy which provides favourable environment for the growth of *Candida spp* (Garcia Heredia *et al.,* 2006). Nonetheless, this high rate calls for urgent attention to the infection since it causes a lot of discomfort to the pregnant woman who is already experiencing some level of discomfort. Moreover, the mother could infect the baby during perinatal period. In addition VVC could be an indication of an underlying infection such as diabetes mellitus.

Furthermore, recent emerging evidence suggests that screening for and eradication of candida during pregnancy may reduce the risk of preterm delivery (Kiss *et al.,* 2004; Roberts *et al.,* 2011). Further studies needs to be carried to confirm these findings.

The over 58% rate of asymptomatic infections of VVC is quite worrying. A similar study in pregnant women in Nigeria reported 65% of VVC in asymptomatic women (Akerele *et al.*, 2002).

The study data suggest a significant association between BV and vaginal symptoms (Discharge, lower abdominal pains, pruritus and dysuria) ($p>0.05$) in the unadjusted analysis. The probability of having BV with any of the vaginal symptoms was almost twice as that of not having the symptom. In a longitudinal study carried out in the USA, vaginal discharge and odor individually were significantly associated with BV whiles the remaining symptoms were not associated. The present study after adjusting for other variables, found no correlation between any of the vaginal symptoms and BV.

In both univariate and multivariate analysis for VVC and vaginal symptoms, pruritus was the only symptoms significantly associated. This finding is consistent with a study in India, where pruritus was the most common symptom to VVC with or without vaginal discharge (Ahmad & Khan, 2009). Eckertet *et al.*, study found significant association between VVC and pruritus or burning (Eckert *et al.*, 1998).

For trichomoniasis, none of the vaginal symptoms were associated in both the adjusted and unadjusted analysis. This might be due to the low number of *Trichomonas vaginalis* detected in the present study.

The study assessed the risk factors for the vaginal infections (BV, VVC, TV) using age groups, trimester, gravidae, douching, sex frequency per week and recent antibiotic use.

After adjusting for other confounding variable, the third trimester of pregnancy was shown to be protective for BV ($p=0.003$) and having had less than 3 pregnancies

(<gravidae 3) was a significant risk factors for BV. From the data, pregnant women in the third trimester were 35% and 53% less likely to have BV compared with the second and first trimesters respectively. BV appeared to decrease with increasing gestational age. The protective nature in third trimester might be due to the physical nature of the pregnant women which makes her sexually unattractive to the opposite sex thereby reducing multiple sexual partners and sex frequency which are the main determinant of vaginal infections. In addition, health education and care during antenatal visit could prevent the pregnant women from vaginal infections.

The study's findings of significant association between BV and having had less than 3 pregnancies (<gravidae 3) was in direct contradiction to that of Balla *et al.,* who reported a positive correlation between BV and having a parity of more than 2 (Bhalla & Kaushika, 1994). From the results (table 7.0), both unadjusted and adjusted odds ratio reveals increasing age group is inversely proportional to BV status. Therefore, there is a higher chance of having those with less than 3 previous pregnancies being in the lower or younger age groups thereby increasing the possibility of having BV. In addition, health education provided during antennal visits coupled with personal experiences in previous pregnancies might give those with three or more pregnancies better knowledge and experience concerning vaginal infections. Women with less than three pregnancies are less likely to have such education and experiences making them more prone to vaginal infections.

Douching which has been known to be a major risk factor to BV in several cross-sectional and longitudinal studies (Trabert & Misra, 2007) did not show any correlation in the present study. Demba et al and Bukusi et al studies showed no significant relationship between BV and douching which is consistent with the present study findings (Bukusi *et al.,* 2006; Demba *et al.,* 2005). Ness et al in his study showed

significant association between douching and BV, however in his findings the reason for douching by many women is because of abnormal vaginal symptoms (Ness *et al.*, 2002). It is possible douching might not be a risk factor for BV but rather a means by which women think they could treat the infection.

Risk factors considered for VVC and TV in this study were not significant in both univariate and multivariate analysis (P>0.05). Studies have shown recent antibiotic intake and douching have a positive correlation to VVC (Ahmad & Khan, 2009). This happens when antibiotic/douching substance kills or suppresses the lactobacillus species which serves as a protective organism making way for the yeast to thrive and colonise the vagina. This negative correlation between antibiotic use and VVC in the present study could be because of the low level of intake of antibiotic by the study participants. The study, however, did not demonstrate any association between TV and any of the risk factors being considered.

5.2 Limitations of the study

1. The diagnosis of TV was done using wet preparation for motile trichomonads which could have accounted for the low prevalence. This diagnosis method is about 60-70% sensitive but due to lack of financial resources the study could not use a more sensitive method such as culture.
2. The risk factors used for the analysis were self-report by study participants; there is the possibility of underreporting or misclassification of risky behaviour.

5.3 Conclusion

The study area had very high prevalence of vaginal infections among pregnant women especially VVC and BV. VVC was the most predominant (36.5%, 215/589) vaginal infection followed by BV (30.9%, 182/589) and the least was TV (1.4%, 8/589).

The study shows that pruritus as a key symptom in VVC infection and this should be considered during clinical diagnosis alongside laboratory confirmation. However, dysuria, lower abdominal pain and vaginal discharge were not associated with VVC and are therefore unspecific.

Having less than three pregnancies was a potential risk factor for BV in this study whiles being in the third trimester of pregnancy was protective of BV.

5.4 Recommendation

In view of the high prevalence of vaginal infections, pregnant women attending antenatal clinic should have prompt and adequate investigations with appropriate treatment to prevent adverse effect of the infection on mother and foetus.

In addition, a comprehensive program on reproductive healthcare education with the aim of reducing vaginal infection prevalence should be put in place.

The uncertainty surrounding how the infection affects the mother and foetus needs to be unraveled. Therefore, I recommend further longitudinal and follow-up studies to investigate the effect of the infection.

Further studies are needed to understand the potential role of BV and TV in HIV and HSV transmission to aid in STI/HIV prevention programs.

REFERENCES

Achkar, J. M., & Fries, B. C. (2010). Candida infections of the genitourinary tract. *Clinical microbiology reviews, 23*(2), 253-273. doi: 10.1128/CMR.00076-09

Adu-Sarkodie, Y., Opoku, B. K., Danso, K. A., Weiss, H. A., & Mabey, D. (2004). Comparison of latex agglutination, wet preparation, and culture for the detection of Trichomonas vaginalis. *Sexually transmitted infections, 80*(3), 201-203.

Agyarko-Poku, T. (2011). Aetiological agents of infective vaginal discharge among women attending a STD clinic in Kumasi, Ghana. *Sexually transmitted infections, 87*(Suppl 1), A305-A305.

Ahmad, A., & Khan, A. U. (2009). Prevalence of Candida species and potential risk factors for vulvovaginal candidiasis in Aligarh, India. *European journal of obstetrics, gynecology, and reproductive biology, 144*(1), 68-71. doi: 10.1016/j.ejogrb.2008.12.020

Ajani, G., Oduyebo, O., Haruna, M., & Elikwu, C. (2012). Nugent Scores of Pregnant Women in a Tertiary Institution in Nigeria.

Akah, P., Nnamani, C., & Nnamani, P. (2010). Prevalence and treatment outcome of vulvovaginal candidiasis in pregnancy in a rural community in Enugu State, Nigeria.

Akerele, J., Abhulimen, P., & Okonofua, F. (2002). Prevalence of asymptomatic genital infection among pregnant women in Benin City, Nigeria. *African journal of reproductive health*, 93-97.

Akinbiyi, A., Watson, R., & Feyi-Waboso, P. (2008). Prevalence of Candida albicans and bacterial vaginosis in asymptomatic pregnant women in South Yorkshire, United Kingdom. *Archives of gynecology and obstetrics, 278*(5), 463-466.

Alderete, J. F., & Garza, G. E. (1988). Identification and properties of Trichomonas vaginalis proteins involved in cytadherence. *Infection and immunity, 56*(1), 28-33.

Alli, J., Okonko, I., Odu, N., Kolade, A., & Nwanze, J. (2011). Detection and prevalence of Candida isolates among patients in Ibadan, Southwestern Nigeria. *Journal of Microbiology and Biotechnology Research, 1*(3), 176-184.

Allsworth, J. E., & Peipert, J. F. (2007). Prevalence of bacterial vaginosis: 2001-2004 National Health and Nutrition Examination Survey data. *Obstetrics and gynecology, 109*(1), 114-120. doi: 10.1097/01.AOG.0000247627.84791.91

Amsel, R., Totten, P. A., Spiegel, C. A., Chen, K. C., Eschenbach, D., & Holmes, K. K. (1983). Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. *The American journal of medicine, 74*(1), 14-22.

Anh, P. K., Khanh, N. T. N., Ha, D. T., Chien, D. T., Thuc, P. T., Luong, P. H., Kilmarx, P. H., Wongchotigul, V., Kitayaporn, D., & Rowe, P. J. (2003). Prevalence of lower genital tract infection among women attending maternal and child health and family planning clinics in Hanoi, Vietnam.

Anukam, K., Osazuwa, E., Ahonkhai, I., Ngwu, M., Osemene, G., Bruce, A. W., & Reid, G. (2006). Augmentation of antimicrobial metronidazole therapy of bacterial vaginosis with oral probiotic Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14: randomized, double-blind, placebo controlled trial. *Microbes and infection / Institut Pasteur, 8*(6), 1450-1454. doi: 10.1016/j.micinf.2006.01.003

Apalata, T., Longo-Mbenza, B., Sturm, A., Carr, W., & Moodley, P. (2014). Factors Associated with Symptomatic Vulvovaginal Candidiasis: A Study among Women Attending a Primary Healthcare Clinic in Kwazulu-Natal, South Africa. *Annals of medical and health sciences research, 4*(3), 410-416. doi: 10.4103/2141-9248.133470

Apea-Kubi, K. A., Sakyi, B., Yamaguchi, S., & Ofori-Adjei, D. (2006). Bacterial vaginosis, Candida albicans and Trichomonas vaginalis infection in antenatal and gynaecological patients in Ghana. *Tropical Journal of Obstetrics and Gynaecology, 22*(2), 108-112.

Arbabi, M., Fakhrieh, Z., Delavari, M., & Abdoli, A. (2014). Prevalence of Trichomonas vaginalis infection in Kashan city, Iran (2012-2013). *Iranian journal of reproductive medicine, 12*(7), 507-512.

Ashraf Ganjoei, T. (2005). Risk factors for bacterial vaginosis in women attending a hospital in Kerman, Islamic Republic of Iran.

Atashili, J., Poole, C., Ndumbe, P. M., Adimora, A. A., & Smith, J. S. (2008). Bacterial vaginosis and HIV acquisition: a meta-analysis of published studies. *Aids, 22*(12), 1493-1501. doi: 10.1097/QAD.0b013e3283021a37

Aubyn, G. B., & Tagoe, D. N. A. (2013). Prevalence of vaginal infections and associated lifestyles of students in the University of Cape Coast, Ghana. *Asian Pacific Journal of Tropical Disease, 3*(4), 267-270.

Bahram, A., Hamid, B., & Zohre, T. (2009). Prevalence of bacterial vaginosis and impact of genital hygiene practices in non-pregnant women in zanjan, iran. *Oman medical journal, 24*(4), 288.

Bhalla, P., Chawla, R., Garg, S., Singh, M., Raina, U., Bhalla, R., & Sodhani, P. (2007). Prevalence of bacterial vaginosis among women in Delhi, India. *Indian Journal of Medical Research, 125*(2), 167.

Bhalla, P., & Kaushika, A. (1994). Epidemilogical and microbiological correlates of bacterial vaginosis. *Indian Journal of Dermatology, Venereology, and Leprology, 60*(1), 8.

Brabin, L., Fairbrother, E., Mandal, D., Roberts, S., Higgins, S., Chandiok, S., Wood, P., Barnard, G., & Kitchener, H. (2005). Biological and hormonal markers of chlamydia, human papillomavirus, and bacterial vaginosis among adolescents attending genitourinary medicine clinics. *Sexually transmitted infections, 81*(2), 128-132.

Bradford, L. L., Ravel, J., & Bruno, V. (2013). Understanding vulvovaginal candidiasis through a community genomics approach. *Current Fungal Infection Reports, 7*(2), 126-131.

Bradshaw, C. S., Morton, A. N., Hocking, J., Garland, S. M., Morris, M. B., Moss, L. M., Horvath, L. B., Kuzevska, I., & Fairley, C. K. (2006). High recurrence rates of bacterial vaginosis over the course of 12 months after oral metronidazole therapy and factors associated with recurrence. *Journal of Infectious Diseases, 193*(11), 1478-1486.

Brand, J., & Galask, R. (1986). Trimethylamine: the substance mainly responsible for the fishy odor often associated with bacterial vaginosis. *Obstetrics & Gynecology, 68*(5), 682-685.

Bukusi, E. A., Cohen, C. R., Meier, A. S., Waiyaki, P. G., Nguti, R., Njeri, J. N., & Holmes, K. K. (2006). Bacterial vaginosis: risk factors among Kenyan women and their male partners. *Sexually transmitted diseases, 33*(6), 361-367.

Calzolari, E., Masciangelo, R., Milite, V., & Verteramo, R. (2000). Bacterial vaginosis and contraceptive methods. *International Journal of Gynecology & Obstetrics, 70*(3), 341-346.

Cassone, A. (2015). Vulvovaginal Candida albicans infections: pathogenesis, immunity and vaccine prospects. *BJOG : an international journal of obstetrics and gynaecology, 122*(6), 785-794. doi: 10.1111/1471-0528.12994

Cherpes, T. L., Hillier, S. L., Meyn, L. A., Busch, J. L., & Krohn, M. A. (2008). A delicate balance: risk factors for acquisition of bacterial vaginosis include sexual activity, absence of hydrogen peroxide-producing lactobacilli, black race, and positive herpes simplex virus type 2 serology. *Sexually transmitted diseases, 35*(1), 78-83. doi: 10.1097/OLQ.0b013e318156a5d0

Cherpes, T. L., Meyn, L. A., Krohn, M. A., Lurie, J. G., & Hillier, S. L. (2003). Association between acquisition of herpes simplex virus type 2 in women and bacterial vaginosis. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 37*(3), 319-325. doi: 10.1086/375819

Cotch, M. F., Pastorek, J. G., 2nd, Nugent, R. P., Hillier, S. L., Gibbs, R. S., Martin, D. H., Eschenbach, D. A., Edelman, R., Carey, J. C., Regan, J. A., Krohn, M. A., Klebanoff, M. A., Rao, A. V., & Rhoads, G. G. (1997). Trichomonas vaginalis associated with low birth weight and preterm delivery. The Vaginal Infections and Prematurity Study Group. *Sexually transmitted diseases, 24*(6), 353-360.

Cronje, H. S., Joubert, G., Muir, A., Chapman, R. D., Divall, P., & Bam, R. H. (1994). Prevalence of vaginitis, syphilis and HIV infection in women in the Orange Free State. *South African medical journal = Suid-Afrikaanse tydskrif vir geneeskunde, 84*(9), 602-605.

Crucitti, T., Jespers, V., Mulenga, C., Khondowe, S., Vandepitte, J., & Buve, A. (2011). Non-sexual transmission of Trichomonas vaginalis in adolescent girls attending school in Ndola, Zambia. *PloS one, 6*(1), e16310. doi: 10.1371/journal.pone.0016310

de Leon, E. M., Jacober, S. J., Sobel, J. D., & Foxman, B. (2002). Prevalence and risk factors for vaginal Candida colonization in women with type 1 and type 2 diabetes. *BMC Infectious Diseases, 2*, 1.

Deceuninck, G., Asamoah-Adu, C., Khonde, N., Pépin, J., Frost, E. H., Deslandes, S., Asamoah-Adu, A., Bekoe, V., & Alary, M. (2000). Improvement of Clinical Algorithms for the Diagnosis ofNeisseria gonorrhoeaeandChlamydia trachomatisby the Use of Gram-Stained Smears Among Female Sex Workers in Accra, Ghana. *Sexually transmitted diseases, 27*(7), 401-410.

Demba, E., Morison, L., van der Loeff, M. S., Awasana, A. A., Gooding, E., Bailey, R., Mayaud, P., & West, B. (2005). Bacterial vaginosis, vaginal flora patterns and vaginal hygiene practices in patients presenting with vaginal discharge syndrome in The Gambia, West Africa. *BMC infectious diseases, 5*(1), 12.

Domeika, M., Zhurauskaya, L., Savicheva, A., Frigo, N., Sokolovskiy, E., Hallen, A., Unemo, M., Ballard, R. C., Eastern European Network for, S., & Reproductive, H. (2010). Guidelines for the laboratory diagnosis of trichomoniasis in East European countries. *Journal of the European Academy of Dermatology and Venereology : JEADV, 24*(10), 1125-1134. doi: 10.1111/j.1468-3083.2010.03601.x

Donbraye-Emmanuel, O., Donbraye, E., Okonko, I., Alli, J., Ojezele, M., & Nwanze, J. (2010). Detection and prevalence of Candida among pregnant women in Ibadan, Nigeria. *World Applied Science Journal, 10*(9), 986-991.

Donders, G. (2010). Diagnosis and management of bacterial vaginosis and other types of abnormal vaginal bacterial flora: a review. *Obstetrical & gynecological survey, 65*(7), 462-473. doi: 10.1097/OGX.0b013e3181e09621

Donders, G. G., Mertens, I., Bellen, G., & Pelckmans, S. (2011). Self-elimination of risk factors for recurrent vaginal candidosis. *Mycoses, 54*(1), 39-45. doi: 10.1111/j.1439-0507.2009.01754.x

Draper, D., Parker, R., Patterson, E., Jones, W., Beutz, M., French, J., Borchardt, K., & McGregor, J. (1993). Detection of Trichomonas vaginalis in pregnant women with the InPouch TV culture system. *Journal of clinical microbiology, 31*(4), 1016-1018.

Duerr, A., Heilig, C. M., Meikle, S. F., Cu-Uvin, S., Klein, R. S., Rompalo, A., Sobel, J. D., & Group, H. S. (2003). Incident and persistent vulvovaginal candidiasis among human immunodeficiency virus–infected women: Risk factors and severity. *Obstetrics & Gynecology*, *101*(3), 548-556.

Eckert, L., Hawes, S., Stevens, C., Koutsky, L., Eschenbach, D., & Holmes, K. (1998). Vulvovaginal candidiasis: clinical manifestations, risk factors, management algorithm. *Obstetrics & Gynecology*, *92*(5), 757-765.

Eriksson, K., Carlsson, B., Forsum, U., & Larsson, P. (2005). A double-blind treatment study of bacterial vaginosis with normal vaginal lactobacilli after an open treatment with vaginal clindamycin ovules. *Acta dermato-venereologica*, *85*(1), 42-46.

Evans, E. G. V., Lacey, C. J., & Carney, J. A. (1986). Criteria for the diagnosis of vaginal candidosis: evaluation of a new latex agglutination test. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, *22*(5), 365-371.

Fidel, P. L. (2004). History and new insights into host defense against vaginal candidiasis. *Trends in microbiology*, *12*(5), 220-227.

Francis, S. C., Ao, T. T., Vanobberghen, F. M., Chilongani, J., Hashim, R., Andreasen, A., Watson-Jones, D., Changalucha, J., Kapiga, S., & Hayes, R. J. (2014). Epidemiology of Curable Sexually Transmitted Infections among Women at Increased Risk for HIV in Northwestern Tanzania: Inadequacy of Syndromic Management. *PloS one*, *9*(7), e101221.

Fredricks, D. N., Fiedler, T. L., & Marrazzo, J. M. (2005). Molecular identification of bacteria associated with bacterial vaginosis. *The New England journal of medicine*, *353*(18), 1899-1911. doi: 10.1056/NEJMoa043802

Garcia-Tamayo, J., Castillo, G., & Martinez, A. (1981). Human genital candidiasis: histochemistry, scanning and transmission electron microscopy. *Acta cytologica*, *26*(1), 7-14.

Garcia Heredia, M., Garcia, S. D., Copolillo, E. F., Cora Eliseth, M., Barata, A. D., Vay, C. A., de Torres, R. A., Tiraboschi, N., & Famiglietti, A. M. (2006). [Prevalence of vaginal candidiasis in pregnant women. Identification of yeasts and susceptibility to antifungal agents]. *Revista Argentina de microbiologia*, *38*(1), 9-12.

Gardner, H. L., & Dukes, C. D. (1955). Haemophilus vaginalis vaginitis: a newly defined specific infection previously classified non-specific vaginitis. *American journal of obstetrics and gynecology*, *69*(5), 962-976.

Gatski, M., Martin, D. H., Clark, R. A., Harville, E., Schmidt, N., & Kissinger, P. (2011). Co-occurrence of Trichomonas vaginalis and bacterial vaginosis among HIV-positive women. *Sexually transmitted diseases*, *38*(3), 163.

Gatski, M., Martin, D. H., Levison, J., Mena, L., Clark, R. A., Murphy, M., Henderson, H., Schmidt, N., & Kissinger, P. (2011). The influence of bacterial vaginosis on the response to Trichomonas vaginalis treatment among HIV-infected women. *Sexually transmitted infections*, sti. 2010.046441.

Georgijevic, A., Cjukic-Ivancevic, S., & Bujko, M. (2000). [Bacterial vaginosis. Epidemiology and risk factors]. *Srpski arhiv za celokupno lekarstvo*, *128*(1-2), 29-33.

Ginocchio, C. C., Chapin, K., Smith, J. S., Aslanzadeh, J., Snook, J., Hill, C. S., & Gaydos, C. A. (2012). Prevalence of Trichomonas vaginalis and coinfection with Chlamydia trachomatis and Neisseria gonorrhoeae in the United States as determined by the Aptima Trichomonas vaginalis nucleic acid amplification assay. *Journal of clinical microbiology*, *50*(8), 2601-2608. doi: 10.1128/JCM.00748-12

Giraldo, P., von Nowaskonski, A., Gomes, F. A., Linhares, I., Neves, N. A., & Witkin, S. S. (2000). Vaginal colonization by Candida in asymptomatic women with and without a history of recurrent vulvovaginal candidiasis. *Obstetrics & Gynecology*, *95*(3), 413-416.

Goldenberg, R. L., Klebanoff, M. A., Nugent, R., Krohn, M. A., Hillier, S., & Andrews, W. W. (1996). Bacterial colonization of the vagina during pregnancy in four ethnic groups. *American journal of obstetrics and gynecology*, *174*(5), 1618-1621.

Gray, R. H., Wabwire-Mangen, F., Kigozi, G., Sewankambo, N. K., Serwadda, D., Moulton, L. H., Quinn, T. C., O'Brien, K. L., Meehan, M., Abramowsky, C., Robb, M., & Wawer, M. J. (2001). Randomized trial of presumptive sexually transmitted disease therapy during pregnancy in Rakai, Uganda. *American journal of obstetrics and gynecology*, *185*(5), 1209-1217. doi: 10.1067/mob.2001.118158

Guzel, A. B., Ilkit, M., Burgut, R., Urunsak, I. F., & Ozgunen, F. T. (2011). An evaluation of risk factors in pregnant women with Candida vaginitis and the diagnostic value of simultaneous vaginal and rectal sampling. *Mycopathologia*, *172*(1), 25-36.

Hardy, P., Nell, E. E., Spence, M., Hardy, J., Graham, D., & Rosenbaum, R. (1984). Prevalence of six sexually transmitted disease agents among pregnant inner-city adolescents and pregnancy outcome. *The Lancet*, *324*(8398), 333-337.

Hashimoto, M., Komori, A., Kuramasu, T., Yokoyama, Y., Kawase, N., & Nakamura, T. (1964). Electron Microscopic Studies on the Fine Structure of Trichomonas Vaginalis. *Journal of the Japanese Obstetrical & Gynecological Society*, *11*, 162-166.

Hay, P. E., Lamont, R. F., Taylor-Robinson, D., Morgan, D. J., Ison, C., & Pearson, J. (1994). Abnormal bacterial colonisation of the genital tract and subsequent

preterm delivery and late miscarriage. *British Medical journal, 308*(6924), 295-298.

Hegazi, M. M., Makhlouf, L. M., Elbahey, M. A., El-Hamshary, E. M., Dawoud, H. A., & El-Gayar, E. K. (2009). Polymerase chain reaction versus conventional methods in the diagnosis of vaginal trichomoniasis. *Journal of the Egyptian Society of Parasitology, 39*(1), 11-21.

Hester, R. A., & Kennedy, S. B. (2003). Candida infection as a risk factor for HIV transmission. *Journal of Women's Health, 12*(5), 487-494.

Hillier, S. L. (1993). Diagnostic microbiology of bacterial vaginosis. *American journal of obstetrics and gynecology, 169*(2 Pt 2), 455-459.

Hillier, S. L., Nugent, R. P., Eschenbach, D. A., Krohn, M. A., Gibbs, R. S., Martin, D. H., Cotch, M. F., Edelman, R., Pastorek, J. G., 2nd, Rao, A. V., & et al. (1995). Association between bacterial vaginosis and preterm delivery of a low-birth-weight infant. The Vaginal Infections and Prematurity Study Group. *The New England journal of medicine, 333*(26), 1737-1742. doi: 10.1056/NEJM199512283332604

Hobbs, M. M., Lapple, D. M., Lawing, L. F., Schwebke, J. R., Cohen, M. S., Swygard, H., Atashili, J., Leone, P. A., Miller, W. C., & Sena, A. C. (2006). Methods for detection of Trichomonas vaginalis in the male partners of infected women: implications for control of trichomoniasis. *Journal of clinical microbiology, 44*(11), 3994-3999. doi: 10.1128/JCM.00952-06

Holzman, C., Leventhal, J. M., Qiu, H., Jones, N. M., Wang, J., & Group, B. V. S. (2001). Factors linked to bacterial vaginosis in nonpregnant women. *American journal of public health, 91*(10), 1664-1670.

Honigberg, B. M., & King, V. M. (1964). Structure of Trichomonas Vaginalis Donn'e. *The Journal of parasitology, 50*, 345-364.

Jefferson, K. K. (2012). The bacterial etiology of preterm birth. *Advances in applied microbiology, 80*, 1-22. doi: 10.1016/B978-0-12-394381-1.00001-5

Jespers, V., Crucitti, T., Menten, J., Verhelst, R., Mwaura, M., Mandaliya, K., Ndayisaba, G. F., Delany-Moretlwe, S., Verstraelen, H., & Hardy, L. (2014). Prevalence and correlates of bacterial vaginosis in different sub-populations of women in Sub-Saharan Africa: a cross-sectional study. *PloS one, 9*(10), e109670.

Johnson, A., Ison, C., Hetherington, C., Osborn, M., Southerton, G., London, W., Easmon, C., & Taylor-Robinson, D. (1984). A study of the susceptibility of three species of primate to vaginal colonization with Gardnerella vaginalis. *British journal of experimental pathology, 65*(3), 389.

Kengne, P., Veas, F., Vidal, N., Rey, J. L., & Cuny, G. (1994). Trichomonas vaginalis: repeated DNA target for highly sensitive and specific polymerase chain reaction diagnosis. *Cellular and molecular biology, 40*(6), 819-831.

KHRC. (2010). Kintampo Health Research Centre Annual Report.

Kilmarx, P. H., Limpakarnjanarat, K., Mastro, T. D., Saisorn, S., Kaewkungwal, J., Korattana, S., Uthaivoravit, W., Young, N. L., Weniger, B. G., & St Louis, M. E. (1998). HIV-1 seroconversion in a prospective study of female sex workers in northern Thailand: continued high incidence among brothel-based women. *Aids, 12*(14), 1889-1898.

Kirakoya-Samadoulougou, F., Nagot, N., Defer, M.-C., Yaro, S., Fao, P., Ilboudo, F., Langani, Y., Meda, N., & Robert, A. (2011). Epidemiology of herpes simplex virus type 2 infection in rural and urban Burkina Faso. *Sexually transmitted diseases, 38*(2), 117-123.

Kirakoya-Samadoulougou, F., Nagot, N., Defer, M.-C., Yaro, S., Meda, N., & Robert, A. (2008). Bacterial vaginosis among pregnant women in Burkina Faso. *Sexually transmitted diseases, 35*(12), 985-989.

Kiss, H., Petricevic, L., & Husslein, P. (2004). Prospective randomised controlled trial of an infection screening programme to reduce the rate of preterm delivery. *British Medical Journal, 329*(7462), 371.

Klebanoff, M. A., Hillier, S. L., Nugent, R. P., MacPherson, C. A., Hauth, J. C., Carey, J. C., Harper, M., Wapner, R. J., Trout, W., & Moawad, A. (2005). Is bacterial vaginosis a stronger risk factor for preterm birth when it is diagnosed earlier in gestation? *American journal of obstetrics and gynecology, 192*(2), 470-477.

Koumans, E. H., Kendrick, J. S., & Group, C. D. C. B. V. W. (2001). Preventing adverse sequelae of bacterial vaginosis: a public health program and research agenda. *Sexually transmitted diseases, 28*(5), 292-297.

Koumans, E. H., Sternberg, M., Bruce, C., McQuillan, G., Kendrick, J., Sutton, M., & Markowitz, L. E. (2007). The prevalence of bacterial vaginosis in the United States, 2001–2004; associations with symptoms, sexual behaviors, and reproductive health. *Sexually transmitted diseases, 34*(11), 864-869.

Krieger, J. N., Tam, M. R., Stevens, C. E., Nielsen, I. O., Hale, J., Kiviat, N. B., & Holmes, K. K. (1988). Diagnosis of trichomoniasis. Comparison of conventional wet-mount examination with cytologic studies, cultures, and monoclonal antibody staining of direct specimens. *Journal of the American Medical Association, 259*(8), 1223-1227.

Kurth, A., Whittington, W. L., Golden, M. R., Thomas, K. K., Holmes, K. K., & Schwebke, J. R. (2004). Performance of a new, rapid assay for detection of Trichomonas vaginalis. *Journal of clinical microbiology, 42*(7), 2940-2943. doi: 10.1128/JCM.42.7.2940-2943.2004

Laga, M., Manoka, A., Kivuvu, M., Malele, B., Tuliza, M., Nzila, N., Goeman, J., Behets, F., Batter, V., & Alary, M. (1993). Non-ulcerative sexually transmitted diseases as risk factors for HIV-1 transmission in women: results from a cohort study. *Aids, 7*(1), 95-102.

Lan, P., Lundborg, C. S., Phuc, H., Sihavong, A., Unemo, M., Chuc, N., Khang, T., & Mogren, I. (2008). Reproductive tract infections including sexually transmitted infections: a population-based study of women of reproductive age in a rural district of Vietnam. *Sexually transmitted infections*, *84*(2), 126-132.

Larsson, P., Fåhraeus, L., Carlsson, B., Jakobsson, T., & Forsum, U. (2007). Predisposing factors for bacterial vaginosis, treatment efficacy and pregnancy outcome among term deliveries; results from a preterm delivery study. *BMC women's health*, *7*(1), 20.

Lassey, A. T., Adanu, K. R., Newman, M. J., & Opintah, J. A. (2004). Potential pathogens in the lower genital tract at manual vacuum aspiration for incomplete abortion in Korle Bu Teaching Hospital, Ghana. *East African medical journal*, *81*(8), 398-401.

Lawing, L. F., Hedges, S. R., & Schwebke, J. R. (2000). Detection of trichomonosis in vaginal and urine specimens from women by culture and PCR. *Journal of clinical microbiology*, *38*(10), 3585-3588.

Leitich, H., Bodner-Adler, B., Brunbauer, M., Kaider, A., Egarter, C., & Husslein, P. (2003). Bacterial vaginosis as a risk factor for preterm delivery: a meta-analysis. *American journal of obstetrics and gynecology*, *189*(1), 139-147.

Leitich, H., & Kiss, H. (2007). Asymptomatic bacterial vaginosis and intermediate flora as risk factors for adverse pregnancy outcome. *Best practice & research. Clinical obstetrics & gynaecology*, *21*(3), 375-390. doi: 10.1016/j.bpobgyn.2006.12.005

Limia, O. F., & Lantero, M. I. (2004). Prevalence of Candida albicans and Trichomonas vaginalis in pregnant women in Havana City by an immunologic latex agglutination test. *Medscape General Medicine*, *6*(4).

Livengood, C. H., 3rd, McGregor, J. A., Soper, D. E., Newton, E., & Thomason, J. L. (1994). Bacterial vaginosis: efficacy and safety of intravaginal metronidazole treatment. *American journal of obstetrics and gynecology*, *170*(3), 759-764.

Livengood III, C. H. (2009). Bacterial vaginosis: an overview for 2009. *Reviews in obstetrics and Gynecology*, *2*(1), 28.

Lossick, J. G. (1980). Single-dose metronidazole treatment for vaginal trichomoniasis. *Obstetrics and gynecology*, *56*(4), 508-510.

Lossick, J. G., Muller, M., & Gorrell, T. E. (1986). In vitro drug susceptibility and doses of metronidazole required for cure in cases of refractory vaginal trichomoniasis. *The Journal of infectious diseases*, *153*(5), 948-955.

Macdonald, F. (1984). Secretion of inducible proteinase by pathogenic Candida species. *Sabouraudia*, *22*(1), 79-82.

Madhivanan, P., Krupp, K., Chandrasekaran, V., Karat, C., Arun, A., Cohen, C., Reingold, A., & Klausner, J. (2008). Prevalence and correlates of bacterial vaginosis among young women of reproductive age in Mysore, India. *Indian journal of medical microbiology*, *26*(2), 132.

Manorama, H. T., & Shenoy, D. R. (1978). Single-dose oral treatment of vaginal trichomoniasis with tinidazole and metronidazole. *The Journal of international medical research*, *6*(1), 46-49.

Marrazzo, J. M. (2011). Interpreting the epidemiology and natural history of bacterial vaginosis: are we still confused? *Anaerobe*, *17*(4), 186-190. doi: 10.1016/j.anaerobe.2011.03.016

Martin, H. L., Richardson, B. A., Nyange, P. M., Lavreys, L., Hillier, S. L., Chohan, B., Mandaliya, K., Ndinya-Achola, J. O., Bwayo, J., & Kreiss, J. (1999). Vaginal lactobacilli, microbial flora, and risk of human immunodeficiency virus type 1 and sexually transmitted disease acquisition. *The Journal of infectious diseases*, *180*(6), 1863-1868. doi: 10.1086/315127

Martinez-de-Oliveira, J., & Fonseca, A. (1990). Diagnosing vaginal candidosis by a slide latex agglutination test. *Infectious Diseases in Obstetrics and Gynecology. Bologna: Monduzi Editore*, 171-172.

Matini, M., Rezaie, S., Mohebali, M., Maghsood, A., Rabiee, S., Fallah, M., & Rezaeian, M. (2012). Prevalence of Trichomonas vaginalis Infection in Hamadan City, Western Iran. *Iranian journal of parasitology*, *7*(2), 67.

McClelland, R. S., Richardson, B. A., Hassan, W. M., Graham, S. M., Kiarie, J., Baeten, J. M., Mandaliya, K., Jaoko, W., Ndinya-Achola, J. O., & Holmes, K. K. (2009). Prospective study of vaginal bacterial flora and other risk factors for vulvovaginal candidiasis. *The Journal of infectious diseases*, *199*(12), 1883-1890. doi: 10.1086/599213

McClelland, R. S., Sangare, L., Hassan, W. M., Lavreys, L., Mandaliya, K., Kiarie, J., Ndinya-Achola, J., Jaoko, W., & Baeten, J. M. (2007). Infection with Trichomonas vaginalis increases the risk of HIV-1 acquisition. *The Journal of infectious diseases*, *195*(5), 698-702. doi: 10.1086/511278

McCourtie, J., & Douglas, L. J. (1985). Extracellular polymer of Candida albicans: isolation, analysis and role in adhesion. *Journal of general microbiology*, *131*(3), 495-503.

Minkoff, H., Grunebaum, A. N., Schwarz, R. H., Feldman, J., Cummings, M., Crombleholme, W., Clark, L., Pringle, G., & McCormack, W. M. (1984). Risk factors for prematurity and premature rupture of membranes: a prospective study of the vaginal flora in pregnancy. *American journal of obstetrics and gynecology*, *150*(8), 965-972.

Mohanty, S., Xess, I., Hasan, F., Kapil, A., Mittal, S., & Tolosa, J. E. (2007). Prevalence & susceptibility to fluconazole of Candida species causing vulvovaginitis. *The Indian journal of medical research, 126*(3), 216-219.

Moodley, P., Connolly, C., & Sturm, A. W. (2002). Interrelationships among human immunodeficiency virus type 1 infection, bacterial vaginosis, trichomoniasis, and the presence of yeasts. *Journal of Infectious Diseases, 185*(1), 69-73.

Muvunyi, C. M., & Hernandez, T. C. (2009). Prevalence of Bacterial vaginosis in women with vaginal symptoms in South Province, Rwanda. *African Journal of Clinical and Experimental Microbiology, 10*(3).

Myer, L., Denny, L., Telerant, R., Souza, M., Wright, T. C., Jr., & Kuhn, L. (2005). Bacterial vaginosis and susceptibility to HIV infection in South African women: a nested case-control study. *The Journal of infectious diseases, 192*(8), 1372-1380. doi: 10.1086/462427

Nagot, N., Ouedraogo, A., Defer, M. C., Vallo, R., Mayaud, P., & Van de Perre, P. (2007). Association between bacterial vaginosis and Herpes simplex virus type-2 infection: implications for HIV acquisition studies. *Sexually transmitted infections, 83*(5), 365-368. doi: 10.1136/sti.2007.024794

Neale, K., & Alderete, J. (1990). Analysis of the proteinases of representative Trichomonas vaginalis isolates. *Infection and immunity, 58*(1), 157-162.

Ness, R. B., Hillier, S. L., Richter, H. E., Soper, D. E., Stamm, C., McGregor, J., Bass, D. C., Sweet, R. L., & Rice, P. (2002). Douching in relation to bacterial vaginosis, lactobacilli, and facultative bacteria in the vagina. *Obstetrics and gynecology, 100*(4), 765.

Nugent, R. P., Krohn, M. A., & Hillier, S. L. (1991). Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *Journal of clinical microbiology, 29*(2), 297-301.

Nyirjesy, P., Seeney, S. M., Grody, M. H., Jordan, C. A., & Buckley, H. R. (1995). Chronic fungal vaginitis: the value of cultures. *American journal of obstetrics and gynecology, 173*(3 Pt 1), 820-823.

Odds, F. C. (1988). *Candida and candidosis: a review and bibliography*: Bailliere Tindall, London.

Okonko, I., Okerentugba, P., Adejuwon, A., & Onoh, C. (2012). Prevalence of sexually transmitted infections (STIs) among attendees of lead city university medical centre in Ibadan, Southwestern, Nigeria. *Archives of Applied Science Research, 4*(2), 980-987.

Okonkwo, N., & Umeanaeto, P. (2010). Prevalence of vaginal candidiasis among pregnant women in Nnewi Town of Anambra State, Nigeria. *African Research Review, 4*(4).

Olowe, O., Makanjuola, O., Olowe, R., & Adekanle, D. (2014). Prevalence of vulvovaginal candidiasis, trichomoniasis and bacterial vaginosis among pregnant women receiving antenatal care in Southwestern Nigeria. *European Journal of Microbiology and Immunology, 4*(4), 193-197.

Ovcinnikov, N. M., Delektorskij, V. V., Turanova, E. N., & Yashkova, G. N. (1975). Further studies of Trichomonas Vaginalis with transmission and scanning electron microscopy. *The British journal of venereal diseases, 51*(6), 357-375.

Paavonen, J., Mangioni, C., Martin, M. A., & Wajszczuk, C. P. (2000). Vaginal clindamycin and oral metronidazole for bacterial vaginosis: a randomized trial. *Obstetrics and gynecology, 96*(2), 256-260.

Pappas, P. G., Rex, J. H., Sobel, J. D., Filler, S. G., Dismukes, W. E., Walsh, T. J., & Edwards, J. E. (2004). Guidelines for treatment of candidiasis. *Clinical Infectious Diseases, 38*(2), 161-189.

Pépin, J., Deslandes, S., Giroux, G., Sobéla, F., Khonde, N., Diakité, S., Demeule, S., Labbé, A.-C., Carrier, N., & Frost, E. (2011). The complex vaginal flora of West African women with bacterial vaginosis. *PloS one, 6*(9), e25082.

Pepin, J., Deslandes, S., Giroux, G., Sobela, F., Khonde, N., Diakite, S., Demeule, S., Labbe, A. C., Carrier, N., & Frost, E. (2011). The complex vaginal flora of West African women with bacterial vaginosis. *PloS one, 6*(9), e25082. doi: 10.1371/journal.pone.0025082

Perez, S., Fernandez-Verdugo, A., Perez, F., & Vazquez, F. (2001). Prevalence of 5-nitroimidazole-resistant trichomonas vaginalis in Oviedo, Spain. *Sexually transmitted diseases, 28*(2), 115-116.

Petrin, D., Delgaty, K., Bhatt, R., & Garber, G. (1998). Clinical and microbiological aspects ofTrichomonas vaginalis. *Clinical microbiology reviews, 11*(2), 300-317.

Pindak, F., Gardner, W., & De Pindak, M. M. (1986). Growth and cytopathogenicity of Trichomonas vaginalis in tissue cultures. *Journal of clinical microbiology, 23*(4), 672-678.

Portillo, F., & Gancedo, C. (1986). Purification and properties of three intracellular proteinases from Candida albicans. *Biochimica et Biophysica Acta (BBA)-General Subjects, 881*(2), 229-235.

Purwar, M., Ughade, S., Bhagat, B., Agarwal, V., & Kulkarni, H. (2001). Bacterial vaginosis in early pregnancy and adverse pregnancy outcome. *Journal of obstetrics and gynaecology research, 27*(4), 175-181.

Ralph, S., Rutherford, A., & Wilson, J. (1999). Influence of bacterial vaginosis on conception and miscarriage in the first trimester: cohort study. *Britist Medical Journal, 319*(7204), 220-223.

Reed, B. D., Zazove, P., Pierson, C. L., Gorenflo, D. W., & Horrocks, J. (2003). Candida transmission and sexual behaviors as risks for a repeat episode of Candida vulvovaginitis. *Journal of Women's Health, 12*(10), 979-989.

Riggs, M., Klebanoff, M., Nansel, T., Zhang, J., Schwebke, J., & Andrews, W. (2007). Longitudinal association between hormonal contraceptives and bacterial vaginosis in women of reproductive age. *Sexually transmitted diseases, 34*(12), 954-959.

Riley, D. E., Roberts, M. C., Takayama, T., & Krieger, J. N. (1992). Development of a polymerase chain reaction-based diagnosis of Trichomonas vaginalis. *Journal of clinical microbiology, 30*(2), 465-472.

Rivers, C. A., Adaramola, O. O., & Schwebke, J. R. (2011). Prevalence of bacterial vaginosis and vulvovaginal candidiasis mixed infection in a southeastern american STD clinic. *Sexually transmitted diseases, 38*(7), 672-674.

Roberts, C. L., Rickard, K., Kotsiou, G., & Morris, J. M. (2011). Treatment of asymptomatic vaginal candidiasis in pregnancy to prevent preterm birth: an open-label pilot randomized controlled trial. *BMC pregnancy and childbirth, 11*(1), 18.

Rodrigues, A., Mårdh, P.-A., Pina-Vaz, C., Martinez-de-Oliveira, J., & Fonseca, A. (1999). Germ tube formation changes surface hydrophobicity of Candida cells. *Infectious diseases in obstetrics and gynecology, 7*(5), 222-226.

Røttingen, J.-A., Cameron, D. W., & Garnett, G. P. (2001). A systematic review of the epidemiologic interactions between classic sexually transmitted diseases and HIV: how much really is known? *Sexually transmitted diseases, 28*(10), 579-597.

Ryu, J. S., Chung, H. L., Min, D. Y., Cho, Y. H., Ro, Y. S., & Kim, S. R. (1999). Diagnosis of trichomoniasis by polymerase chain reaction. *Yonsei medical journal, 40*(1), 56-60.

Sawyer, P. R., Brogden, R. N., Pinder, R. M., Speight, T. M., & Avery, G. S. (1976). Tinidazole: a review of its antiprotozoal activity and therapeutic efficacy. *Drugs, 11*(6), 423-440.

Schmid, G., Narcisi, E., Mosure, D., Secor, W. E., Higgins, J., & Moreno, H. (2001). Prevalence of metronidazole-resistant Trichomonas vaginalis in a gynecology clinic. *The Journal of reproductive medicine, 46*(6), 545-549.

Schwebke, J. R., & Barrientes, F. J. (2006). Prevalence of Trichomonas vaginalis isolates with resistance to metronidazole and tinidazole. *Antimicrobial agents and chemotherapy, 50*(12), 4209-4210. doi: 10.1128/AAC.00814-06

Schwebke, J. R., & Weiss, H. (2001). Influence of the normal menstrual cycle on vaginal microflora. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 32*(2), 325. doi: 10.1086/318464

Sebitloane, H., Moodley, J., & Esterhuizen, T. (2011). Pathogenic lower genital tract organisms in HIV-infected and uninfected women, and their association with postpartum infectious morbidity. *SAMJ: South African Medical Journal, 101*(7), 463-466.

Sena, A. C., Miller, W. C., Hobbs, M. M., Schwebke, J. R., Leone, P. A., Swygard, H., Atashili, J., & Cohen, M. S. (2007). Trichomonas vaginalis infection in male sexual partners: implications for diagnosis, treatment, and prevention. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 44*(1), 13-22. doi: 10.1086/511144

Simhan, H. N., Caritis, S. N., Hillier, S. L., & Krohn, M. A. (2005). Cervical anti-inflammatory cytokine concentrations among first-trimester pregnant smokers. *American journal of obstetrics and gynecology, 193*(6), 1999-2003.

Simon, V., Ho, D. D., & Karim, Q. A. (2006). HIV/AIDS epidemiology, pathogenesis, prevention, and treatment. *The Lancet, 368*(9534), 489-504.

Sobel, J., Myers, P., Kaye, D., & Levison, M. (1981). Adherence of Candida albicans to human vaginal and buccal epithelial cells. *Journal of Infectious Diseases, 143*(1), 76-82.

Sobel, J., Peipert, J. F., McGregor, J. A., Livengood, C., Martin, M., Robbins, J., & Wajszczuk, C. P. (2001). Efficacy of clindamycin vaginal ovule (3-day treatment) vs. clindamycin vaginal cream (7-day treatment) in bacterial vaginosis. *Infectious diseases in obstetrics and gynecology, 9*(1), 9-15. doi: 10.1155/S1064744901000035

Sobel, J. D. (1997). Vaginitis. *The New England journal of medicine, 337*(26), 1896-1903. doi: 10.1056/NEJM199712253372607

Sobel, J. D. (2000). Bacterial vaginosis. *Annual review of medicine, 51*, 349-356. doi: 10.1146/annurev.med.51.1.349

Sobel, J. D. (2005). What's new in bacterial vaginosis and trichomoniasis? *Infectious disease clinics of North America, 19*(2), 387-406. doi: 10.1016/j.idc.2005.03.001

Sobel, J. D. (2007). Vulvovaginal candidosis. *Lancet, 369*(9577), 1961-1971. doi: 10.1016/S0140-6736(07)60917-9

Sobel, J. D., Subramanian, C., Foxman, B., Fairfax, M., & Gygax, S. E. (2013). Mixed vaginitis—more than coinfection and with therapeutic implications. *Current infectious disease reports, 15*(2), 104-108.

Sunday-Adeoye, I., Adeoye, J., Umeora, O., & Okonta, P. (2009). The prevalence of Trichomonas vaginalis and Candida albicans infection in the lower genital tracts of antenatal patients in Abakaliki, Southeastern Nigeria. *Nepal Journal of Obstetrics and Gynaecology, 4*(1), 11-14.

Sutton, M., Sternberg, M., Koumans, E. H., McQuillan, G., Berman, S., & Markowitz, L. (2007). The prevalence of Trichomonas vaginalis infection among reproductive-age women in the United States, 2001–2004. *Clinical Infectious Diseases, 45*(10), 1319-1326.

Sutton, M. Y., Sternberg, M., Nsuami, M., Behets, F., Nelson, A. M., & Louis, M. E. S. (1999). Trichomoniasis in pregnant human immunodeficiency virus–infected and human immunodeficiency virus–uninfected Congolese women: Prevalence, risk factors, and association with low birth weight. *American journal of obstetrics and gynecology, 181*(3), 656-662.

Tolosa, J. E., Chaithongwongwatthana, S., Daly, S., Maw, W. W., Gaitan, H., Lumbiganon, P., Festin, M., Chipato, T., Sauvarin, J., Goldenberg, R. L., Andrews, W. W., & Whitney, C. G. (2006). The International Infections in Pregnancy (IIP) study: variations in the prevalence of bacterial vaginosis and distribution of morphotypes in vaginal smears among pregnant women. *American journal of obstetrics and gynecology, 195*(5), 1198-1204. doi: 10.1016/j.ajog.2006.08.016

Trabert, B., & Misra, D. P. (2007). Risk factors for bacterial vaginosis during pregnancy among African American women. *American journal of obstetrics and gynecology, 197*(5), 477 e471-478. doi: 10.1016/j.ajog.2007.03.085

Turovskiy, Y., Sutyak Noll, K., & Chikindas, M. L. (2011). The aetiology of bacterial vaginosis. *Journal of applied microbiology, 110*(5), 1105-1128.

Valadkhani, Z. (2004). Role of pH on Adhesion of Trichomonas Vaginalis Isolated from Symptomatic & Asymptomatic Women to Vaginal Epithelial Cells in Vitro [hplimg].

WHO, W. H. O. (2005). Sexually transmitted and other reproductive tract infections: a guide to essential practice.

Workowski, K. A., Berman, S., Centers for Disease, C., & Prevention. (2010). Sexually transmitted diseases treatment guidelines, 2010. *MMWR. Recommendations and reports : Morbidity and mortality weekly report. Recommendations and reports / Centers for Disease Control, 59*(RR-12), 1-110.

Yen, S., Shafer, M.-A., Moncada, J., Campbell, C. J., Flinn, S. D., & Boyer, C. B. (2003). Bacterial vaginosis in sexually experienced and non–sexually experienced young women entering the military. *Obstetrics & Gynecology, 102*(5), 927-933.

Young, G., & Jewell, D. (2001). Topical treatment for vaginal candidiasis (thrush) in pregnancy. *Cochrane Database Systematic Review, 4*.

APPENDICES

APPENDIX 1 INFORMED CONSENT FORM

Introduction

The Kintampo Health Research Centre is carrying out a study on the prevalence of bacterial vaginosis and other reproductive tract infections in pregnant women in Kintampo North Municipality and South District. We would like to invite you to participate in the study.

Purpose of the Study

Bacterial Vaginosis (BV) is a common disorder of the genital tract in women characterized by an alteration of the normal acidic vaginal environment dominated by the organism *lactobacilli* to a vaginal environment dominated by pathogens such as *Gardneralla vaginallis*, *Mycoplasma species* and anaerobes, with a corresponding increase in pH. Certain practices, medications and sexual life style have been known to be associated with this disorder. Also reproductive tract infections such as Candidiasis, Trichomoniasis and Chlamydia infections are dominant among female in their reproductive age. This disorder/infections have been known to be associated with many adverse pregnancy outcomes such as spontaneous abortion, still birth, prematurity, low birth weight, postpartum endometritis, premature rapture of membrane, infertility, postpartum sepsis, spontaneous miscarriage and sequelae in surviving neonates. BV management is of little or no consideration in ante-natal clinics but these infections could cause very serious complication in pregnancy. Also the uncertainties surrounding the role of BV in acquisition of sexually transmitted infections require a better understanding of the epidemiology of the disease. The aim of the study seeks to determine the prevalence of Bacterial vaginosis and other Reproductive tract infections in the Kintampo North Municipal and Kintampo South District. The results of this study will be important in planning intervention studies aimed at reducing bacterial vaginal infections and improving birth outcomes.

Study Procedure

We are inviting you to take part in this study because you/your child are (is) pregnant and you resident in this municipality/district. If you agree to (or for your child to) be part of this study, you will sign this form and I will ask you a few questions on your socio-

demographics, lifestyle, sexual and reproductive history, contraceptive use, current and past medical history of genital infection symptoms. HIV, Hepatitis B and Syphilis results will be copied from your (child) maternal health record book. A midwife will examine you/your child and take two high vagina swabs (HVS) and an endocervical swab. This sample will be examined in the laboratory for bacterial vaginosis and other reproductive tract infections.

Risks or Discomforts

A trained midwife will collect the specimen which will be examined in the laboratory for possible infections. The procedure will be carried out under aseptic condition in order not to introduce any microorganism into your sex organ. This will not cause any harm to you or the unborn baby.

Compensation

You will not be paid for your participation in this study. However, if you get injured as a result of your participation in the study, the study will take care of you.

Benefits

For participants that would be found to have reproductive tract infection would be referred to the health facility for treatment. The information given to us will also help to contribute to improve reproductive health services to persons like you and several others in the future.

Confidentiality

The information that is collected from you will be used only for the purpose of this study. We will not use your name or any information that will make it possible to identify you when we are talking or writing about this study. The forms on which we will take information from you will be kept under lock and key during and after the study period. Our staff who will be speaking to you have been well trained and will not give out your information to anyone outside the study.

Voluntariness and Right to Withdraw

Being part of this study is completely voluntary. You have the right to leave the study at any time, or to decide not to answer any question. If you decide not to take part in this

study, your decision will not affect your relationship with the interviewers and the KHRC in anyway.

Persons to Contact

If at any time, you have questions related to this study, you may contact the following persons**;** Mr. Dennis Konadu Gyasi on telephone number 0243440760, Ms. Zuwera Yidana (0244973090), Mr. Farrid Boadu (0247561998) and Ms Louisa Iddrisu (0244469868) at the Kintampo Health Research Centre, or write to them through this address: Kintampo Health Research Centre, Ghana Health Service, Post Office Box 200, Kintampo B/A. If you have questions as regards your rights as a study participant, please contact the chairman of the KHRC ethics committee; Dr. Eyison, at the College of Health and Wellbeing, Kintampo or you can call him on telephone numbers 020 9121255.

Statement of Consent

I confirm that (I have read) the information and consent form or (has been read to me). I have asked questions and received answers concerning the areas I did not understand. I have voluntarily given consent to participate in this study. By signing this form, I have not waived any of my legal rights. I have the right to withdraw from the study at anytime without it affecting me in any way. I will be given a copy of this consent sheet.

.....	.....	……/……/……
Name of participant	Signature/left thumb print	Date
.....	.....	……/……/……
Name of Witness	Signature/left thumb print	Date

Certification by Individual Seeking Consent

I certify that I have explained to the individual, the nature and purpose of this study. I have answered all questions that have been raised and have witnessed the above signatures on the date indicated below:

.....	.....	……/……/……
Name of Staff	Signature/left thumb print	Date

APPENDIX 2

QUESTIONNARE

Topic: Prevalence of Bacterial Vaginosis, Trichomoniasis and Vulvovaginal Candidiasis in Pregnant Women Attending Antenatal Clinic at the Kintampo Municipal Hospital, Kintampo

Study Participant's ID…… [ ]

Date of Interview……..[/ /]

1.0 Demographic Characteristics

1.1 Age:...……..……[ ]

1.2 Community:……[_____]

1.3 Marital Status:……..Married [ ] Single [ ] Cohabiting [ ]

1.4 Educational Level:.....……..Nil [ ] Elementary [ ] Secondary [ ] Tertiary [ ]

1.5 Employment status:..……..Employed [ ] Unemployed [ ]

1.6 Religious Status:……...Christian [ ] Muslim [ ] Traditional [ ] Others [ ]

2.0 Obstetric Characteristics

2.1 Number of pregnancies (Include present pregnancy)…….……[ ]

2.2 Trimester of pregnancy…….……First [ ] Second [ ] Third [ ]

3.0 Clinical presentation

3.1 Do you have any sign/symptom of vaginal infection?……..…Yes [ ] No [ ]

3.2 If Question 3.1 is "Yes", do you have any of the following?

3.2.1 Lower abdominal pains……. Yes [ ] No [ ] NA [ ]

3.2.2 Vaginal Discharge……. Yes [ ] No [ ] NA [ ]

3.2.3 Pruritus……... Yes [ ] No [ ] NA [ ]

3.2.4 Dysuria……... Yes [ ] No [ ] NA [ ]

3.2.5 Genital sores……..…. Yes [ ] No [ ] NA [ ]

3.2.6 Genital Warts…….…… Yes [ ] No [ ] NA [ ]

4.0 Behavioural Characteristics

4.1 Do you douche? …… Yes [ ] No [ ]

4.2 If Question 4.1 is "Yes" what do you use in douching?

1. Antiseptics	2. Bathing soap	3. Water
4. Salty water	5. Lime water	6. Herbs/spices
7. Other.....		8. NA

4.3 Have you taken any antimicrobial in the past three weeks?…….. Yes [ ] No [ ]

4.4 On average, how many times to you have sex per week? ……..……..……[ ]

4.5 How many sexual partners have you had in the past one month……[ ]

Interviewer's name_____

APPENDIX 3

GRAM STAINING PROCEDURE

Equipment: Bunsen burner, alcohol-cleaned microscope slide, water

Reagents: Crystal violet, Gram's iodine solution, Acetone/ethanol, 25% Safranine

Procedure

1. Prepare and heat-fix smear.

2. Stain the slides as follows:

    a. Flood the slide with crystal violet for 30 - 60 seconds.

    b. Pour off excess dye and wash gently in tap water and drain the slide against a paper towel.

    c. Flood the slide with Gram's iodine for 30 – 60 seconds.

    d. Wash with tap water and drain carefully. (Do not blot.)

    e. Add a few drops of decolorizer so the solution trickles down the slide. Rinse it off with water after 5 seconds. Further delay will cause excess decolorization in the gram-positive cells, and the purpose of staining will be defeated.

    f. Counterstain with 0.25% safranin for 30 – 60 seconds.

    g. Wash, drain, blot, and examine under oil.

3. Examine under oil immersion (X100 magnification)

Quality Control

Daily prepare the smears of *S. aureus* and *E. coli*. Follow the Gram staining procedure and observe for Gram positive reaction (*S. aureus*) and Gram negative reaction (*E. coli*) under oil immersion.

APPENDIX 4

NUGENT CRITERIA

Laboratory examination of vaginal smears and the determination of the Nugent Score N Score = The sum of the scores for each bacterial morphotype listed below. (Note Number of Organisms seen / 100X objective)

Lactobacilli	**SCORE**	**Gardnerella, Bacteroides**	**SCORE**	**Curved gram-negative bacilli**	**SCORE**	**Sum=*N-SCORE**
> 30	0	0	0	0	0	0
5-30	1	<1	1			3
1-4	2	1-4	2	1-4	1	5
<1	3	5-30	3	5-30	2	8
0	4	>30	4	>30 or	2	10

***Interpretation of Nugent Score**

If N Score is:	**Then Report**	**Status**
0-3	BV- Negative	Normal Flora
4-6	BV-Inter-mediate	Inter-mediate Flora
7-10	BV-Positive	BV

APPENDIX 5 ETHICAL APPROVAL

Kintampo Health Research Centre (KHRC) Institutional Ethics Committee (IEC)
P.O Box 200
Kintampo, B/A
Ghana, West Africa

Tel: +233(3520)92037 (Ext 117)
E-mail: fred.kanyoke@kintampo-hrc.org

FULL ETHICAL APPROVAL CERTIFICATE

Dennis Gyasi Konadu
Kintampo Health Research Centre
Box 200
Kintampo, B/A
Ghana, West Africa

Date: 19th May 2014

Study File Number: 2014-10

Title of study: Prevalence of Bacterial Vaginosis and other selected Reproductive Tract Infections in pregnant women attending antenatal care in Central Ghana

Principal Investigator(s): Dennis Gyasi Konadu

Co-Investigator(s): Farrid Boadu, Zuwera Yidana, Louisa Iddrisu, Sabastina Amoako, David Kwame Dosoo, Robert Awuley Lartey, Dr. Kwaku Poku Asante

Type of Review: Full Board Meeting

Approval Date: 19th May, 2014

Expiration Date: 19th May, 2015

1. The Kintampo Health Research Centre Institutional Ethics Committee (IEC) is constituted and operates in conformance with requirements of 45 CFR 46, 21 CFR 50, 21 CFR 56 and section 3 of the International Council on Harmonization Guidelines. The OHRP Federal wide Assurance number for the committee is 00011103; the IRB registration number is 0004854.

2. The study in title was reviewed by the IEC on 29th April 2014 and a Conditional Ethical Approval (CEA) was granted to the study.

3. The IEC subsequently granted Full Ethical Approval (FEA) for implementation of the study after condition/concerns were addressed in a revised protocol (version 4.0, dated 16th May, 2014).

4. The following documents were reviewed and approved;
   4.1 Prevalence of Bacterial Vaginosis and other selected Reproductive Tract Infections in pregnant women attending antenatal care in Central Ghana, Version 4, dated 16 May 2014
   4.2 Information and consent form, Version 4, dated 16 May 2014

File number: 2014-10 Page 1 of 2

THE CHAIRMAN, KINTAMPO
HEALTH RESEARCH CENTRE
INSTITUTIONAL ETHICS
COMMITTEE

Kintampo Health Research Centre (KHRC) Institutional Ethics Committee (IEC)
P.O Box 200
Kintampo, B/A
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Tel: +233(3520)92037 (Ext 117)
E-mail: fred.kanyoke@kintampo-hrc.org

4.3 Information and Assent form (12 – 17 years), Version 2, dated 16 May 2014
4.4 BV study Medical History form, Version 1, dated 16 May 2014
4.5 BV study Laboratory form, Version 1, dated 15 July 2013
4.6 Study Budget
4.7 Curriculum Vitae of study Investigators

5. During study implementation, the IEC must be informed within 72 hours by the principal investigator (PI) of learning of any (a) unexpected, serious, study related adverse events; (b) disclosed adverse events, or (c) unanticipated problems with the study which may pose risk to study participants or others.

6. Changes or modifications to this research activity must be submitted and approved by the IEC before they are implemented.

7. PI(s) would be required to submit application for renewal of this approval certificate (if necessary) plus a progress report.

8. PI(s) is required to notify the IEC of study completion (end of data collection/last follow-up)

9. Submit final report of the study three months after approval certificate expires (study closure)

10. Before conduct of the study, submit original/final copy of your informed consent/assent forms for an **authentication stamp** before making photocopies for your consent process.

11. Regulated study records, including IEC approvals and signed consent forms, must be securely maintained by PI(s) and available for audits for three years after the study is closed with the IEC.

Sincerely,

THE CHAIRMAN, KINTAMPO
HEALTH RESEARCH CENTRE
INSTITUTIONAL ETHICS
COMMITTEE

.....
Mrs. Charlotte Agyemang-Tawiah
Vice-Chair
Institutional Ethics Committee
Kintampo Health Research Centre

File number: 2014-10