PROFILING OF BACTERIA IN BACTERIAL VAGINOSIS INFECTION AMONG PRETERM BIRTHS IN KISUMU COUNTY

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DECLARATION

I hereby declare this dissertation is my original work under the guidance of the supervisors listed above and has not been presented before any institution for the purpose of research and knowledge acquisition.

I comprehensively understand what plagiarism is and the University of Nairobi policy on this matter. The work submitted herein has undergone a plagiarism check.

In this document, all references made to previous works have been duly recognized and acknowledged as required.

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DEDICATIONS

I dedicate this work to all preterm babies, the unborn, my loving mum Florence Gichohi, all mothers and mothers to be. To God be all the Glory

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ACRONYMS

BV	-Bacterial Vaginosis
ERC	-Ethics Research Committee
H_2O_2	- Hydrogen peroxide
IL-β	-Interleukin beta
IL-4	-Interleukin 4
IL-6	-Interleukin 6
IL-7	-Interleukin 7
INF-γ	-Interferon gamma
JOOTRH	-Jaramogi Oginga Odinga Teaching Referral Hospital
KCH	-Kisumu County Hospital
KNH	-Kenyatta National Hospital
LMP	-Last Menstruation Period
MMPs	- Matrix Metalloproteinase
MOH	-Ministry of Health
PGs	-Prostaglandins
PID	-Pelvic inflammatory disease
PIN	-Participants Identification Number
PPROM	-Pre-term Premature Rupture of Membranes
PTB	-Pre-term Birth
TNα	-Tumor Necrotic alpha
Ultra-S	-Ultra sound
UoN	-University of Nairobi
WHO	-World Health Organization

LIST OF DEFINITIONS

Preterm births- births below 37th week of gestation

Term births - births from 37th week of gestation and above.

Cervicitis- inflammation of the cervix

Endometritis- inflammation of the endometrium

Singleton pregnancy- a pregnancy with one fetus

Multiple gestation- a pregnancy with more than one fetus

Neonatal- newborn child

Sepsis-blood infection

Chorioammnionitis- inflammation of amnio and chrorio (outer membrane)

Periventricular leukomalacia – a form of white-matter brain injury

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ABSTRACT

Background: Preterm birth (PTB) is defined as a live birth (singleton or multiple) before 37 weeks of completed gestation. PTB is the topmost cause of morbidity and death of pretem babies internationally and the second foremost cause of death for children below five years globally. In spite the determinations to avoid PTB in most nations the incidence of PTB has been on the rise. Common causes of PTB include multiple fetal, infections, genetic influence and chronic diseases such diabetes and hypertension. Among the infectious agents, bacteria attribute to the largest number of microorganisms associated with preterm births. The most prevalent lower genital infection that causes pre-term births is bacterial vaginosis and several researches have shown a positive association between the two. This study therefore highlights the profile of bacteria associated with BV related with pre-term birth in Kisumu County.

Broad Objective: To profile bacteria associated with bacterial vaginosis related to preterm deliveries in Kisumu County Hospital.

Methodology: This was a comparative study conducted in Kisumu County. The study population included expectant women recruited at Kisumu County hospital. A vaginal specimen was collected from the study participants. The specimen was run through Amsel and Nugent score laboratory diagnostic test to determine the BV status of the study participants. In addition identification of different bacteria was done under Nugent scoring. A pre-tested questionnaire was used to collect risk factors. Analysis was done using SPSS version 20.

Results: Of the 228 pregnant women were enrolled in the study, 181 study participants had complete data thus could be categorized if they delivered at term or preterm. Last menstruation Period (LMP) and ultra-sound (Ultra-s) were used to determine gestational age. Of the 181 who had LMP results, 64 had ultra-S. BV prevalence based on LMP was found to be 12% (22/181). *Gardenerella vaginalis/Bacteroides* were dominant among bacterial vaginosis (BV) positive with a frequency of 50% in both term and preterm under LMP. However under ultra-S a frequency of 46% and 55% was recorded among term and preterm respectively. Risk factors that were significant under ultra-S were age 31-40 (p=0.002), use of nylon panty material (p=0.045), history of PTB (p=0.049), condom use in the last sexual act (p=0.022), parity 3+0 (p=0.013), parity 4+0 (p=0.003, gravidae 1 and 2 (p=0.034 and 0.001) respectively. Under LMP hormonal and non-hormonal contraceptive (p=0.046) and parity (p=0.031) were significant.

Conclusion: The dominant bacteria were G.*vaginalis*/Bacteroides in both term and preterm deliveries, under BV positives and the frequencies were slightly higher among PTB in ultra-S.

Key words: Bacterial vaginosis and preterm birth

1.0 INTRODUCTION

PTB is defined as all births (singleton or multiple) before 37th weeks of completed gestation (Quinn *et al.*, 2016) or less than 259 days from the time when the woman had her first day of her last menstruation and after 28 weeks gestation (Blencowe *et al.*, 2013; Quinn *et al.*, 2016). Common causes of PTB are multi-fetal (e.g. twin, triplets) infections, genetic factors and chronic diseases such diabetes and hypertension (Blencowe *et al.*, 2013)

PTB can be stratified into mild preterm (32-36 weeks), very preterm (28-31 weeks) and extremely preterm (<28 weeks) (Moutquin, 2003). This is based to gestational age. PTB can also be categorized into spontaneous (idiopathic) or provider-initiated pre-term birth (Turienzo et. al, 2016). Spontaneous preterm birth (SPB) is the untimely rupture of membranes or start of labour before 37 completed gestation weeks (Bala et al., 2017). Provider initiated preterm birth as a result of stimulation of labour or noncompulsory caesarian birth before 37 completed weeks of gestation. This is for both maternal (high risk pregnancies) and fetal indications (Bala et al, 2017). Provider-initiated pre-term births are subdivided into medically indicated (iatrogenic) that attributes 25% and preterm premature rupture of membranes (PPROM) which attributes 25% (Goldenberg *et al.*, 2009). Spontaneous preterm births attributes two thirds of all preterm births (Lydon *et al.*, 2018). This study focused on spontaneous preterm births.

Spontaneous preterm birth is caused by a number of factors, leading to the uterus to change from inactive state to active or latent phase of labour and PTB. The predisposing factors to spontaneous preterm bith differ by gestation age (Blencowe *et al.*, 2013), different populations (Bala et al., 2017), communities and environs. However, 50% of the causes of PTB are not known (Blencowe *et al.*, 2013; Hernández-Díaz *et al.*, 2014)

Maternal history of preterm birth has been strongly associated with PTB. Maternal history of PTB is determined by relation with genetic, non-genetic and environmental risk factors (Blencowe *et al.*, 2013). In addition, maternal age (either under 17 or over 40), are prone to preterm deliveries (WHO, 2012). This is as a result of increase in the maternal age, there is increased incidences of cardiovascular diseases, over weight, uterine leiomyoma, multiparity, increased risk of hypertensive disorders of pregnancy, gestational diabetes, caesarean delivery and maternal

mortality. In addition, heightened risk of chromosomal defects in the fetus (Bala et. al, 2017). Increased risk of spontaneous PTB has ben associated with low body mass index and a short duration of less than 18 months between conception and preceding pregnancy. (Goldenberg *et al.*, 2009).

Multiple pregnancies (twins, triplets, etc.) is another documented risk factor that carries nearly 10 fold increase risk of preterm birth in relation to singleton births (Blencowe *et al.*, 2013)

A number of life style factors that contribute to spontaneous preterm birth include pressure, standing for long hours, too much physical work (Blencowe *et al.*, 2013), caffeine intake, taking spicy food and skipping meals (Hernández-Díaz *et al.*, 2014). Peridontal diseases, too much alcohol consumption and smoking have attributed to increase risk of PTB (Barros *et al.*, 2010). In addition, late start of prenatal care or no prenatal care predisposes one to preterm delivery (Dijk et al., 2010)

Preterm birth is more likely to occur when a mother is expecting a male child, among the PTB 55% were found to be male (Barros *et al.*, 2010) and when compared with girls of the same gestation, the male were at a greater likelihood of dying (Barros *et al.*, 2010). The role of ethnicity has been extensively discussed; nevertheless indication that supporte a discrepancy in normal gestational period with ethnic cluster has been conveyed in numerous population-based studies. The variations are associated to socioeconomic and way of life factors (Blencowe *et al.*, 2013). For instance, African babies incline to be born earlier than Caucasian babies. Nevertheless, for a particular gestational age, African babies tend to have less repiratory distress, fewer neonatal deaths compared to Caucasian babies. Babies with genetic deformities are eliminated from studies recording preterm rates yet high chances the babies are born preterm (Blencowe *et al.*, 2013)

Clinical conditions that predispose mothers to preterm birth can be separated into maternal and fetal. However, some more important direct causes recognized include severe pre-eclampsia, uterine rupture, cholestasis, hormonal disruption fetal distress, fetal growth restriction with abnormal tests and placental abruption (Agrawala and Hirscha, 2012; Blencowe *et al.*, 2013) Placenta abruption may present with absence or presence of vaginal bleeding. Vaginal bleeding in more than one trimester indicate higher risks of preterm birth (Ekmekci and Gencdal, 2018) Core

maternal conditions (e.g. renal disease, hypertension, obesity, and diabetes) rise the threat of maternal malaise (e.g., pre- eclampsia) and medically-indicated preterm birth. The global widespread of obesity and diabetes is, hence possibly to develop a progressively more significant contributor to universal preterm birth. In addition women with moderate to severe anemia, early in gravidity are at a greater exposure to preterm births (Zhang *et al.*, 2009).Assisted fertility treatment is predisposing factor to PTB since both maternal and fetal risk factors have identified after the treatment (Blencowe *et al.*, 2013)

Infection contribute a vital role in preterm birth, mostly attributing to extreme preterm (Moutquin, 2003). HIV, malaria, urinary tract infections, bacterial vaginosis, and syphilis have been found to be linked with predisposion to preterm birth (Barros *et al.*, 2010). Morover, other disorders have more recently been reported to be related with infection, e.g., "cervical incompetency"[cervix thins out (effaces) or opens (dilates) without contraction] leading to swelling with secondary premature cervical shortening (less than twenty five milliners) and ascending infection within the uterus (Blencowe *et al.*, 2013).

Moreover, pregnancies with oligohydramnios are at higher exposure to PTB (Bala et al., 2017)

Bacterial vaginosis (BV) has been consistently linked to PTB (Bahram et al., 2009). A study done in 2012 at Kenyatta National Hospital documented the prevalence of BV to be 26% among mothers with new born (Martha, 2012) while a research done in western Kenya documented a BV prevalence of 18% among adolescent schoolgirls. BV topped the list of infections seconded by *Candida albicans* at 9%, *Chlamydia trachomatis* and *Trichomonas vaginalis*, both at 3% and *Neisseria gonorrhea* at 1% (Kerubo *et al.*, 2016). Another study done the same year, same region among 18 years and above documented the prevalence of BV to be 39% (Okuku *et al.*, 2015).

BV is defined as the imbalance of the vaginal microbiome, characterized by a shift from dominant Lactobacillus to a polymicrobial flora (Aldunate *et al.*, 2015; Onderdonk et al., 2016). A balanced microbiome constitutes a ratio of anaerobe to aerobe to be 2:1 and 5:1. In a normal vaginal ecosystem estradiol stimulates glycogen from the vaginal epithelium. The glycogen undergoes hydrolysis into glucose. The glucose is then broken down into lactic acid by lactobacilli, generating hydrogen peroxide (H_2O_2). H_2O_2 is a bacteriacin and an antibacterial (Ranjit et al., 2018)

(Lactobacillus accounts for 95% of the normal flora). However, when BV is present, the quality and quantity of(H_2O_2) producing lactobacilli decreases, vaginal pH increases to more than 4.5. This is followed by a modification in the ratio of anaerobe to aerobe (100:1 and 1000:1) (Guaschino *et al.*, 2006; Wein, 2011; Bitew *et al.*, 2017). Metabolic by-products of the anaerobic bacteria, which include amines increase the vagina pH resulting to exfoliation of epithelial cells in the vaginal (clue cells) (Tebes et al., 2003). H_2O_2 produced by lactobacilli may have a critical role in preventing fetal membrane degradation, prostaglandin release and ascending infection (Donders *et al.*, 2000; Kelly et al., 2003). BV is linked to severe hostile upshots, such as pre-term births, neonatal mortality, infection on the upper part of the genital tract, pelvic inflammatory disease (PID), inflammation on the endometrium, low birth weight, increase in the frequency of abnormal Papanicolaou (Pap) smears, cervicitis, salpingitis, post-operative infections, oophoritis with or without tubo-ovarian abscess, obstetric complications, such as premature rapture of membranes, and increase in the acquisition of sexually spread diseases (e.g. Herpes simplex virus-2 and human papillomavirus, *Neisseria gonorrhoea* and *Chlamydia trachomatis*) (Hebb *et al.*, 2004; Koumans *et al.*, 2007; Rodrigo, 2013; Baljinder Kaur, 2015). Vaginal cuff cellulitis can also occur if invasive gynecological procedures or surgeries are performed when a patient has BV(Easmon et al., 1992). In addition BV has been shown to have a wide array of medical, psychological and social consequences on patients (Hebb *et al.*, 2004).

Therefore, in this study we identified the most common bacteria among those that cause BV and the risk factors associated with BV linked PTB.

2.0 LITERATURE REVIEW

2.1 Epidemiology

Pre-term birth is the principal cause of perinatal mortality and morbidity globally (WHO, 2012). In spite of inputs to avoid PTB in several nations, the projected figures of PTBs have intensified. In 1990, 2.0 million incidences of PTB were reported 2.2 million PTB incidences were reported in 2010 (Howson *et al.*, 2013) with BV being steadily associated with pre-term delivery. Among the reproductive age, BV is the most prevalent vaginal infection (Bahram et al, 2009). BV prevelence ranges from 8-75% dependent on the geographical, race and medical characteristics of the study population (Bitew *et al.*, 2017). Interestingly, variations on BV prevalence have been captured within similar population groups (Bitew *et al.*, 2017). Despite BV prevalence being elevated in parts of Africa and lowest Europe, some regions in Africa have very low BV prevalence while in Europe have very high rates (Nejad and Shafaie, 2008) However, population approximates for BV is blurred since 50-75% of the infections are asymptomatic, hence many women with BV do not pursue clinical care and thus many are not included in the clinical population estimates (Hoffmann *et al.*, 2014).

2.2 Etiology of Pre-term births

Despite of several factors related with PTB, infections are the leading causes of PTB. Microbes comprising bacteria, parasites, viruses and fungal have been associated as the causal agents for spontaneous PTB (Agrawala and Hirscha, 2012). The infection can either be intrauterine or extrauterine. Intaruetrauterine infections initiated by bacteria, are echoed to be the main cause of infection linked to preterm labor (Agrawala and Hirscha, 2012). A study done by Golden *et al* (2000) indicated bacterial infection, in the amniotic fluid accounts to 80% of women who delivered < 30 gestation weeks. This was compared to 30% less than or equal to 37 gestational week (Friese, 2003) Researches that have used standard microbiological procedures propose that chorioamnionitis with bacteria contributes up to 45% of spontaneous PTB (Zhou *et al.*, 2010) However, putting in place molecular techniques, bacteria detection level raises up to 60% of preterm delivering women (Mendz et al., 2013; Romero *et al.*, 2016)

BV is the utmost prevalent lower genital infection that causes pre-term births, neonatal mortality and low birth weight (Okuku *et al*., 2015) BV is linked with a double increase probability of PTB with the greatest risk when BV is present 16 weeks earlier of gestation (Hebb *et al.*, 2004; Margolis

and Fredricks, 2014) Among pregnant women, BV is linked with the occurrence of fibronectin, which relates with a 16-fold rise in clinical chorioamnionitis, 6 fold rise in neonatal sepsis, 1.8 and 1.9-fold increase in acquisition of *Neisseria gonorrhea* and *Chlamydia trachomatis* respectively (Bautista *et al.*, 2016) and also associated with intra-amniotic infection. Chorio-amnionitis is associated with neonatal sepsis in both term and preterm infants (Kaur, 2015). Women with BV at 23-26 weeks of their gestation have an association with intra-amniotic fluid infection at term (Easmon et al., 1992) and a 6 times increased threat of neonatal death, when a woman has BV between 14-24 week (Kaur, 2015). Neonates born to mothers with BV suffer long term neurological consequences such as hyperactivity, academic difficulties in school, severe handicaps such as cerebral palsy, periventricular leukomalacia, low Apgar score, damage in the dopaminenergic and brain injury due the toxins produced by *G.vaginalis* crossing the placenta (Kaur, 2015)

2.2.1 Bacterial vaginosis

Bacterial vaginosis (BV) is vaginal discharge, common among childbearing women, caused by non-specific bacteria. BV occurs as a result of the reduction of the normal flora (lactobacillus), resulting in an alkaline pH and rise in BV causing bacteria, mostly anaerobic gram-negative rods (Holst et al., 1994) Major bacteria detected are *Gardenerella vaginalis (G. vaginalis), Streptococcus viridans (S. viridans), Atopobium vaginae (A. vaginae), Porphyromonas asaccharolytica (P. asaccharolytica), Prevotella* species and anaerobic including *Bacteroides, Fusobacteria, Peptostreptococcus, Mobiluncus* species, *Veillonella* and *Eubacteria* (Money, 2005).

Gardenerella vaginalis was formerly called *Corynebacterium vaginale* (due to its variable gram stain reaction) or *Haemophilus vaginalis (*due to its requirement for haemin and NAD*)* (Margolis and Fredricks, 2014) *Gardenerella vaginalis* is of the family bifidobacteriaceae, genus Gardenerella and species *G. vaginalis* (Esbroeck *et al.*, 1996) *G. vaginalis* is nonspore-forming, gram-variable staining facultative anaerobe, nonmotile-forming, coccobacilli and survives at pH of 5-11 (Esbroeck *et al.*, 1996) However, under an electron microscope appears as gram-positive, although the peptidoglycan layer can be thinner than many gram positive organisms, resulting in negative gram staining (Harwich *et al.*, 2010). *G. vaginalis* are found in the urinary tract,

endometrium, fetal membranes and newborn infants as a result of maternal infections, neonatal infections and suppurative lesions. *G.vaginalis* can also be transmitted sexually (Catlin, 1992). *G. vaginalis* attaches well to urogenital squamous epithelial cells due to the exopolysaccharide layer and pili. *G. vaginalis* forms biofilms that are resistant to H_2O_2 and antibiotic treatment. The biofilm provides a platform for other pathogenic bacteria to cling to (Bagnall and Rizzolo, 2017) *G. vaginalis* also stimulate inflammatory processes thus displace indigenous lactobacilli from its habitat (Catlin, 1992). *G. vaginalis* secretes 60-kDa and vaginolysin, a potential virulence factor that, lyses neutrophils, only human erythrocytes (dependent on the presence of CD59, a complementary regulatory molecule) and endothelial cells (Jarosik *et al.,* 1998). *G. vaginalis* alters the microbial environment as a result of erythrocyte lysis freeing iron metabolites. These toxins induce interleukin-8 production from human epithelial cells (Margolis and Fredricks, 2014). *G.vaginalis* has been associated with cervical cancer, vertebral osteomyelitis, infertility, retinal vasculitis and acute hip arthritis (Yeoman *et al.*, 2010; Jayaprakash et al., 2012). Other virulent factors include sialidase, prolidase, phospholipase C and protease (Moncla and Pryke, 2009; Margolis and Fredricks, 2014). Phospholipase C degrades placental tissues by breaking down phospholipids to arachidonic acid. This results in production of prostaglandin triggering the onset of premature labor. Protease breaks down tissue proteins, resulting in the release of amine that support the growth of *G. vaginalis* and other bacteria in the vagina (Dennise F, Mandell, 2015). Nevertheless, little is well-known regarding how *G. vaginalis* cause diseases (Moncla and Pryke, 2009). Sialidase plays a role in impairing the turgidity and elasticity of the fetal membrane leading to PTB (Briselden *et al.*, 1992).

Streptococcus viridans is of the family streptococcacea, genus streptococcus and species *S. viridans. S.viridans* mostly gives an alpha or gamma hemolytic in blood agar and rarely beta hemolysis. *S.viridan* is resistant to optochin test. Other characteristics include, non-motile, aerobic to facultative anaerobe, non-capsulated and no solubility in bile (Hardie and Whiley, 1997) *S. viridians* are commensals of low virulence with binding to platelets, binding to fibrin, exopolysaccharide production and binding to fibronectin identified as virulence factors (Tunkel and Sepkowitz, 2002). *S viridans* commonly causes of native valve endocarditis and late onset prosthetic valve endocarditis, severe pyogenic infections, bacteremia in neutropenic patients, neonatal sepsis and septicemia/shock syndrome also known as "α strep shock syndrome" (Dhotre

et al., 2016). *S. viridian* has been isolated from amniotic fluid among women with clinical amniotitis (Rabe et al., 1988; Mazor *et al.*, 1994). To date there has been no documentation on how *S.viridans* causes PTB.

Atopobium vaginae are of the kingdom bacteria, phylum actinobacteria, class actinobacteria, order coriobacteriales, family coriobacteriaceace, genus atopobium and species. *A. vaginae* are a gram-positive, facultative anaerobes, rod shaped or coccobacilli. *A. vaginae* give grey-white colonies after forty-eight hours culture in anaerobic conditions. *A. vaginae* are gram-positive, short chains, in pairs or singly (Rodriguez Jovita *et al.*, 1999; De Backer *et al.*, 2007) *A. vaginae* give positive reaction in acid phosphates, arginine dihydrolase, arginine arylamidase, histidine arylamidase, leucine arylamidase, proline arylamidase, glycine arylamidase and serine arylamidase. Negative reactions are recorded in alanine arylamidase, B-Galactosidase, pyroglutamic acid arylamidase and thyroxine arylamidase (Polatti, 2012). *A.vaginae* has been shown to cause tuboovarian abscess after transvaginal oocyte recovert, salpingitis and endometritis (Mazor *et al.*, 1994). Just like *G. vaginalis*, *A. vaginae* forms biofilms that are resistant to H_2O_2 and antibiotic treatment thus supporting the growth of other anaerobic bacteria (Ferris *et al.*, 2007). A. vaginae is suggested to be pathogenic that can lead to maternal bacteremia and fetal death (Mazor *et al.*, 1994).

Porphyromonas asaccharolytica is of the kingdom bacteria, phylum bacteroidetes, class bacteroidetes, order bacteroidales, family porphyromonadaceae, and genus porphyromonas and species *P. asaccharolytica* (Ng *et al.*, 1994). *P. asaccharolytica* is gram-negative, obligate anaerobe, non-spore forming, non-motile rods or coccobacilli and catalase negative (Ng *et al.*, 1994). Proteinase is produced by *P. asaccharolytica*, which enables hydrolyzation of gelatin, casein, coagulated protein, plasma protein, azacol and collagen. Protease by-products weaken the collagen structure in the gestational sac thus leading to premature rapture of membranes (Friese, 2003).

Prevotella species are of the family prevotellaceae and genus prevotella. *Prevotella spp*. form circular, convex, 1-2 mm and shiny gray colonies. On Gram stain, they form short gram-negative rods which may adopt coccobacilli shape (Margolis and Fredricks, 2014). *Prevotella spp.* are gram negative, bile sensitive, catalase negative, however variations have been reported (Dorn et al., 1998) *Prevotella spp*. is in a position to form biofilm that resist host defense mechanism, resulting

to chronic infection (Yamanaka *et al.*, 2009) *Prevotella spp.* produces collagenase as a virulence factor. Collagenase facilitates raptures of the membranes leading to PTB (Doust and Mobarez, 2004). *Prevotella spp.* provide vital nutrients to G.vaginalis and Peptostreptococcus spp., their association with BV could be through expedition of growth of other causative species(Margolis and Fredricks, 2014). *Prevotella spp.* are also known to cause genital infections, bacteremia, wound infection, bite infections, abscesses and periodontitis (Zhang *et al.*, 2015).

Bacteroides species are in the family bacteroidaceae and genus bacteroides. *Bacteroides* are gram positive, obligate anaerobes, bacilli, non-endospores and some are motile while others are non-motile (Moore et al., 2016). Neuraminidase (sialidase) is a virulence factor produced by bacteroides. The enzyme changes neuraminic acid-containing glycoprotein of human plasma (Briselden *et al.*, 1992). Other virulence factors produced by bacteroides are hyluronidase, DNase, phospholipase A2 and heparinase. *Bacteroides* are known to degrade complement factors of Immunoglobulin G and M. Capsule is an important virulence factor of *Bacteroides fragilis*. Other virulence factors used in the adherence of *Bacteroides* are pili (fimbriae) and lectinlike adhesins. In addition *Bacteroides* produce butyrate and succinate, which give a cytotoxic effect. Sialidase alter the immune signals and damage host mucosal epithelial barrier thus permitting bacteria to access the uterus as well as impairing fetal membrane's strength and elasticity resulting to PTB (Briselden *et al.*, 1992). Phospholipase A 2 induces prostaglandin synthesis resulting to PTB (Briselden *et al.*, 1992).

Fusobacteria species are of the family fusobacteriaceae and genus fusobacterium (Bolstad et al., 1996) *Fusobacterium spp.* Are gram negative, spindle shaped or may have parallel sides, 5-10 μm long, tapered ends, often seen in pairs (end to end), indole positive and fluoresce under ultraviolet light (Bennett and Eley, 1993; Avila-campos *et al.*, 2006; Margolis and Fredricks, 2014). *F. nucleatum* show hemolytic activity in human and sheep erythrocytes. Fusobacteria species has adhesion which enable them to coaggregate with the widest range to other genera tested so far, however, it does not coaggregate with other fusobacteria (Avila-campos *et al.*, 2006). High levels of phospholipase A2 produced by *Fusobacteria*, induce synthesis of prostaglandin resulting to PTB (Briselden *et al.*, 1992), early onset of neonatal sepsis (Han, 2013) and colorectal cancer (Mcguire *et al.*, 2014)

Peptostreptococcus species are of the family clostridiaceae and genus peptostreptococcus (Murdoch, 1998) *Peptostreptococcus* are gram-positive cocci, non-spore forming, obligate anaerobes and found singly, in pairs or in chains (Riggio and Lennon, 2002) Gas liquid chromatography is used in the identification of *Peptostreptococccus spp*. In addition peptone and amino acid is metabolized to isobutyric, butyric, acetic, caproic and isocaproic acid. *Peptostreptococcus spp.* is commonly associated with necrotizing soft tissue infections and commonly associated with polymicrobial infection. *Peptostreptococcus* are the second most commonly associated anaerobe in clinical infection after *Bacteroides spp.*(Riggio and Lennon, 2002). However, there is no record of how Peptostreptococcus causes PTB (Krepel *et al.*, 2018).

Eubacteria species is of the family Eubacteriacea and genus Eubacterium (Hill, Ayers and Kohan, 1987). *Eubacterium* are either gram negative or gram positive and some are non-motile while others are motile (Hill et al., 1987). Some strains of *Eubacteria* are known to produce DNase and phosphatase (Margaret et al, 1990). Phosphatase initiates prostaglandin production which stimulate to contractions leading to PTB (Friese, 2003).

Mobiluncus species are of the family actinomycetaceae and genus mobiluncus. *Mobiluncus* are gram-negative and curved in shape (Vetere *et al.*, 1987). *Mobiluncus species* are indole, catalase, hydrogen peroxide negative and metabolize succinate enzyme thus raising the vaginal pH to alkaline levels and preventing chemotaxis of cells that are immunocompetent (Mirmonsef *et al.*, 2012; Spiegel, 2012). This leads to proliferation of infectious organisms leading to formation of an NF-κB coordinated inflammatory state thus recruiting pro-inflammatory cytokines (IL-β, IL-4, IL-6, IL-7, TNF-α and INF-γ) and chemokine (IL-8 and RANTES), exciting the production of matrix metalloproteins (MMPs) and arachidonic acid metabolites such as prostaglandins (PGs) and hydroxyeicosatetraenoic acids (HETE) (Witkin *et al.*, 2013). MMPs destroy and digest the cervical extracellular matrix and fetal membranes, while the arachidonic acid metabolites moderate myometrial contractility leading to cervical maturation leading to PTB (Witkin *et al.*, 2013).

Veillonella species are of the family Vellonellacease genus veillonella.Veilonella are strictly anaerobic, gram negative cocci which form part of the normal flora of the oral, genitourinary, respiratory and intestinal tracts of humans' and animals (Rovery *et al.*, 2005; Marriott et al., 2007)

Veillonella are gram-positive cocci, oxidase positive, catalase negative, indole negative, urease negative and nitrate reduction positive (Brook and Brook, 1996) Veillonella are also known to be associated with endocarditis, periodontitis, dental carriers and osteomyelitis (Mashima et al., 2016). Lipopolysaccharide is the main virulent factor for Veillonella species and are known to form biofilms that are resistant to H_2O_2 thus enabling the growth of other anaerobic bacteria (Rovery *et al.*, 2009).

2.2.1.1 BV and HIV

A study done in Uganda found BV increases the risk of HIV infection by more than 2-fold. A study done in Malawi among women attending antenatal clinic conferred a 3 fold increased risk of HIV infection (Myer *et al.*, 2018). Particularly, high concentration *G.vaginalis* and *M.hominis* has been associated with increased HIV shedding (Koumans *et al.*, 2007; Margolis and Fredricks, 2014). Microbes such as Bacteroides, Prevotella, Gardenerella have been shown to attract CD4 cells to the mucosa, thus increasing HIV acquisition (Africa et al., 2014). A number of hypotheses suggest how BV increases HIV acquisition. One, a healthy vaginal makes healthy lactobacillus that produces hydrogen peroxide. Hydrogen peroxide can inactivate HIV. When there is a shift from a lactobacillus dominated vaginal flora, the absence of the H_2O_2 makes it possible for the HIV to stay alive longer in the vagina, thus increase the rate of transmission. Secondly, BV has shown to stimulate cells containing the virus thus increasing HIV replication. Thirdly, BV disrupts the skin cells at the surface of the vagina. This makes it easy for the virus to reach a deep layer of cells that are susceptible infection. Fourthly, BV increases intravaginal levels of interleukin-10, which increases susceptibility of macrophages to HIV. In addition studies have shown that a stable protein produced by *G.vaginalis* increases production of HIV by HIV infected cells by as much as 77-fold (Schmid *et al.*, 1995; Mirmonsef *et al.*, 2013). Interestingly, women with BV have three time likelihood of transmitting HIV to their male sexual partners compared to women with a normal vaginal microbiome (Schmid *et al.*, 1995). In addition, observational data and physiology of the vaginal epithelium suggest those post-menopausal women are at higher risk of HIV acquisition. However, studies focusing on post-menopausal women with atrophic vaginitis have not yet been done (Myer *et al.*, 2018). Moreover, BV increases the risk of acquiring *Neisseria gonorrhea* by 1.7, *Chlamydia trachomatis* by 3.4, Herpes simplex virus -2 by 2.1 and Trichomonal genital infection by 1.8 (Margolis and Fredricks, 2014).

2.2 2 Diagnosis of bacterial vaginosis

Diagnosis of BV is based on clinical and laboratory diagnosis. Clinical diagnosis is based on the nature of vaginal discharge.

2.2.2.1 Amsel's criteria

Diagnosis of BV is based on the presence of the three of the following four findings; increased vaginal pH>4.5, presence of white adherent discharge that contains numerous exfoliated epithelial cells with bacteria (Gram-variable polymorphic rods) attached to their surface (clue cells) that has a characteristic fishy odor, more so when 10% of potassium hydroxide is added (whiff test) and a characteristic thin, gray or white homogeneous discharge (Rao *et al.*, 2016).

However, BV can be asymptomatic in about 50% and this is why Nugent's scoring system is preferred in scientific community (Carr et al., 1998).

2.2.2.2 Nugent scoring

Nugent, whom it was named after, first described Nugent scoring in 1991 (Nugent *et al.,* 1991)

Nugent score is the gold standard for diagnosis BV, which is based on bacteria counting in Gram stained slides of vaginal secretions (Chawla *et al.*, 2013). In Nugent score criteria, the vaginal (Lateral swab or posterior fonicle of the vagina) swab is smeared on a clear glass slide, air dried, heat fixed then Gram stained (Rao *et al.*, 2016). The bacteria morphology is observed under an oil immersion objective (×100) using the following scheme:

1+, < 1 per field

2+, 1-4 per field

3+, 5-30 per field

4+, >30 per field

Large gram positive rod are considered to be lactobacillus morphotypes, small gram negative to gram variable are considered to be *G.vaginalis* and *Bacteroides spp*. morphotypes while curved gram negative curved are considered to be *Mobiluncus spp*. morphotypes (Rao *et al.*, 2016).

Table 1: Nugent scoring

Determination of the Nugent score through laboratory examination of vaginal smears Nugent score = The total sum of the scores for every bacteria morphotype is listed below (Note the quantity of organisms seen/100 × objective)						
Lactobacillus	**Score**	***G. vaginalis*, Bacteroides**	**Score**	**Curved gram variable bacilli**	**Score**	**Nugent score**
30 or more	0	0	0	0	0	0
5-30	1	Less than 1	1	Less than 1	1	3
1-4	2	1-4	2	1-4	1	5
Less than 1	3	5-30	3	5-30	2	8
0	4	30 or more	4	30 or more	2	10

The Nugent scores are interpreted as follows:

A score of 0-3 – Normal (B.V negative)

A score of 4-6 – Intermediate

A score of 7-10 – B.V positive (Mohanty *et al.*, 2010)

2.2.2.3 Cytology-Papanicolaou smear

A wet mount is done and examined under a microscope for the presence of clue cells. An epithelial cell is termed as a clue cell, when more than 20% of the epithelial cells have stripped appearance due to adherent cocco bacilli and whose edges are obscured or fuzzy (Vandana *et al.*, 2018).

2.2.2.4 Culture

Vaginal cultures have excellent sensitivity for BV diagnosis. However, cultures are not recommended because the predictive value for *G. vaginalis* is less than 50%. Thus vaginal gram stains are more useful than culture since BV is a polymicrobial infection (Nenadi and Pavlovi, 2015).

2.2.2.5 Rapid test kit

The BV Blue kit is a rapid test kit used to diagnose bacterial vaginosis. The kit detects vaginal fluid sialidase activity, which is produced by causative agents of bacterial vaginosis such as *G.vaginalis, Bacteroides, Prevotella and Mobiluncus*. The BV Blue kit detects bacterial vaginal fluid sialidase at levels of ≥7.8 (Gad *et al.*, 2014). Affirm VP III is rapid test used for the detection of Candida species, *G. vaginalis* and *Trichomonas vaginalis* from a vaginal swab (Brown *et al.*, 2004).

2.2.2.6 Molecular assay

In the diagnosis of BV molecular assays are used, although not commonly since they are expensive. However molecular assays are the most effective. Specifically, quantitative molecular tool using a specific real-time polymerase chain (Bretelle *et al.*, 2018).

2.2.2.7 Sensitivity and specificity

Nugent scoring being the gold standard for BV, Amsel has recorded a sensitivity of 0.91 and specificity of 0.91, positive predictive value of 0.86, negative predictive value of 0.94 and accuracy of 0.91 diagnosis (Mohammadzadeh *et al.*, 2014). Vaginal pH recoded a sensitivity of 100% and specificity of 58.9%, while whiff test recoded a sensitivity of 100% and a specificity of 97.3% (Mohammadzadeh *et al.*, 2014). While another study Amsel recorded positive predictive values 80%, negative predictive value 94%, sensitivity 78% and specificity 95.6%, Nugent scoring as the gold standard (Bhat et al., 2011). A similar study was done and Amsel recorded a sensitivity, specificity, positive predictive value, negative predictive of 100%, 91.20% 84.12% and 100 % respectively (Bhat et al, 2011). In comparison with Nugent scoring, Amsel criteria recorded a specificity of 78.72%, sensitivity of 92.35%, negative predictive value of 93.54% and positive predictive value of 75.51% (Rao *et al.*, 2016). A study was done with Amsel criteria as the gold standard for BV and Nugent scoring recoded a sensitivity of 65% and specificity of 97.3%, positive predictive value of 80.8%, negative predictive value of 94.2% and an accuracy of 92.7% (Mohammadzadeh *et al.*, 2014).

Pap smear and vaginal culture were compared to Nugent scoring as gold standard. Pap smear had a sensitivity and specificity of 43.1 and 93.6%. Vaginal culture had a sensitivity and specificity 77.8 and 97.7%. Positive and negative predictive values for Pap smear were 73.8 and 93.3%

respectively. Vaginal culture had a positive and negative predictive value of 78.8 and 91.8% respectively (Aktepe et al., 2004). BV Blue kit was compared to Amsel criteria and Nugent scoring. In comparison to Nugent scoring and Amsel criteria, BV Blue kit recorded a sensitivity and specificity of 91.7, 97.8, 91.7 and 97.8% respectively. While the positive and the negative predictive value for BV Blue kit versus Nugent scoring and Amsel criteria were 50.0,100,100 and 88.2%, respectively (Myziuk et al., 2003). PCR has recorded a sensitivity of 99% and specificity of 89% for diagnosis of BV, in comparison to Amsel criteria. In comparison to Nugent scoring, a sensitivity of 95.9 and 93.7% was recorded (Fredricks *et al.*, 2007).

2.2.2.8 Advantages and Disadvantages of Amsel and Nugent scoring

Amsel criteria is reliable, cheap, fast, simple and easy, while Nugent scoring is complex and require expertise (Mohammadzadeh *et al.*, 2014). Moreover, Nugent scoring can identify a few bacteria species and the exact number of Lactobacillus can be influenced by the variability of the methods. Depending on how you spread the sample on the slide the homogeneity and thickness of the sample on the slide may vary. Thus it is important to adhere to the basic standards of quality control (Mohammadzadeh *et al.*, 2014) However PCR has been credited to be more sensitive compared to Amsel criteria and Nugent scoring (Fredricks *et al.*, 2007; Mohammadzadeh *et al.*, 2014).

2.3 Pathogenesis of pre-term birth

Studies have been done in both animal models and humans as to how the bacteria cause PTB (Salminen *et al.*, 2008; Mendz et al., 2013). These studies have suggested that bacteria is likely to be having a more direct role in the pathogenesis of PTB by secreting enzymes such as collagens (Tebes et al., 2003) that degrade fetal membranes, or by inducing the synthesis and release of uterotonins such as prostaglandins(Koucký *et al.*, 2009), able to stimulate uterine contractions or their presence can lead to the production of pro-inflammatory cytokines such as phospholipase A2 thus causing preterm labor (Agrawal and Hirsch, 2012; Madianos et al., 2013; Rimawi, 2013)

Bacteria and diverse microbes are recognized by pattern recognition receptors such Toll-like receptors that activate innate immune system, prompting a proinflammatory cascade orchestrated by several elements such, the transcription factor NF-Ƙb (Agrawala and Hirscha, 2012) This cascade result in amplification of effector molecules such as cytokines (e.g. In tumor necrosis

factor-α, Interleukin-1) (Agrawala and Hirscha, 2012), prostaglandins, proteases, chemokine's such Interleukin-8 and other enzymes, to produce a coordinated response featuring placental detachment, infiltration of inflammatory cells into gestational tissues, cervical ripening and weakening of the fetal membranes leading to PTB (Agrawala and Hirscha, 2012).

In addition, metabolic by-products of the anaerobic bacteria, which include amines increase the vagina pH resulting to exfoliation of the vaginal epithelial cells (clue cells) (Tebes et al., 2003). H_2O_2 produced by lactobacilli may have a critical role in preventing fetal membrane degradation, prostaglandin release and ascending infection. Alternative mechanism that has been proposed consist of the incidence rates and the bacterial loads of these bacteria are more among women with preterm low birth weight delivery (Monga and Blanco, 1995; Goldenberg et al., 2002; Kataoka *et al.,* 2006).

However many questions in relation to the pathogenesis of BV remain unanswered. In relation to how *Lactobacillus* reduce in number (Donders *et al.,* 2000).

2.4 Treatments of BV to prevent PTB

Metronidazole and clindamycin are the first-line treatment for BV. However recurrent rate are high, approximated to 50% at 3 months (Ferris *et al.*, 2007; Bagnall and Rizzolo, 2017). *A. vaginae* are resistant to metronidazole but susceptible to clindamycin. Unfortunately, clindamycin destroys lactobacilli (Ferris *et al.*, 2007). However, recurrence is common in 30 % at 3 months and later recurrence in 50 % at 6 months (Margolis and Fredricks, 2014).

2.5 Problem statement

Pre-term birth is the sole largest cause of perinatal mortality and morbidity in the world, (Agrawala and Hirscha, 2012; WHO, 2012) with a prevalence rate of 5-18% in 184 countries (Wagura *et al.*, 2018). Kenya is ranked position 48 among the top 50 countries with the highest proportion of PTB globally and position 13 with the highest deaths which result from pre-term birth complications (Blencowe *et al.*, 2012; Gebreslasie, 2016)

Microorganisms comprising bacteria, parasites, viruses and fungal have been associated as the causative agents for spontaneous PTB. Bacteria constitute the prime number of microorganisms linked with PTB (Zhou *et al.*, 2010) with molecular techniques detecting bacteria in 60% of women delivering preterm (Mendz et al., 2013; Romero *et al.*, 2016). BV which has been the most prevalent lower genital infection is postulated to be one of the causes PTB and neonatal mortality (Guaschino *et al.*, 2006; Bahram et al., 2009). However little has been done to profile the bacteria associated with BV leading to PTB. Yet the numbers of PTB are on the rise.

2.5.1 Justification

A number of studies have been done in Africa investigating an association between BV and PTB with varied findings. A study done in Nigeria,2010 (Aderoba *et al.*, 2016), found an association between BV and PTB unlike a study done in Tanzania (Shayo *et al.*, 2012). Through the study, we will be in a position to identify the most prevalent bacteria that causes BV after profiling the identified bacteria species, which may be associated with PTB. If that is the case as per our prediction, then this may offer an oppurtunity to formulate a policy or policies regarding management of BV in pregnancies for the purposes of controlling BV and reducing PTB. However, should we find that a particular bacteria or bacterium associated with pregnant women in Kisumu, there may be need to repeat a similar study in different parts of Kenya to confirm if what is found in Kisumu is similar to what we have in other parts of Kenya. More over the study will recommend on whether it is important to screen women coming for attenatal clinic for BV, since BV is assymtomatic in 50-75% of the infections, thus resulting in the reduction of PTBs (Hoffmann *et al.*, 2014). In addition the study will form a foundation for further studies to be done, in the optimization of BV treatment based on specific bacteria associated with PTB.

2.6 Research questions

1. What is prevalence of Bacterial vaginosis among pregnant women in Kisumu County?
2. Which bacterium among Bacterial vaginosis species is strongly associated with pre-term birth in Kisumu County?
3. What are the risks factors associated with BV infections and linked to pre-term births?

2.7 Null hypothesis and objectives

2.7.1 Null hypothesis

There is no association between bacterial vaginosis and pre-term births.

2.7.2 Broad Objective

To profile bacteria associated with bacterial vaginosis related to preterm deliveries in Kisumu County Hospital.

2.7.3 Specific Objectives

1. To determine the prevalence of BV among pregnant women in Kisumu County
2. To characterize bacteria species in bacteria vaginosis infection associated with preterm births in Kisumu County
3. To determine the risk factors associated with bacteria vaginosis and preterm births

3.0 METHODOLOGY

3.1 STUDY DESIGN

This was a comparative cross sectional analytical study conducted for one year. Comparative studies allow comparison of proportions in exposed group and in the unexposed group. In this study, the exposed group were pregnant women diagnosed with BV, while unexposed were BV negative pregnant women.

To examine the association between a supposed risk factor and a health outcome, analytical cross-sectional studies may be used. The risk factor and the health outcome are measured simultaneously. This makes it difficult to know whether the disease or the exposure came first, thus the study design limits to draw valid conclusions about an association or predicted causality. Causality should be confirmed by more rigorous studied (Antay-bedregal and Camargo-revello, 2015) .

3.2 STUDY SITE

Kisumu County is one of the new established counties. This county borders Siaya County to the west, Vihiga County to the north, Nandi County to the north east, Kericho County to the east, Nyamira County to the south and Homa Bay County to the south west. The county lies within longitudes 33° 20’ East and 35° 20’ East and latitudes 0° 20’ South and 0° 50’ South of the equator. Kisumu is situated 1174 meters above sea level.

The study enrolled participants who were in maternity ward at Kisumu County Hospital (KCH) and Jaramogi Oginga Odinga Teaching and Referral Hospital in Kisumu County. However the data collection took place only at KCH and not the two sites because the sample size was attained within a shorter duration than expected. KCH was established in the year 1900 during the building of Kenya-Uganda Railway. The obstetrics and gynecology department in the hospital offers patient care in major and minor obstetric and gynecological surgeries as well as specialized gynecology clinic, antenatal clinic (ANC), Family planning and cervical cancer screening.

3.3 STUDY POPULATION

The study involved two cohorts. One cohort, comprise of women delivering before 37^{th} week of gestation while the other group, women delivering from 37^{th} week of gestation and above

(comparative group). The second cohort involved pregnant women visiting or admitted at KCH. Kisumu County hospital receives deliveries from all over Kisumu. Therefore giving a general representation of the whole Kisumu County. The total number of deliveries KCH receives per month is between 200-250 per month and 10-20 pre-term births.

3.3.1 Inclusion criteria

Table 2: Inclusion criteria of both term and preterm cohorts

Pre-term deliveries	**Term deliveries**
Singleton pregnancy Women delivering before 37thweek of gestation Women who willingly consented to participate in the study 18 years and above Residence of Kisumu county	Singleton pregnancy Women delivering at 37th week of gestation and above Women who willingly consented to participate in the study 18 years and above Residence of Kisumu county

3.3.2 Exclusion criteria

Table 3: Exclusion criteria of both term and preterm cohorts

Pre-term deliveries	**Term deliveries**
Assisted surgery Use of antibiotics in the past two weeks Obstetric emergencies e.g. antepartum hemorrhage, preeclampsia, eclampsia	Use of antibiotics during the previous two weeks Obstetric emergencies e.g. antepartum hemorrhage, preeclampsia, eclampsia

3.4: SAMPLE SIZE CALCULATION

The study majored in the comparison of two proportions. The study design allows comparison of two proportions. Thus the used sample size formula is the most appropriate because it permits getting the minimum sample size in a comparative study of two proportions.

$$n_1 \geq \frac{1+r}{r}\frac{\left(Z_\alpha + Z_\beta\right)^2 . \bar{P}(1-\bar{P})}{(P_1 - P_2)^2}$$

n_1= minimum sample size for cases (women with pre-term births)

Z_β= critical value corresponding to 70% power (β=0.30; Z_β= 0.53)

$Z_{\alpha/2}$= critical value corresponding to 0.05 α-level of significance ($Z_{\alpha/2}$=1.96)

p_1= Proportion exposed among control group- women with full term births (p_1=0.39 based on the prevalence of bacterial vaginosis (BV) among women in Western Kenya (Okuku et al., 2015)

r= ratio of controls to cases (women with full term births to women with preterm births. Due to the few number of preterm births per month in the facility a ratio of 4 full term birth women to 1 preterm birth was used to maintain the power (r=4)

p_2= Proportion exposed among the cases (proportion of women with bacterial vaginosis among those that had full term births)

p_1- p_2= Expected difference in the prevalence (p1-p2=-0.20)

$\bar{\rho}$ = pooled prevalence = (P_1+ P_2) / 2 ($\bar{\rho}$=0.49)

Using the formula and defined parameters, minimum sample size for women with preterm births is 59 and women with full-term births 117

59+117=176

3.5 SAMPLING PROCEDURE OF STUDY PARTICIPANTS

Preterm deliveries are not predictable. This poses a limitation in recruitment of participants for the study within the limited study period. Consecutive sampling was therefore used to select participants until the required sample size was achieved. Participants, who met the inclusion criteria and willingly gave consent, were included in the study.

3.5.1 Recruiting and consenting procedures

Before the study nurse consented the participants who had met the inclusion criteria, she explained to the identified participant about the study procedures, expected results, risks and benefits. After which the study nurse had a one on one questionneiring session with the participant.

3.6 DATA COLLECTION PROCEDURES

3.6.1 Pretesting of interview tools

The questionnaires that was used to gather information in this study was pretested in Kisumu County Hospital. The pretesting was done by the principal investigator and the study nurse. This was done after ethical approval and before study kick off. A total of 10 different participants were subjected to the face-to-face interviews to test the questionnaire to determine whether the questions are understandable and are being answered correctly. Changes were be made accordingly to any question that required clarity.

3.6.2 Administering qualitative interviews

A questionnaire was used to collect socio-demographic information and medical history of the study participants. The socio-demographic information included age, family history, education level, marital status and socio-economic status. Medical history included previous pre-term deliveries, period between pregnancies, infertility treatment and sexually transmitted diseases. The questionnaire was first validated before use after which the participants filled the form. The form was then be taken back for tabulation and analysis

3.6.3 Laboratory analysis

3.6.3.1 Sample collection

A trained clinician collected the samples. Figure 1 show how the swab sample was collected. Three vaginal swabs were collected from each of the consenting participants. One was reserved for further studies while the two were used in Amsel criteria and Nugent scoring. Prior to swab collection, the study nurse ensured his/her hands are properly clean and the participants' bladder is empty. The participant lay in a dorsal position, knees flexed; hips abducted and head on pillow. The study nurse examined the vaginal for any vaginal discharge and the nature of the discharge, abnormal skin condition, lesions and evident of female genital mutilation. Warm a sterilized

speculum at 37°C. Clean away any cervical mucus if necessary. The speculum was inserted in the vaginal. Dalcon vaginal swab were inserted about 1-2 cm and a posterior or lateral vaginal wall swab was taken in a rotating motion. The stick was inserted back in the tube and corked tightly. This was repeated with the other Dalcon vaginal swab. Labeling of the dalcon vaginal swabs was done before examination, with the participant's name, number, time and date of collection. The two tubes were transported to the laboratory in Gas pak anaerobic chamber.

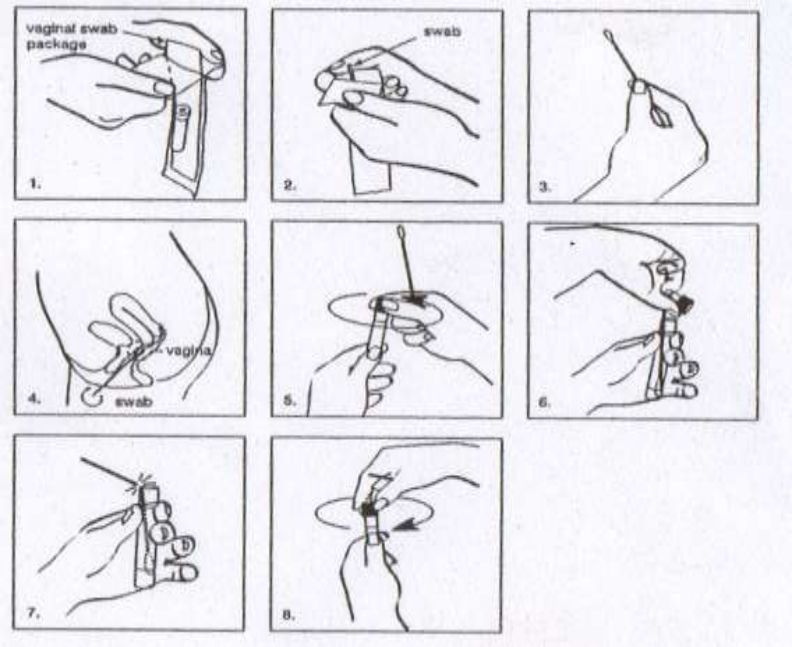

Figure 1:Steps for collection of high vaginal swab

3.6.3.2 Diagnosis

Clinical and laboratory diagnosis are used in the diagnosis of BV. However, clinical diagnosis has more to do with laboratory. In this study we dealt with both clinical and laboratory diagnosis. Amsel criteria and Nugent scoring were used in the diagnosis of BV. Nugent scoring is the gold standard. This study used Amsel and Nugent scoring.

3.6.3.2.1 Clinical diagnosis (Amsel criteria)

The clinical diagnosis of BV was based on the Amsel criteria, which is defined by three of the following criteria.

Clinical diagnosis of BV is based on the presence of the three of the following four findings:

- Homogeneous vaginal discharge, which appears to adhere to the wall of the vaginal in a thin film and can vary from white to grey.

- Increased vaginal pH > 4.5.

Litmus paper was used to determine the pH of the discharge. The colour change was compared with a pH standard colour chart.

- Presence of an amine odor (fishy smell) – positive whiff test.

A swab was placed in small size test tubes. In the test tube a few drops of 10% potassium hydroxide were added. Flapping was done on the opening of the test tube to capture the presence of the amine odor. This was for the positive whiff test. In the case of negative whiff test, amine odor was absent.

- Presence of clue cell.

A smear was prepared on a slide using the third swab. Normal saline was added on the slide and a cover slip placed on the smear and examined at a magnification power ×40. Clue cells were identified for positive samples of BV. The clue cells are identified as vaginal epithelial cells with heavy coating of bacteria, in that the peripheral boarders are obscured(Rao *et al.*, 2016).

3.6.3.2.2 Laboratory diagnosis (Nugent score criteria)

In Nugent score criteria, the vaginal swab were smeared onto a clear and clean glass slide and air-dried before Gram staining. The bacteria morphology was observed under an oil immersion objective (×100)

Large gram positive rod are considered to be lactobacillus morphotypes, small gram negative to gram variable are considered to be *G.vaginalis* and *Bacteroides spp.* morphotypes while curved Gram variable rods are considered to be *Mobiluncus spp*. Morphotypes(Rao *et al.*, 2016)

1+, < 1 per field

2+, 1-4 per field

3+, 5-30 per field

4+, >30 per field

Table 4: Nugent scoring

Determination of the Nugent score through laboratory examination of vaginal smears **Nugent score = The total sum of the scores for every bacteria morphotype is listed below** **(Note the quantity of organisms seen/100 × objective)**						
Lactobacillus	**Score**	***G. vaginalis, Bacteroides***	**Score**	**Curved gram variable bacilli**	**Score**	**Nugent score**
30 or more	0	0	0	0	0	0
5-30	1	Less than 1	1	Less than 1	1	3
1-4	2	1-4	2	1-4	1	5
Less than 1	3	5-30	3	5-30	2	8
0	4	30 or more	4	30 or more	2	10

The Nugent scores are interpreted as follows:

A score of 0-3 – Normal (B.V negative)

A score of 4-6 – Intermediate

A score of 7-10 – B.V positive(Mohanty *et al.*, 2010)

3.7 QUALITY ASSURANCE PROCEDURES

Standard operating procedures were developed and used in all procedures that involved sample collection, sample transportation and analysis. Equipment operation was done according to manufacturer's instructions. Reading of slides was re-confirmed by the supervising microbiologist before results are signed out to the participant's records.

3.8 QUALITY CONTROL

Professionals (study nurse and principal investigator) who are well trained in the field did the study. In addition the principal investigator was thoroughly trained from sample collection to

sample storage for three consecutive months on BV at University of Nairobi Tropical and Infectious Diseases Institute. After which a quality control was done at Kenya Medical Research Institute with a staff who has worked on BV for the last 30 years.

The Patient identification number was to label the specimen and identify the consent forms and questionnaires, rather than participant's name. The study nurse and principal investigator wore proper protective equipment's e.g. gloves, lab coat, when handling a participant or a specimen. Before running any sample the bench was sanitized.

3.9 ETHICAL CONSIDERATION

The protocol and informed consent form was reviewed and approved by the Kenyatta National Hospital-University of Nairobi Ethics and Research Committee (KNH-UoN ERC) prior to any protocol-related procedures (e.g., recruitment efforts) being conducted. The principal investigator informed the KNH-UON ERC the progress of the study on a regular basis per the ERC requirements, but at a minimum once a year. Written informed consent was obtained from the potential study participants prior to any protocol-specified procedures being conducted. To maintain confidentiality, initials and coded numbers were used to identify the participants' laboratory specimens, source documents, and study reports. All study records were maintained in a secured location. Participant information was not obtained or released without written permission from the participant/participant's legally authorized representative except as necessary for monitoring of the study. Permission to carry out this study was also obtained from the hospital's administrator and the county health department.

Participation in this study was completely voluntary and the participants could withdraw even after accepting to participate (Appendix A and B). The interviewee was also be informed that apart from having a direct benefit to an individual participant (by knowing their BV status -Thus providing proper management) and receiving BV treatment, this study will go a long way in helping both BV testing policy during pregnancy but also designing ways to prevent and manage BV to avoid preterm births.

3.10 DATA MANAGEMENT

Laboratory results were entered in a data collection form (Appendix D) and the documented socio demographic data from the corresponding questionnaire were entered into Microsoft Excel 2013 and stored in a password protected computer. All the collection forms and questionnaires filled were kept in a lockable filing cabinet located in a restricted-access room. The slides were stored in slide mailers in a lockable cabinet with restricted access.

Data was cleaned, coded and analyzed using SPSS. Data cleaning was done to identify extreme and missing records in the data set. For extreme values, counterchecking was done with the questionnaires/data collection forms, and replacement done in case of data entry errors. For missing values, the information was recovered from the questionnaires/data collection forms. If the information is not available the pattern of missingness was be assessed as whether completely missing at random or not. Unless the data is missing data is completely missing at random, pairwise deletion was adopted in the analysis. Evaluation for completeness and accuracy was done daily. Data was backed up daily.

3.10.1 Data analysis

Univariate analysis was done to summarize the data/variables. For continuous/discrete variables, histograms were plotted to show the distribution; measures of central tendency (means/medians/mode) and dispersion (SD/ IQR) were reported depending on the distribution. For categorical data such as marital status of the woman, BV test results, bacteria isolated etc. bar/pie charts were plotted to show the distribution; frequencies and proportions were reported.

In bivariate analysis, a test of associations (Pearson chi-square/ test) was used to check for association between the socio-demographic characteristics, clinical history of women with preterm births and the BV status in relation to the gestation at birth. Pearson chi-square statistics and corresponding p-values were reported. The study was conducted at 0.05 α-level of significance.

4.0 RESULTS

A total of 228 pregnant women were enrolled in the study through, systematic random sampling. Out of the 228, only 209 were followed up to delivery stage, while 19 had incomplete results.

4.1 Last Menstruation Period and Ultra Sound as determinants of preterm

The participants were categorized as preterm or term based on Last Menstruation Period (LMP) and Ultra-sound (Ultra-S). This is as illustrated in table 4. Last Menstruation Period (LMP) captured 209 pregnant women. Out of the 209 pregnant women under LMP, 76 (36.4%) went for Ultra sound (Ultra-S).

Table 4: Preterm births by LMP and Ultra-S

Deliveries	**Term n (%)**		**Preterm n (%)**	
	Normal delivery	Cs delivery	Normal delivery	Cs delivery
LMP	120 (57.4%)	19 (9.1%)	61 (29.2%)	9 (4.3%)
Ultra-S	35 (46.1%)	5 (5.3%)	29 (39.5%)	7 (9.2%)

Ultra-S being very accurate, it was used as the gold standard. Only those who had a normal delivery were included in the analysis the rest were excluded. A comparison was done between LMP and Ultra-S. The comparison was done to bring the participants who had a discrepancy and those who were at par in the two modes of classification. The comparison is presented in table 5. A total of 64 study participants qualified for the comparison. The 64 comprised of women who had both LMP and Ultra-S data and they delivered normally. Of the sixty-four, 34 (97.1%) and 28 (96.6%) had term and preterm deliveries respectively. These were captured both in LMP and Ultra-S. A slight discrepancy was evident between the two modes of classification. Of the 64 study participants, 1 (2.9%) classified as preterm under LMP, was reclassified as term under Ultra-S. In addition, 1 (3.4%) classified as term under LMP was reclassified as preterm under Ultra-S.

Table 5: Comparison between LMP and Ultra-sound for determination of preterm births

		Gestation LMP n (%)		
	Gestation	Term svd	Preterm svd	TOTAL
Gestation Ultra-S n (%)	Term svd	34 (97.1%)	1 (3.4%)	35 (54.7%)
	Preterm svd	1 (2.9%)	28 (96.6%)	29 (45.3%)
	TOTAL	35 (100.0%)	29 (100.0%)	64 (100.0%)

4.2 Demographic characteristics of the study participants

Different social demographic characteristics of mothers that are risk factors to preterm births were outlined as illustrated in table 6. A chi-square was run on each variable, in both LMP and Ultra-S mode of gestation classification. Majority of the study participants were aged 21-25 years in both LMP and Ultra-S. In the different categories of marital status, marital status steady partner living together were the majority under LMP, while married monogamous were the majority under Ultra-S. In the different categories of marital status, housewives were the majority. Both age and housewife were captured to have a statistical difference (p-value 0.016 and 0.005 respectively), under Ultra-S. However the rest of the risk factors there was no statistical significance.

Table 6: Different social demographic characteristics of mothers

		BIRTH OUTCOME							
	DEMOGRAPHICS	LMP		p-value	OR (95% CI of OR)	Ultra-S		p-value	OR (95% CI of OR)
Variables		Term	Preterm			Term	Preterm		
i. Age	21-25	56 (46.7%)	29 (47.5%)	0.053	0.906 (0.392-2.097)	14 (40.0%)	11 (37.9%)	0.339	1.886 (0.510-6.978)
	26-30	30 (25.0%)	10 (16.4%)	0.292	0.583 (0.213-1.598)	9 (25.7%)	6 (20.7%)	0.529	1.6 (0.369-6.946)
	31-40	13 (10.8%)	10 (16.4%)	0.592	1.346 (0.454-3.994)	0 (0.0%)	7 (24.1%)	0.002	0.294 (0.141-0.614)
	18-20	21 (17.5 %)	12 (19.7%)		Ref	12 (34.3%)	5 (17.2%)		Ref
ii. Marital status	Steady partner living together	43 (35.8%)	23 (37.7%)	0.882	0.936 (0.392-2.237)	8 (22.9%)	10 (34.5%)	0.098	3.750 (0.754-18.641)
	Steady partner not living together	9 (7.5%)	3 (4.9%)	0.475	0.583 (0.132-2.580)	2 (5.7%)	1 (3.4%)	0.770	1.5 (0.098-23.069)
	Married monogamous	42 (35.0%)	21 (34.4%)	0.767	0.875 (0.362-2.113)	13 (37.1%)	13 (44.5%0	0.147	3.0 (0.659-13.662)
	Married polygamous	4 (3.3%)	1 (1.6%)	0.472	0.438 (0.044-4.378)	3 (8.6%)	1 (3.4%)	1.000	1.0 (0.073-13.664)
	Other (Widowed/Divorced)	1 (0.8%)	1 (1.6%)	0.698	1.75 (0.1-30.592)	0 (0.0%)	1 (3.4%)	0.118	0.250 (0.094-0.666)
	Single (Never married)	21(17.5%)	12 (19.7%)		Ref	9 (25.7%)	3 (10.3%)		Ref
iii. Occupation	Housewife	50 (41.7%)	31 (50.8%)	0.172	1.798 (0.771-4.194)	12 (34.5%)	18 (62.1%)	0.005	7.0 (1.65-29.697)
	Small-scale business (fishing)	25 (20.8%)	14 (23.0%)	0.326	1.624 (0.614-4.292)	7 (20.0%)	7 (24.1%)	0.055	4.667 (0.916-23.785)
	Farmer	0 (0.0%)	0 (0.0%)	-	-	0 (0.0%)	0 (0.0%)	-	-
	Civil servant	7 (5.8%)	0 (0.0%)	0.130	0.744 (0.618-0.894)	1 (2.9%)	0 (0.0%)	0.645	0.824 (0.661-1.026)
	Casual labourer	7 (5.8%)	4 (6.6%)	0.484	1.657 (0.399-6.878)	1 (2.9%)	1 (3.4%)	0.288	4.667 (0.223-97.497)
	Students	2 (1.7%)	2 (1.7%)	0.301	2.9 (0.360-23.390)	0 (0.0%)	0 (0.0%)		-
	Unemployed	29 (24.2%)	10 (16.4%)		Ref	14 (40.0%)	3 (10.3%)		Ref
iv. Main source of income	Business	34 (28.3%)	19 (31.1%)	0.838	1.118 (0.385-3.248)	10 (28.6%)	8 (27.6%)	1.000	1.000 (0.2-5.004)
	Husband	52 (43.3%)	29 (47.5%)	0.833	1.118	13 (37.1%)	14 (48.3%)	0.700	1.346

					(0.404-3.077)				(0.296-6.131)
	Remittance	2 (1.7%)	0 (0.0%)	0.328	0.667 (0.493-0.902)	1 (2.9%)	0 (0.0%)	0.389	0.556 (0.310-0.997)
	Relatives	18 (15.0%)	6 (9.8%)	0.538	0.667 (0.183-2.434)	6 (17.1%)	3 (10.3%)	0.629	0.625 (0.093-4.222)
	Self service	14 (11.7%)	7 (11.5%)		Ref	5 (14.3%)	4 (13.8%)		Ref
v. Education	Secondary	50 (41.7%)	27 (44.3%)	0.708	0.880 (0.45-1.719)	17 (48.6%)	11 (37.9%)	0.346	0.644 (0.211-1.728)
	Tertiary	26 (21.7%)	7 (11.5%)	0.089	0.439 (0.168-1.149)	4 (11.4%)	3 (10.3%)	0.674	0.700 (0.132-3.699)
	Primary	44 (36.7%)	27 (44.3%)		Ref	14 (40.0%)	15 (51.6%)		Ref

P<0.05=significant; OR = odds ratio; CI = Confidence interval; ref = reference category

4.3 Association between BV and preterm birth

A total of 228 study participants were diagnosed for bacterial vaginosis under Amsel criteria and Nugent scoring (gold standard). Two modes of gestational classification were used i.e. LMP and Ultra-S. This is as illustrated in table 7. However only 181 study participants had complete data, thus could be categorized if the delivered term or preterm. Majority of the study participants turned negative in both Amsel and Nugent.

Table 7: Comparison between bacterial vaginosis and gestation at birth

		BIRTH OUTCOME					
		LMP			**Ultra-S**		
	Diagnosis	Term (%)	Preterm (%)	p-value (CI)	Term (%)	Preterm (%)	p-value (CI))
BV Amsel	Negative	109 (90.8%)	50 (82.8%)	0.084 (0.09-0.07)	30 (85.7%)	20 (69.0%)	0.107 (0.135-0.095)
	Positive	11 (9.2%)	11 (18%)		5 (14.3%)	9 (31.0%)	
	TOTAL	120(100%)	61(100%0		35(100%)	29(100%	
BV Nugent	Intermediate	39 (32.5%)	24 (39.4%)	0.080 (0.07-0.08)	9 (25.7%)	12 (41.4%)	0.211 (0.23-0.246)
	Negative	70 (58.3%)	26 (42.6%)		21 (60.0%)	11 (37.9%)	
	Positive	11 (9.2%)	11 (18.0%)		5 (14.3%)	6 (20.7%)	
	TOTAL	120(100%)	61(100%)		35(100%)	29(100%)	
CI: Confidence interval							

4.3.1 Profiling bacteria associated with bacterial vaginosis among preterm births

Different bacteria were profiled in relation to whether study participants delivered term or preterm, as illustrated in figure 2. This was general irrespective of one being negative or intermediate or positive for BV. Under LMP, the highest bacterium among term deliveries was *Lactobacilli* 56% seconded by *G.vaginalis*/*Bacteroides* 23%. These corresponded to preterm deliveries. This is as illustrated in figure 3 and figure 6

Diverse bacteria were profiled in relation to whether study participants delivered term or preterm and if they were positive or intermediate for BV. Under LMP-BV positive, the highest bacteria among term and preterm deliveries were *G.vaginalis*/*Bacteroides,* both taking 50%. This is as illustrated in figure 5. Under Ultra-S-BV positive, *G.vaginalis*/*Bacteroides* had the highest percentage in both term and preterm deliveries. However, slightly higher among preterm deliveries

55%, compared to term deliveries 46%. This is as illustrated in figure 8. Profiling of bacteria under intermediate diagnosis of BV is as illustrated in figure 4 and 7.

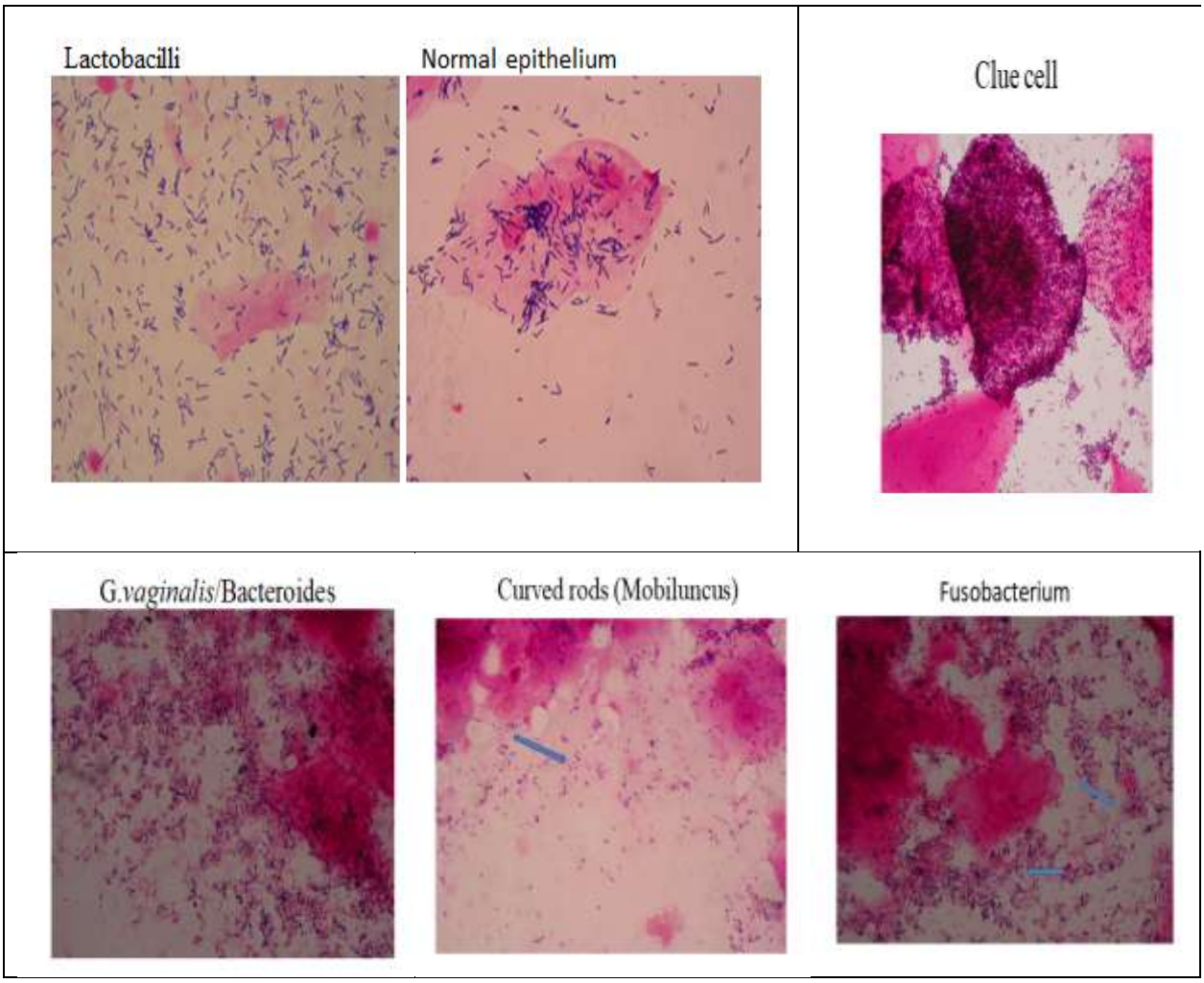

Figure 2: Bacteria identified and morphology of normal epithelium and a clue cell

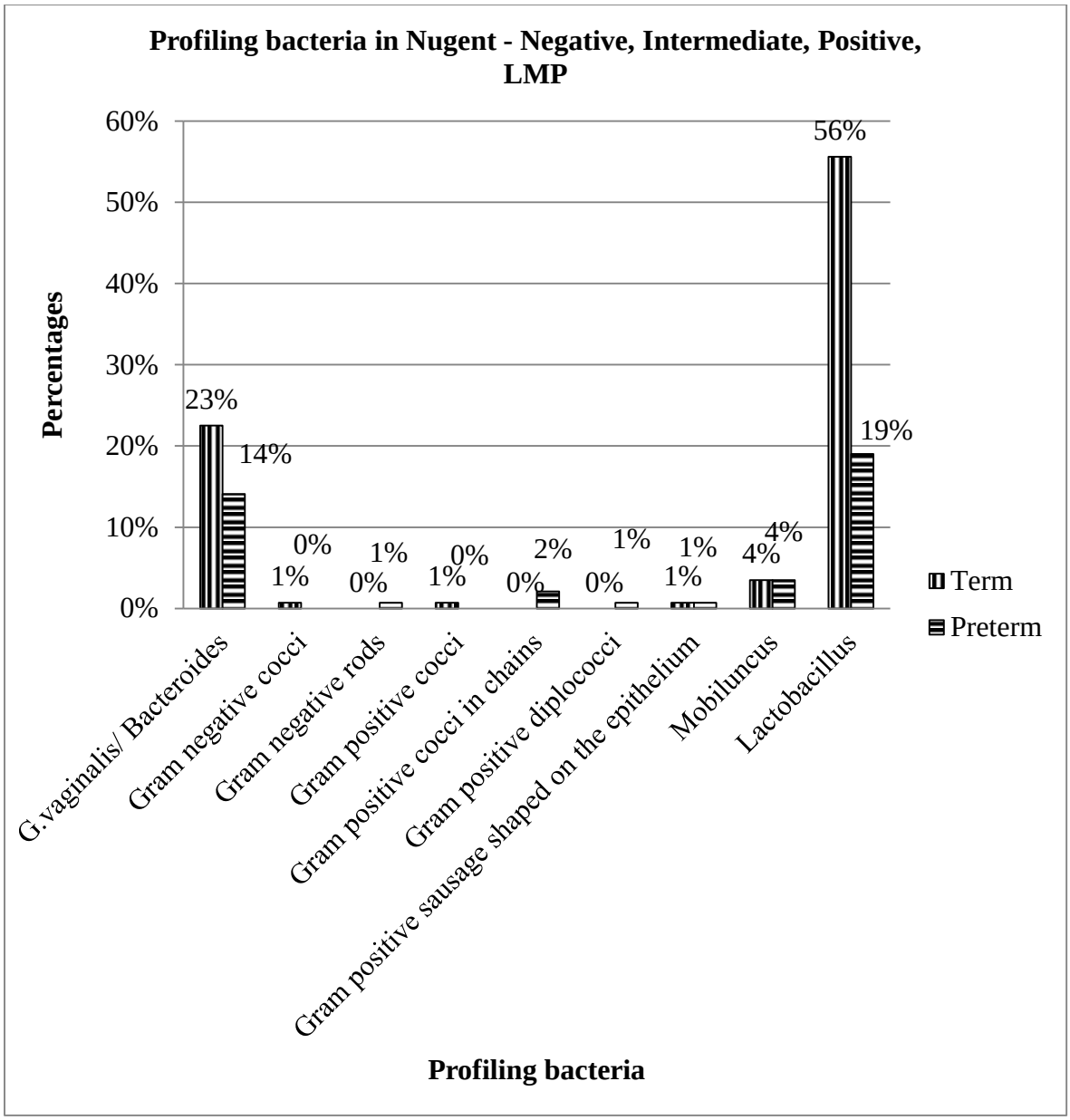

Figure 3: Profiling different of bacteria associated with bacterial vaginosis with regard to normal delivery, gestation at birth (LMP)-in negative, intermediate and positive diagnosis of BV

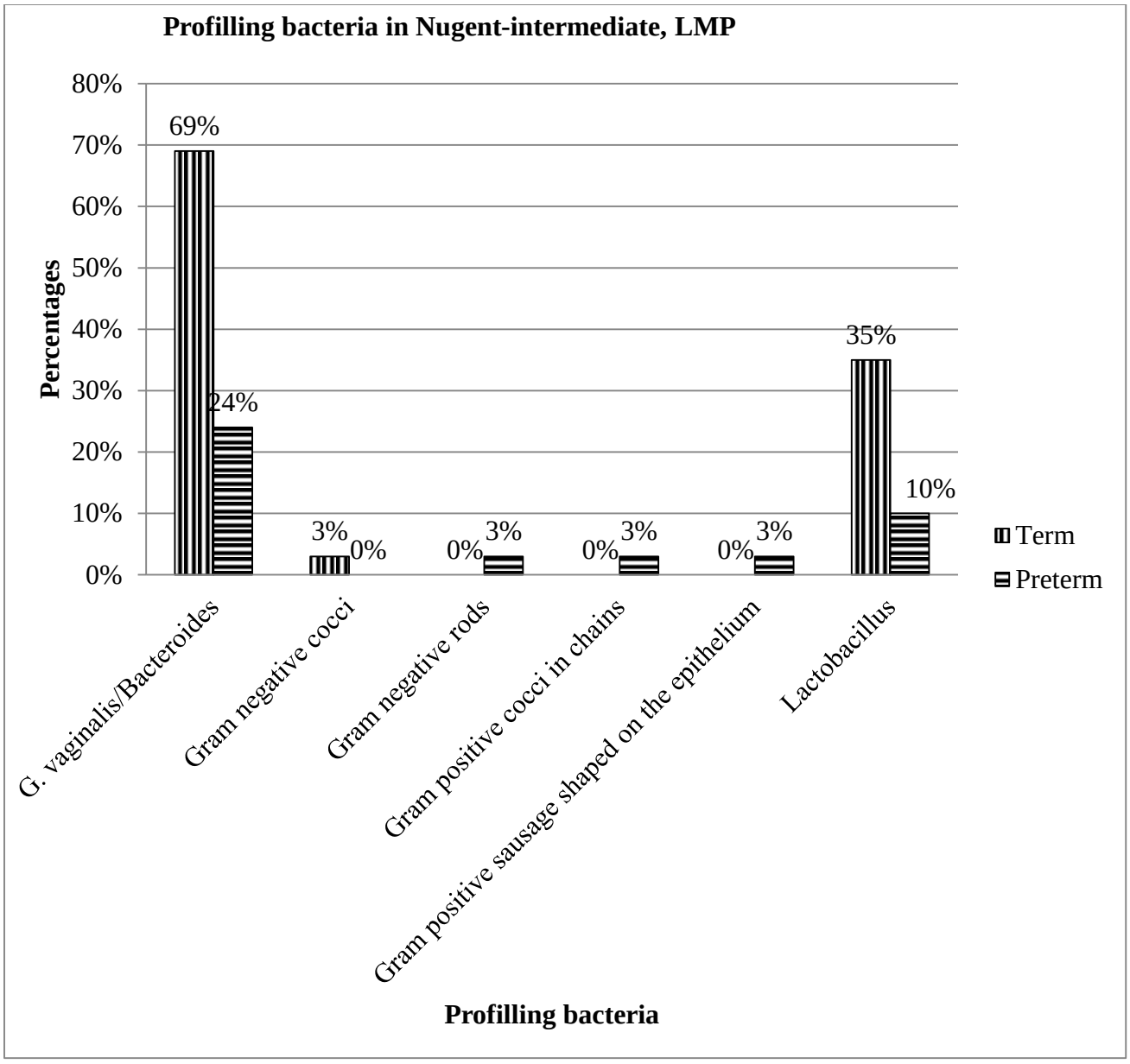

Figure 4: Profiling different bacteria associated with bacterial vaginosis with regard to intermediate diagnosis, gestation at birth (LMP)

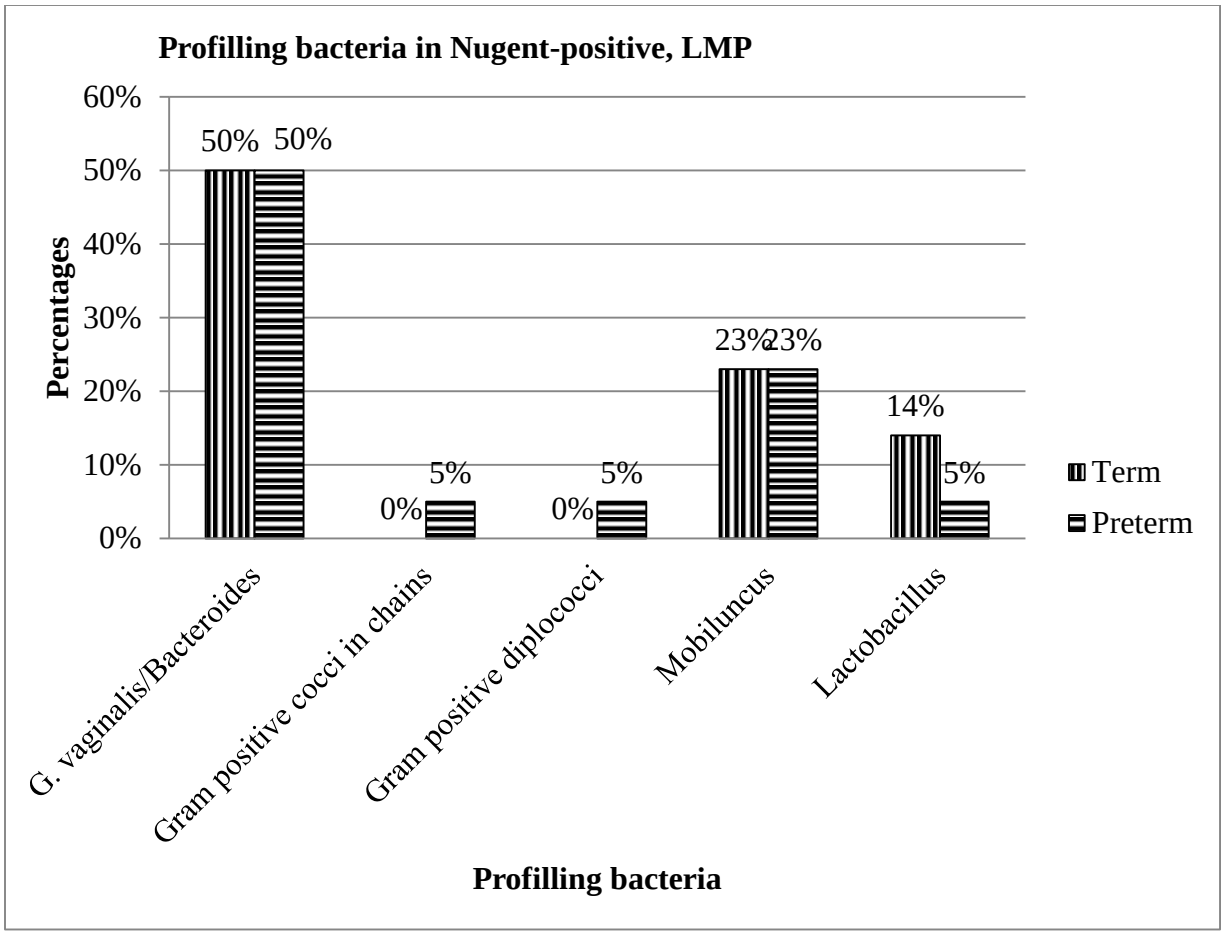

Figure 5: Profiling different bacteria associated with bacterial vaginosis with regard to positive diagnosis, gestation at birth (LMP)

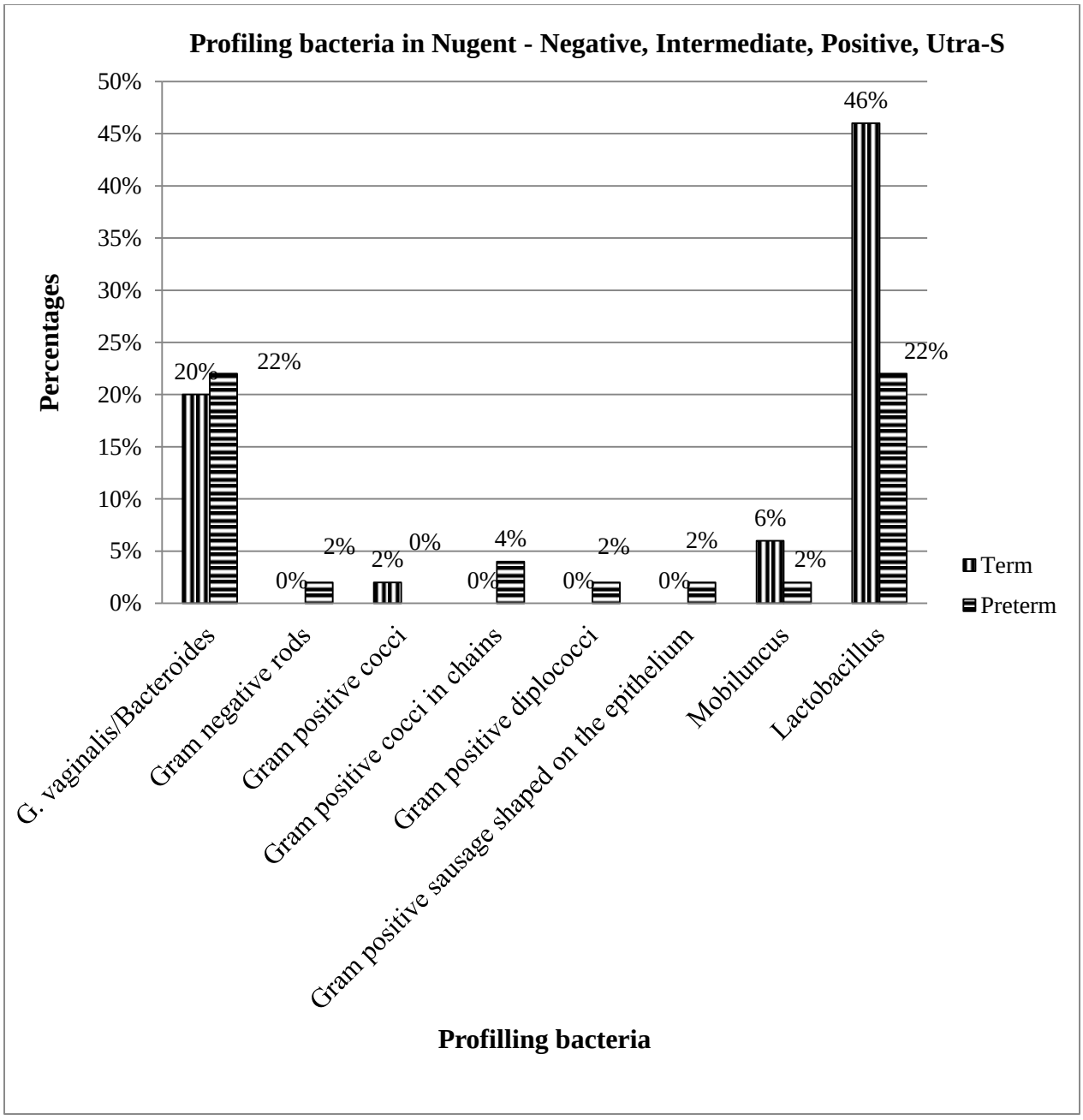

Figure 6: Profiling different bacteria associated with bacterial vaginosis with regard to normal delivery, gestation at birth (Ultra-S)-in negative, intermediate and positive diagnosis of BV

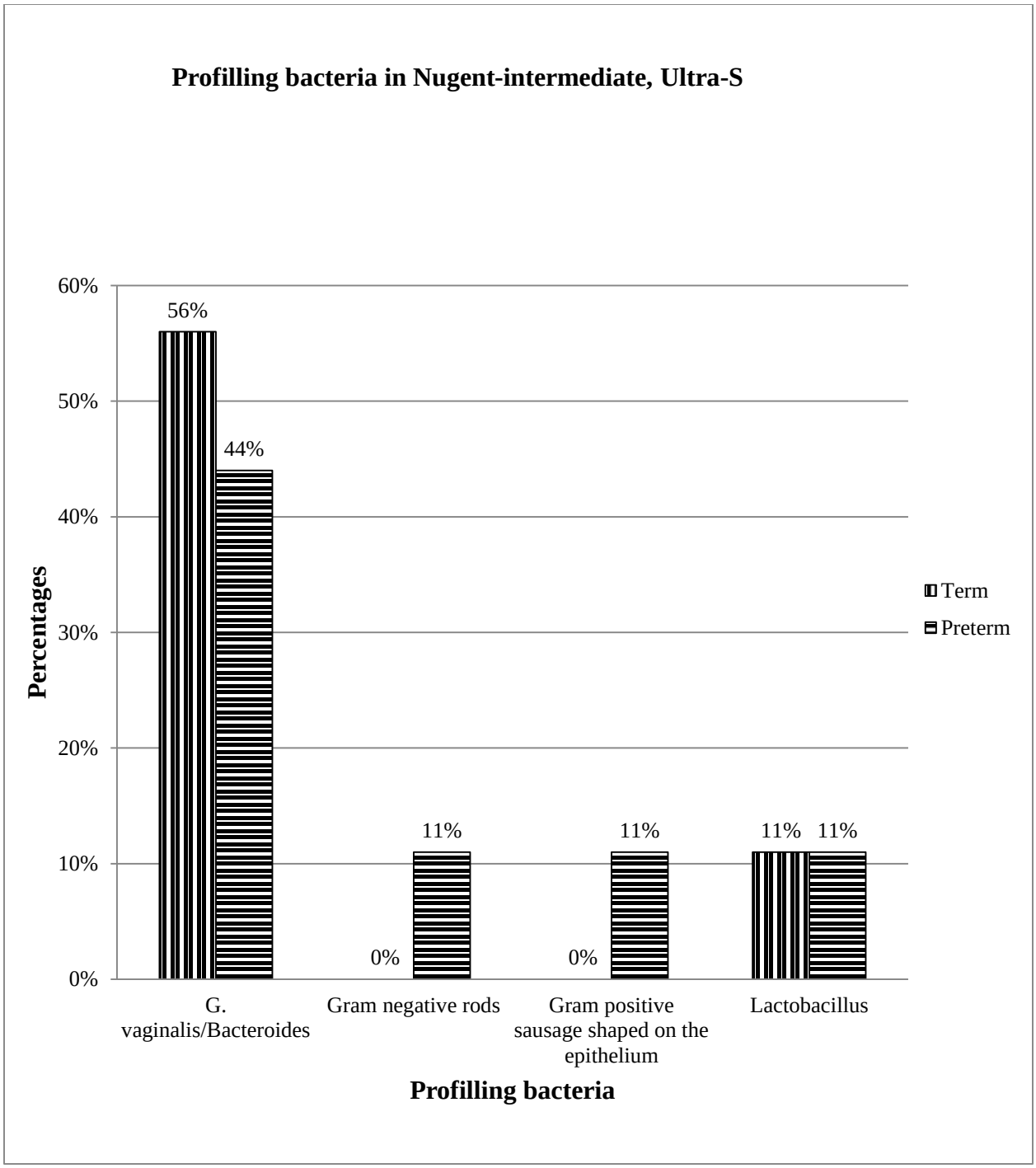

Figure 7: Profiling different bacteria associated with bacterial vaginosis with regard to intermediate diagnosis, gestation at birth (Ultra-S)

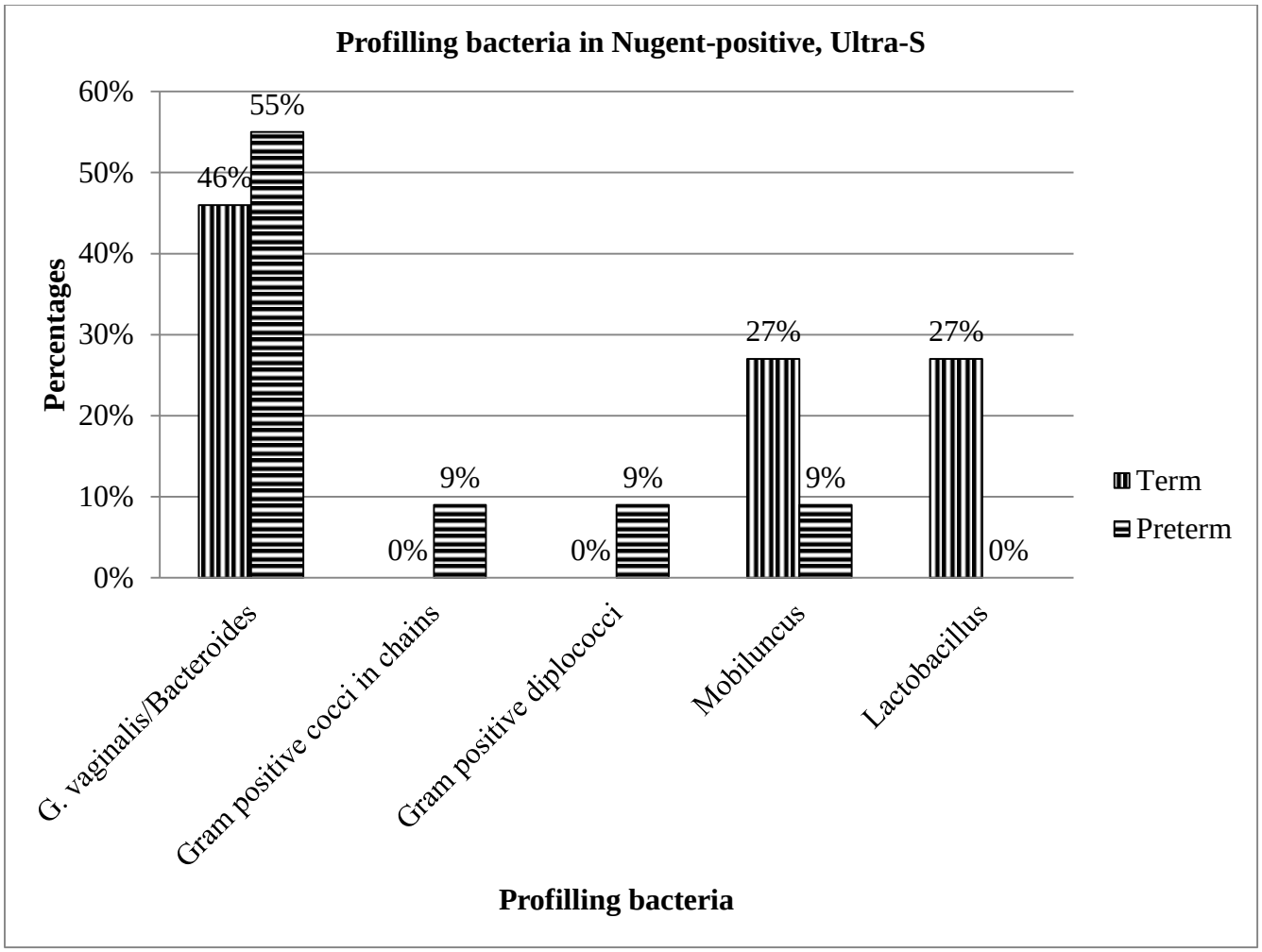

Figure 8: Profiling different bacteria associated with bacterial vaginosis with regard to positive diagnosis, gestation at birth (Ultra-S)

4.3.2 Performance characteristic of Amsel against Nugent

Amsel recorded a sensitivity and specificity of 58.6% and 98.3% respectively, as illustrated in table 8.

Table 8: Performance characteristic of Amsel against Nugent

		BV Amsel criteria			
	Diagnosis	Negative	Positive	Total	p-value
BV Nugent criteria	Negative	119 (60.7%)	2 (6.2%)	121 (53.1%)	0.000
	Positive	12 (6.1%)	17 (53.1%)	29 (12.7%)	
	Intermediate	65 (33.2%)	13 (40.6%)	78 (34.2%)	
	Total	196 (100%)	32 (100%)	228 (100%)	

Sensitivity	Specificity
(119/121)*100=98.3%	(17/29)*100= 58.6%

4.4 Proportions of preterm deliveries among bacterial vaginosis infections

The classification of delivering term or preterm was based on Ultra-S. A comparison was done in relation to infections and outcome of delivery. Figure 2 highlights the results.

Amsel and Nugent scoring were used in the diagnosis of BV. Using Nugent scoring, 11 participants had positive result. Of the 11 who turned positive, 5 (45.5%) delivered term while 6 (54.5%) delivered preterm. A total of 21 turned intermediate under Nugent scoring, of the 21 intermediates, 9 (42.9%) delivered term while 12 (57.1%) delivered preterm.

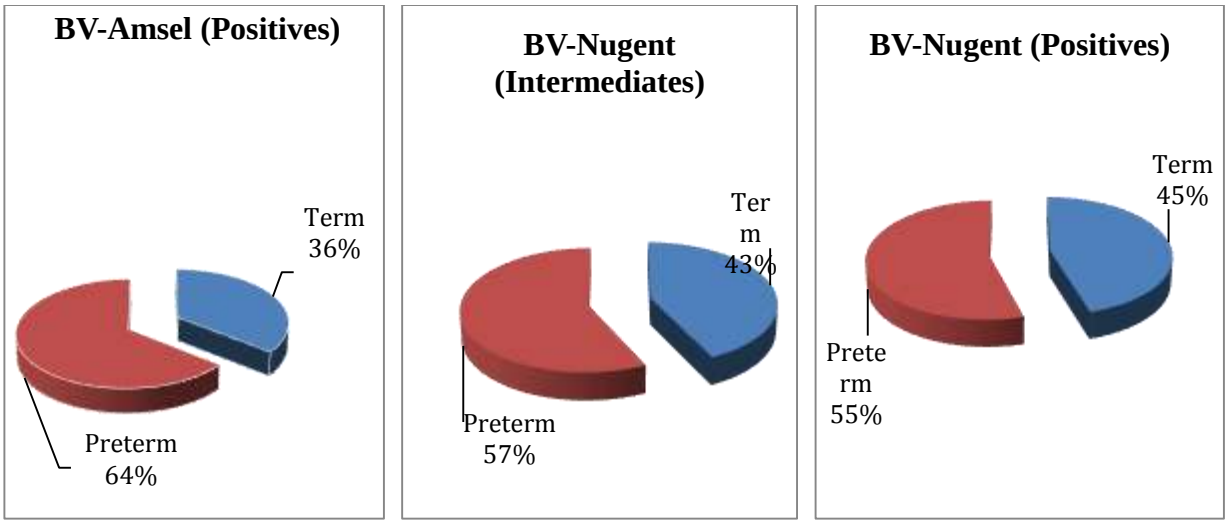

Figure 2: Proportion of preterm births among BV

4.5 Association between syphilis, HIV and PTB

Besides BV, two diseases namely syphilis and HIV were analysed in association of PTB. A total of 181 study participants were analysed for the two infections. In the two infections, majority turned negative, 186 (97.8%) and 147 (87.0%) on syphilis and HIV respectively under LMP.

However, there was no statistical difference in the two infections and missing data was excluded (pearsons chi-square test: p= 0.417 on syphilis and p=0.598 on HIV). The distribution of these infections is shown in Table 11.

Table 9: Prevalence of Syphilis and HIV among LMP and Ultra-S study participants

Results	LMP		Ultra-S	
	Syphilis	HIV at Maternity	Syphilis	HIV at Maternity
Negative	86 (97.9%)	147 (87.0%)	57 (98.3%)	45 (88.2%)
Positive	4 (2.1%)	22 (13.0%)	1 (1.7%)	6 (11.8%)
TOTAL	190 (100%)	169 (100%)	58 (100%)	51 (100%)

4.6. Risk factors to bacterial vaginosis

Factors affecting preterm birth associated with bacterial vaginosis outlined as illustrated in table 12. The factors included; circumcision status of sex partner (p=0.566), vaginal irritation (0.155), lower abdominal pain (p=0.795), infected by any sexually transmitted disease (STI) currently or in the past (p=0.694), what you use for bathing (p=0.887), what you use for scrubbing your body (p=0.572), were found not have statistical difference. However a statistical difference of p=0.045 was captured on use of nylon panty material.

Table 10: Different risk factors to bacterial vaginosis

	Variables	BIRTH OUTCOME							
		LMP				**Ultra-S**			
		Term svd	Preterm svd	p-value	OR (95% CI of OR)	Term svd	Preterm svd	p-value	OR (95% CI of OR)
i Circumcision status of the male partner	Circumcised	83 (69.2%)	39 (63.9%)	0.566	0.790 (0.412-1.515)	27 (77.1%)	20 (69.0%)	0.461	0.658 (0.216-2.006)
	Non circumcised	37 (30.0%)	22 (36.1%)		Ref	8 (22.9%)	9 (31.0%)		Ref
ii Vaginal irritation	Yes	42 (35.0%)	28 (45.9%)	0.155	1.576 (0.841-2.953)	12 (34.3%)	13 (44.8%)	0.390	1.557 (0.566-4.281)
	No	78 (65.0%)	33 (54.1%)		Ref	23 (65.7%)	16 (55.6%)		Ref
iii Lower abdominal pain	Yes	81 (67.5%)	40 (65.6%)	0.795	0.917 (0.478-1.760)	22 (62.9%)	20 (69.0%)	0.609	1.313 (0.462-3.728)
	No	39 (32.5%)	21 (34.4%)		Ref	13 (37.1%)	9 (31.0%)		Ref
iv **Infected with any sexually transmitted disease**	Yes	12 (5.7%)	5 (13.8%)	0.694	0.804 (0.270-2.395)	2 (5.7%)	4 (13.8%)	0.270	2.640 (0.447-15.579)
	No	108 (94.3%)	56 (86.2%)		Ref	33 (94.3%)	25 (86.2%)		Ref
v Type of STI infected with	Gonorrhoea	4 (3.3%)	3 (4.9%)	0.679		1 (2.9%)	2 (6.9%)	0.544	
	HIV	3 (2.5%)	0 (0.0%)			0 (0.0%)	0 (0.0%)		
	HIV and Syphilis	1 (0.8%)	0 (0.0%)			0 (0.0%)	0 (0.0%)		
	Syphilis	4 (3.3%)	2 (3.3%)			1 (2.9%)	2 (6.9%)		
vi Presence of fishy smell from the vaginal	Yes	25 (20.8%)	17 (27.9%)	0.289	1.468 (0.720-2.993)	8 (22.9%)	8 (27.6%)	0.664	1.286 (0.414-3.995)
	No	95 (79.2%)	44 (72.1%)		Ref	27 (77.1%)	21 (72.4%)		Ref
vii Use when bathing	Soap and water	117 (97.5%)	59 (96.7%)	0.995	1.009 (0.090-11.351)	34 (97.1%)	27 (93.1%)	0.267	2.259 (1.705-2.994)
	Water and other detergents(liquid soap, body deodorants)	1 (0.8%)	1 (1.6%)	0.709	2.000 (0.051-78.250)	1 (2.9%)	1 (3.4%)	0.386	2.000 (0.500-7.997)
	Water only	2 (1.7%)	1 (1.6%)		Ref	0 (0.0%)	1 (3.4%)		Ref

viii Use for scrubbing your body	Inner pant	27 (22.5%)	12 (19.7%)	0.871	1.079 (0.429-2.713)	10 (28.6%)	5 (17.2%)	0.200	0.389 (0.090-1.673)
	Sponge	59 (49.2%)	35 (57.4%)	0.339	1.441 (0.681-3.049)	18 (51.4%)	15 (51.7%)	0.478	0.648 (0.195-2.157)
	A piece of cloth	34 (28.3%)	14 (23.0%)		Ref	7 (20.0%)	9 (31.0%)		Ref
ix Material of panties	Nylon	11 (9.2%)	4 (6.6%)	0.614	0.735 (0.222-2.435)	4 (11.4%)	0 (0.0%)	0.045	0.480 (0.360-0.641)
	Both cotton and nylon	16 (13.3%)	11 (18.0%)	0.444	1.390 (0.597-3.236)	7 (20.0%)	3 (10.3%)	0.204	0.396 (0.092-1.707)
	Cotton	93 (77.5%)	46 (75.4%)		Ref	24 (68.6%)	26 (89.7%)		Ref
x Washing genitals	Yes	67 (55.8%)	38 (62.3%)	0.405	1.307 (0.696-2.456)	17 (48.6%)	19 (65.5%)	0.174	2.012 (0.731-5.539)
	No	53 (44.2%)	23 (37.7%)		Ref	18 (51.4%)	10 (34.5%)		Ref
xii What you use to wash vaginal	Soap and water	73 (60.8%)	37 (60.7%)	0.982	0.993 (0.528-1.866)	23 (65.7%)	17 (58.6%)	0.560	0.739 (0.267-2.043)
	Other detergents	0 (0.0%)	0 (0.0%)		-	0 (0.0%)	0 (0.0%)		-
	Water only	47 (39.2%)	24 (39.3%)		Ref	12 (34.3%)	12 (41.4%)		Ref
xiii Washing vaginal immediately after sex	Yes	72 (60.0%)	39 (63.9%)	0.607	1.182 (0.625-2.236)	21 (60.0%)	19 (65.5%)	0.650	1.267 (0.456-3.518)
	No	48 (40.0%)	22 (36.1%)		Ref	14 (40.0%)	10 (34.5%)		Ref

P<0.05=significant; OR = odds ratio; CI = Confidence interval; ref = reference category

4.7 Maternal and obstetrics risk factors for PTB

Different maternal and obstetric factors predisposing mothers to preterm births were outlined as illustrated in table 13. Out of the variables, history of preterm birth (p=0.049), condom use in the last sexual act (p=0.022), parity 3+0 (p=0.013), parity 4+0 (p=0.003), gravidae 1 (p=0.034) and gravidae 2 (p=0.001) under ultra-sound were captured to have a statistical difference. Under LMP hormonal and non-hormonal type of contraceptive (p=0.046) and parity 4+0 (p=0.031) were captured to have a statistical difference. However, the rest of the risk factors there was no statistical difference.

Table 11: Different maternal and obstetric factors predisposing mothers to preterm births

Variables		BIRTH OUTCOME							
		LMP				Ultra-S			
		Term svd	Preterm svd	p-value	OR (95% CI of OR)	Term svd	Preterm svd	p-value	OR (95% CI of OR)
i. Age of sex debut	Less than 18 years	57 (47.5%)	30 (49.2%)	0.831	1.070 (0.577-1.982)	19 (54.3%)	15 (51.7%)	0.838	0.902 (0.337-2.419)
	More than 18 years	63 (52.5%)	31 (50.8%)		Ref	16 (45.7%)	14 (48.3%)		Ref
ii No. of sex partners in the last six months	1	110 (91.7%)	56 (91.8%)	0.284	3.055 (0.359-25.996)	31 (88.6%)	28 (96.6%)	0.065	1.903 (1.493-2.426)
	More than 1	4 (3.3%)	4 (6.6%)	0.143	6.000 (0.478-75.344)	0 (0.0%)	1 (3.4%)		-
	None	6 (5.0%)	1 (1.6%)		Ref	4 (11.4%)	0 (0.0%)		Ref
iii History of PTB	Yes	12 (10.0%)	9 (14.8%)	0.608	1.544 (0.611-3.901)	3 (8.6%)	8 (27.6%)	0.049	3.683 (0.872-15.556)
	No	105 (87.5%)	51 (83.6%)		Ref	29 (82.9%)	21 (72.4%)		Ref
	N/A (primigravida)	3 (2.5%)	1 (1.6%)			3 (8.6%)	0 (0.0%)		
iv Period between last pregnancy	Less than 1 year	21 (17.5%)	12 (19.7%)	0.919	1.126 (0.494-2.565)	4 (11.4%)	5 (17.2%)	0.108	1.125 (0.261-4.848)
	N/A (primigravida)	34 (28.3%)	16 (26.2%)		-	13 (37.1%)	4 (13.8%)		-
	More than 1	65 (54.2%)	33 (54.1%)		Ref	18 (51.4%)	20 (69.0%)		Ref
v Emotional stress	Yes	59 (49.2%)	32 (52.5%)	0.675	1.141 (0.616-2.114)	16 (45.7%)	17 (58.6%)	0.304	1.682 (0.623-4.546)
	No	61 (50.8%)	29 (47.5%)		Ref	19 (54.3%)	12 (41.4%)		Ref.
vi Contraceptive use	Yes	78 (65.0%)	33 (54.1%)	0.155	0.635 (0.339-1.189)	20 (57.1%)	19 (65.5%)	0.494	1.425 (0.515-3.940)
	No	42 (35.0%)	28 (45.9%)		Ref	15 (42.9%)	10 (34.5%)		Ref
vii Type of contraceptive	Hormonal	54 (71.1%)	25 (75.8%)	0.059	1.463 (1.259-1.700)	18 (90.0%)	14 (73.7%)	0.383	1.778 (1.310-2.413)
	Hormonal and Non-hormonal	14 (18.4%)	8 (24.2%)	0.046	1.571 (1.146-2.155)	1 (5.0%)	5 (26.3%)	0.088	6.000 (1.003-35.908)
	Non-hormonal	8 (10.5%)	0 (0.0%)		Ref	1 (5.0%)	0 (0.0%)		Ref

viii Infertility treatment	Yes	5 (4.2%)	3 (4.9%)	0.816	1.190 (0.275-5.152)	1 (2.9%)	0 (0.0%)	0.359	0.540 (0.430-0.678)
	No	115 (95.8%)	58 (95.1%)		Ref	34 (97.1%)	29 (100%)		Ref
ix Condom use in the last sexual act?	Yes	22 (18.3%)	18 (29.5%)	0.087	1.865 (0.909-3.826)	3 (8.6%)	9 (31.0%)	0.022	4.8 (1.159-19.879)
	No	98 (81.7%)	43 (70.5%)		Ref	32 (91.4%)	20 (69.0%)		Ref
x Parity	0+1	4 (3.3%)	2 (3.3%)	0.947	0.941 (0.156-5.673)	1 (2.9%)	1 (3.4%)	0.457	3.000 (0.15-59.890)
	0+3	0 (0.0%)	1 (1.6%)	0.178	0.347 (0.236-0.509)	0(0.0%)	1 (3.4%)	0.110	0.25 (0.107-0.584)
	1+0	39 (32.5%)	18 (29.5%)	0.734	0.869 (0.386-1.955)	15 (42.9%)	10 (34.5%)	0.323	2.000 (0.5-7.997)
	1+1	5 (4.2%)	0 (0.0%)	0.112	0.653 (0.533-0.801)	1 (2.9%)	0(0.0%)	0.567	0.750 (0.565-0.995)
	2+0	28 (23.3%)	12 (19.7%)	0.638	0.807 (0.329-1.977)	5 (14.3%)	5 (17.2%)	0.192	3.000 (0.560-16.071)
	2+1	1 (0.8%)	1 (1.6%)	0.657	1.882 (0.111-32.010)	0(0.0%)	1 (3.4%)	0.110	0.250 (0.107-0.584)
	3+0	7 (5.8%)	2 (3.3%)	0.464	0.538 (0.100-2.880)	1 (2.9%)	5 (17.2%)	0.013	15.00 (1.325-169.870)
	3+1	1 (0.8%)	0 (0.0%)	0.468	0.653 (0.533-0.801)	0(0.0%)	1 (3.4%)	0.110	0.250 (0.107-0.584)
	4+0	2 (1.7%)	6 (9.8%)	0.031	5.647 (1.026-31.066)	0(0.0%)	5 (17.2%)	0.003	0.250 (0.107-0.584)
	5+0	0 (0.0%)	1 (1.6%)	0.178	0.347 (0.236-0.509)	0(0.0%)	1 (3.4%)	0.110	0.250 (0.107-0.584)
	5+1	0 (0.0%)	1 (1.6%)	0.178	0.347 (0.236-0.509)	0(0.0%)	1 (3.4%)	0.110	0.250 (0.107-0.584)
	6+0	0 (0.0%)	0 (0.0%)		-	0(0.0%)	0(0.0%)	-	-
	0+0	32 (26.7%	17 (27.9%)		Ref	12 (34.3%)	4 (13.8%)		Ref
xi Gravidae	1	35 (29.2%)	19 (31.1%)	0.463	1.543 (1.268-1.878)	15 (42.9%)	6 (20.7%)	0.034	1.4 (1.068-1.835)
	2	41 (34.2%)	18 (29.5%)	0.509	1.439 (1.215 (1.704)	6 (17.1%)	9 (31.0%)	0.001	2.5 (1.345-4.646)
	3	31 (25.8%)	13 (21.3%)	0.519	1.419 (1.172-1.719)	1 (2.9%)	5 (17.2%)	0.000	6.000 (1.003-35.908)
	4	8 (6.7%)	3 (4.9%)	0.546	1.375	0(0.0%)	2 (6.9%)		-

				(0.958-1.975)			
	5	3 (2.5%)	6 (9.8%)	0.197	3.000 (1.191-7.558)	0(0.0%)	5 (17.2%)	-
	6	0 (0.0%)	2 (3.3%)	0.083	-	0(0.0%)	2 (6.9%)	-
	7	0 (0.0%)	0 (0.0%)		-	0(0.0%)	0(0.0%)	-
	0	1 (0.8%)	0 (0.0%)		Ref	13 (37.1%)	0 (0.0%)	Ref
	P<0.05=significant; OR = odds ratio; CI = Confidence interval; ref = reference category						

4.6.2 1 Relationship between body mass index of expectant mothers and preterm births

Weight and height measurements were obtained from the records and used to compute body mass index (BMI). BMI was used to determine if one was underweight or obese as these are known risk factors for preterm deliveries.

Body mass index was compared between term and preterm deliveries with the use of independent t-test for LMP and Ultra-S. The results are shown in table 14 and 15. A total of 181 normal deliveries were determined by LMP. Of the 181, a total 64 did an Ultra-S. Under LMP, the mean body mass index for term and preterm were 26.2 and 25.8 respectively, which can be classified as overweight. However there were no statistical differences noted between the two groups (t-test; p=0.853).

Under Ultra-S, the mean body mass index for term and preterm were 26.4 and 26.5 respectively, which can be classified as under overweight. However these means were not statistically different (t-test; p=0.961).

Table 12: Comparison of body mass index between term and preterm under LMP

Gestation classification	N	Mean	p value
Term	120	26.205	0.853
Preterm	61	25.815	

Table 13: Comparison of body mass index between term and preterm under Ultra-S

Gestation classification	N	Mean	p value
Term svd	35	26.416	0.961
Preterm	29	26.491	

5.0 DISCUSSION

PTB is the leading cause of prenatal mortality and morbidity globally. In spite of efforts to prevent PTB in most nations, the incidences of PTB have been on the rise. Infections are the leading causes of PTB. Among the infectious agents, bacteria constitute the largest number of microorganisms associated with PTB. The most prevalent lower genital infection that causes PTB is bacterial vaginosis (BV. In this study, BV prevalence was found to be 9.2% and 18% among the term and preterm deliveries respectively, under LMP. Under Ultra-S, BV prevalence was 14.3% and 20.6% under term and preterm deliveries. The most common bacterium was found to be *lactobacilli* in both term and preterm deliveries. However among BV positive and intermediate, *G. vaginalis/Bacteroides* were dominant in both LMP and ultra-s. A number of risk factors were looked into such as age (31-40), history of preterm births, and use of nylon panty material under ultra-S and hormonal and non-hormonal use of contraceptives under LMP were found to have a statistical significance.

Among the social demographic factors, age was reported to be associated with PTB, in particular age between 31-40. This was similar in other studies despite of differences in geographical location, such as Europe, Canada and Nigeria (Saurel-cubizolles *et al.*, 2004; WHO, 2012; Butali *et al.*, 2016; Cnattingius, Vixner and Norman, 2016; Michael *et al.*, 2017; Fuchs *et al.*, 2018). However in other studies there was no association (Aderoba, Olokor and Olagbuji, 2017; Goisis *et al.*, 2017). The disparity could be as a result to difference in the social economic status. Interestingly, there was variation per study even within the same country on the minimum gestation age of a preterm birth. For some it was 22 weeks while others it was 28 weeks (Butali *et al.*, 2016; Aderoba, Olokor and Olagbuji, 2017). In addition housewife under occupation was reported to be associated with PTB; however no association has been reported from other studies

The prevalence of BV in this study was 12%. A study done in Ethiopia among pregnant women and the same age group of 18-40 had a slightly higher prevalence of 19.4%. The study compared the prevalence of BV between symptomatic and asymptomatic, which was 31.6% and 15.9% respectively. In addition a higher percentage of pregnant women were asymptomatic for BV (Ababa *et al.*, 2014). Moreover some studies had a higher prevalence of 37% among HIV-1 pregnant women in Kenya, 48.6% among normal women in Ethiopia (Bitew *et al.*, 2017) and 29.2% among normal women aged between 14-49 years in USA (Quillan *et al.*, 2007). A similarity

in the two studies done in Ethiopia and USA is that BV prevalence is lower among young girls compared to older women. In this study, BV prevalence was higher among preterm deliveries similar to a study done in Nigeria (Aderoba, Olokor and Olagbuji, 2017).

BV has been consistently associated with PTB. However in this study there was no statistically significant association as documented in quite a number of studies (Shayo *et al.*, 2012; Freitas *et al.*, 2018; Mcelrath *et al.*, 2018). Unlike recent studies, a number of past studies have proclaimed an association between BV and PTB (Obert *et al.*, 2000; Hebb *et al.*, 2004; Bahram et al., 2009; Margolis and Fredricks, 2014; Aderoba et al., 2017). This provides a limelight for more research to be done on what has changed to lead to no association. Interestingly a study done in India and Sweden, reported a significance of BV and PTB in early pregnancy period of 16-20 weeks and no significance in late pregnancy period of 28-32 weeks (Jacobsson *et al.*, 200;Gupta *et al.*, 2016). The difference in the findings could be as a result of difference in the geographic location, race and clinical characteristics. In addition the prevalence of BV also varies within the same study population groups (Bitew *et al.*, 2017).

Different bacteria associated to BV were profiled in association to PTB. Bacteria profiled included Lactobacilli, G.*vaginalis*/Bacteroides, Mobiluncus and Fusobacteria. However, there was no statistical difference between term and preterm deliveries. The most dominant bacteria were lactobacillus, more so among the term deliveries. However, among those who were positive and intermediate for BV, *G.vaginalis*/Bacteroides was the commonest among term and preterm deliveries under gestation by both LMP and Ultra-S. The same findings were evident in Tanzania, despite of the lack of categorization of the common bacteria between term and preterm deliveries (Shayo *et al.*, 2012). A study done in USA reported *Mobiluncus* bacteria associated to BV, to have a significance of about 2-fold increased risk to PTB, despite absence of statistical significance (Foxman *et al.*, 2014).

In this study, bacterial vaginosis was not associated with risk factors such as: circumcision status of the male partner, vaginal irritation, fishy smell from the vaginal, lower abdominal pain, history of STI, washing of vaginal immediately after sex, items used when bathing and washing vaginal. This was an interesting finding since the mentioned factors predispose one to BV yet little has been done in comparison to term and preterm deliveries. Some studies have reported an association with the use of cloth for intravaginal cleansing in 1 to >28 times a week, (Clelland *et al.*, 2008)

and fishy smell from the vaginal (Schwebke and Desmond, 2005). The differences could be attributed to geographical and study population differences. Some studies used pregnant women while others used non-pregnant women female sex workers. However, a statistical difference was captured in use of nylon panties as risk factor to bacterial vaginosis. This was equivalent to other studies (Yu et al., 2011; Bardin *et al.*, 2013).

In our study we found some maternal and obstetrics risk factors that were associated to PTB. These included history of PTB, condom use, parity, gravidae, hormonal and non-hormonal type of contraceptive. Many studies have reported an association of PTB and history of PTB (Saurel-cubizolles *et al.*, 2004; Hamad et al., 2005; Zhang *et al.*, 2012; Phillips *et al.*, 2017). However, a prospective study done in Sweden recorded no association on PTB and history of PTB (Larsson *et al.*, 2007) Association of condom use in the last sexual act and PTB has not been reported from other studies. However, a study done in USA reported consistent use of condom, increased recurrence of BV (Jane R. Schwebke, 2009) while other studies have reported reduction BV (Mart et al., 2004; Horne *et al.*, 2009). Association of gravidae and PTB was reported in this study. However, other studies have reported no association (Horne *et al.*, 2009; Koss *et al.*, 2015). Parity was captured to have a statistical significance in this study just like other studies (Fredricks, 2011; Wagura *et al.*, 2018) Hormonal and non-hormonal use of contraceptive has been associated with PTB like a study done by WHO (Mohamed *et. al.*, 2012)

Besides BV, the study was in a position to capture data on HIV and candidiasis. However, there was no statistical difference in both. HIV prevalence was 13% and 11% under LMP and Ultra-S respectively. This is slightly high compared to the HIV prevalence of 6.1% among women who had at least one live birth in the last 5 years in different counties in Kenya, (Sirengo *et al.*, 2016). Difference in the prevalence could be as a result of dissimilarity in the study populations.

One participant was captured to be positive for candidiasis. The participant delivered term, however she was excluded from the study since she delivered through caesarean.

5.1 STUDY STRENGTHS

The main strength of this study is the vigor with which it arrived at the diagnosis of BV. This is unlike a number of studies. Two main diagnosis were used, Amsel and Nugent (gold standard). Moreover, profiling of the different bacteria associated to PTB was highlighted and a comparison was made between the term and PTB cohorts. Hence, very few studies have profiled bacteria associated with BV in relation to PTB.

In addition, the study extensively highlighted risk factors that predispose women to BV and PTB. This has not been done in any of the studies. Most studies focus on risk factors to BV or PTB separately but not both. Thus through this study more light has been shed.

5.2 STUDY CONCLUSION

The study was in a position to profile different bacterial associated with bacterial vaginosis in relation to term and preterm deliveries. Lactobacillus was dominant in the two cohorts. However, study participants diagnosed for BV positive or intermediate *G.vaginalis*/Bacteroides was the most dominant.

Moreover, *G.vaginalis*/Bacteroides were slightly higher among the preterms compared to terms.

BV prevalence was found to be 12% among women about to deliver. Despite the low prevalence, high percentages (55%) who were diagnosed positive of BV delivered preterm.

Ages, house wife under occupation, history of PTB, nylon panty material, parity, gravidae, hormonal and non-hormonal use of contraceptive, condom use in the last sexual act were found to be significantly associated with preterm deliveries. Vaginal irritation, fishy smell from the vaginal, lower abdominal pain, history of STI, washing of vaginal immediately after sex, items used when bathing and washing vaginal were found not to be significantly associated to PTB.

5.3 STUDY LIMITATION

The study had the limitation of not drawing causality as a result of the study design. In addition microscopy is limited to identifying a wider array of bacteria associated with BV.

5.4 RECOMMENDATION

The study recommends a replication of the study in a longitudinal study that allows drawing causal relationships. In addition use of loop-mediated isothermal amplification (LAMP) or Multiplex Polymerase Chain Reaction (PCR) that helps in the identification of different species of bacteria and their quantity, since BV is an imbalance of the aerobes and anaerobes.

More research needs to be done since a discrepancy is arising on association of BV and PTB. Recent studies are reporting no association while past studies reported BV to be associated with PTB. More needs to be done on what has caused the shift.

Further research is needed in condom use and association to PTB. Some studies have reported consistent use of condom increases recurrence of BV while other studies are reporting reduction of BV.

5.5 STUDY RESULTS DISSEMINATION PLAN

The results of this study will be disseminated through print publication in peer review journal and as well as in conferences and seminars.

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7.0 APPENDIXES

(A) INFORMED CONSENT EXPLANATION DOCUMENT

Title of study: Profiling of bacteria species in bacteria vaginosis associated with preterm births in Kisumu County

Principal investigator: Jelioth Muthoni, MSc. Student, University of Nairobi/KNH (tel. 0792487066)

Introduction

I would like to talk to you about the study being conducted by Jelioth Muthoni, a medical microbiology student at the University of Nairobi/Kenyatta National Hospital. The purpose of this consent is giving you information, which will help you, make up your mind on whether to participate in the study or not. Feel free to as any question concerning the study, any benefits, and risk to ensure that this consent form is clear. This will help you make a concrete decision on whether to participate in the study or not. Ones you decide to participate you will be given a copy of this consent to keep it in your records.

Purpose of the study

This study is about having a better understanding on the risk factors and the bacteria that are associated with pre-births. I hereby kindly request for your permission to use your vaginal specimens for my research, which will be used to identify the bacteria, using most reliable diagnostic method and the questioners will identify the risk factors. High standards of confidentiality of your test will be ensured.

Study procedure

A study nurse in Jaramogi Oginga Odinga teaching referral hospital will consent the participants who meet the inclusion criteria. Then carry a one on one questionneiring session with the participant. After which the study nurse will take a vaginal swab. The vaginal sample will be run in Amsel and Nugent score criteria by the principal investigator.

Role of Participant

Obtaining a small vaginal swab from the participants who obtain the consent.

Type of specimen

Three vaginal swabs will be obtained on each participant who obtains the consent.

Possible storage of specimen

One of the swabs collected will be stored for further analysis.

Expected time in the study

The study is expected to run for one year. However, it will be one time contact with the study participant.

Benefits

The benefits of participating in this research include:

The diagnosis will be used in the identification of Bacterial vaginosis; the tests are of high sensitivity and specificity. Participants' positive for BV will be referred to Obstetric and Gynecological for treatment. In addition the results will be used to guide on the measures to be put in place in the prevention of pre-term deliveries. The participants will incur no cost.

Risks and discomforts

The risks in this research are minimal. A slight discomfort in the collection of the vaginal swab.

Confidentiality

Records relating to you or your patients participation in the study will remain confidential. A signed copy of the consent form will be given to you.

Data dissemination

The data acquired will be presented to the School of Medicine, Department of Medical Microbiology, Jaramogi Oginga Odinga teaching referral hospital and University of Nairobi Tropical and Infectious Diseases. Moreover, it will be published in peer reviews, presented in journal clubs and seminars.

Voluntary participation

Your participation in this study is voluntary. Ones you decline from participating, no services will be denied that are normally available to you.

Contact information

If you have questions now or in future regarding your rights or about this study, you may contact:

- Jelioth Muthoni, MSc student at the University of Nairobi on +254792487066
- Chairperson, KNHUONERC.-Prof. Anastasia Guantai

  P.O BOX 20723-00200 Nairobi. Tel#: 726300-9, Fax 725272/

- KNH-UoN ERC Secretary- Prof. Mark L. Chindia

  Contact telephone numbers 2726300 ext. 44102 email, uonknh_erc@uonbi.ac.ke

- Dr. Julius Oyugi Supervisor Tel +254713898564
- Prof. Walter Jaoko Supervisor Tel +254727555254

(B) CONSENT FORMS

Consent from the patient

The above details about the study and the basis of participation have been explained to me and I agree to give permission for use of my vaginal sample in the proposed study. I understand that at liberty to choose whether my specimen should be used in the study or not. I give my consent for my vaginal specimen to be used in the diagnosis of bacterial vaginosis and the association with pre-term deliveries.

Patient's signature/Thumb mark: ----------

P I's signature: ----------

Date: ----------

Ridhaa ya kukubali kuwa muhusika katika Kiswahili

Title of study: Wasifu aina ya bakteria katika bakteria utoko kuhusishwa na kuzaliwa njiti katika Kisumu County

Mpelelezi mkuu: Jelioth Muthoni, MSc.student, Chuo Kikuu cha Nairobi / KNH (Number ya rununu 0792487066)

Utafiti kuanzishwa

Ningependa kuzungumza na wewe kuhusu utafiti unaofanywa na Jelioth Muthoni, mwanafunzi wa mikrobiolojia katika Chuo Kikuu cha Nairobi / Kenyatta Hospital ya Taifa. Madhumuni ya idhini hii ni kukupa taarifa, ambayo kukusaidia, kufanya uamuzi wako kama kushiriki katika utafiti au la. Jisikie huru kama swali lolote kuhusu utafiti, faida yoyote, na hatari ya kuhakikisha kwamba fomu hii ya idhini ni wazi. Hii itasaidia kufanya uamuzi halisi na kama kushiriki katika utafiti au la. Ukiamua kushiriki utapewa nakala ya idhini hii ya kuitunza katika kumbukumbu zako.

Madhumuni ya utafiti

Utafiti huu ni kuhusu kuelewa mzuri juu ya mambo ya hatari na bakteria ambayo ni kuhusishwa na kabla ya kuzaliwa. I hapa kwa huruma ombi kwa ruhusa ya kutumia sampuli yako ya uke kwa ajili ya utafiti wangu, ambao utatumika kwa kutambua bakteria, kwa kutumia mbinu zinazoaminika kwa uchunguzi na kuuliza maswali yatakayo tambua hatari. Viwango vya hali ya juu vya usiri vitatumika kwa matokeo yako.

Utaratibu kifani

Katika somo hili, kufanya tamu ya uke kwa kupata specimen. Muuguzi katika hospitali ya rufaa ya Jaramogi Oginga Odinga mafunzo ya uhamisho atachukua utaratibu baada ya uchunguzi mkuu amepokea ridhaa kutoka kwa mgonjwa. Baadaye, sampuli ya uke itatumika katika vigezo vya alama za Amsel na Nugent.Pia dakika 15 kikao wa kujaza dodoso.

Wajibu wa Mshiriki

Kupata kidogo ya uke usufi na dodoso kujazwa kutoka washiriki ambao watapeana idhini.
Aina ya sampuli
Usufi uke tatu zitapatikana kwenye kila mshirika ambaye amepeana idhini.

Uwezekano uhifadhi wa sampuli

Moja ya swabs zilizokusanyawa itakuwa kuhifadhiwa kwa ajili ya uchambuzi zaidi.

Inatarajiwa wakati katika utafiti

Utafiti unatarajiwa kuendesha kwa mwaka mmoja

Faida

Faida ya kushiriki katika utafiti huu ni:

Utambuzi zitatumika katika utambuzi wa bakteria utoko, vipimo ni ya unyeti wa juu na maalum. Washiriki watakao kuwa na bacteria utoko wata tumwa kwa Obsteric na Gynecologia kwa matibabu. Aidha matokeo itumika kuongoza hatua za kuwekwa katika nafasi kwa kuzuia uzazi kabla ya muda. Washiriki hawatalipa chochote.

Hatari na usumbufu

Hatari katika utafiti huu ndogo. Usumbufu kidogo kwa ukusanyaji wa usufi uke.

Usiri

Recordi kuhusiana na wewe au mgojwa wako katika kushiriki kwa utafiti utabaki kuwa siri. Anayehusika kwa utafiti atapewa nakala aliyotia saini ya fomu ya idhini.

Usambazaji wa data

Data alipewa yatawasilishwa kwa Shule ya Tiba, Idara ya Medical Microbiology, Jaramogi Oginga Odinga kufundisha hospitali ya rufaa na Chuo Kikuu cha Nairobi Tropical na Magonjwa ya Kuambukiza. Aidha, itachapishwa katika peer mapitio majarida na kuwasilisha katika vilabu jarida na semina.

Ushiriki wa hiari

Kushiriki kwako katika utafiti huu ni ya hiari. Wale watakaoshuka kutokana na kushiriki, hakuna huduma unayopokea kwa kawaida utanyimwa. Utapokea huduma kwa kawaida. Maelezo ya mawasiliano Kama una maswali sasa au katika siku zijazo kuhusu haki zako au utafiti huu, unaweza kuwasiliana na:

- Jelioth Muthoni, MSc Mwanafunzi katika chuo Kikuu cha Nairobi on +254792487066
- Mwenyekiti, KNHUONERC. Prof. Anastasia Guantai

  P.O BOX 20723-00200 Nairobi. Namba ya simu#: 726300-9, Faksi 725272/

- KNH-UoN ERC Katibu Mawasiliano- Prof. Mark L. Chindia

  Nambari za simu 2726300 ext. 44102 email, uonknh_erc@uonbi.ac.ke

- Dr. Julius Oyugi Supervisor Tel +254713898564
- Prof. Walter Jaoko Supervisor Tel +254727555254

NYONGEZA FOMU YA KUKUBALI

Ridhaa kutoka kwa mgonjwa

Maelezo ya hapo juu kuhusu utafiti na msingi wa ushiriki zimeelezwa kwangu na mimi kukubali kutoa ruhusa kwa ajili ya matumizi ya sampuli yangu uke katika utafiti mapendekezo. Naelewa kwamba uhuru wa kuchagua kama sampuli yangu itumike katika utafiti au la. Natoa kibali yangu kwa sampuli yangu uke kutumika katika utambuzi wa vimelea utoko na kushirikiana na kujifungua kabla ya muda.

Sahihi/kidole gumba cha mgonjwa: ----------

Sahihi ya mlinzi wa mgonjwa: ----------

Tarehe: ----------

KITABU MAR RUSA MICHIWO BANG WINJO TIENDE NONRO

Wich mar nonro: Kawo ratiro mar aina mag bacteria makelo tuo e duong mar mine mamiyo ginyuolo nyithindo ka pok kinde mar nyuol ochopo e County mar Kisumu

Janonro Maduong :Jelioth Muthoni, japuonjre mar Masters e mbalarieny mar Nairobi , hospital mar Kenyatta (namba mar sime 0792487066)

Chakruok

De ahero mondo alosie kodi kuom nonro ma itimo kod Jelioth Muthoni, japuonjre mar medical microbiology e mbalarieny mar Nairobi/hospital mar Kenyatta. Gimomiyo rusa ni imiyi en ni mondo iwinj tie nonro ni ma biro konyi ngado bura e pachi ka idwaro bedo e nonro ni kata ka ok idwar. Bed thuolo penjo gimoro amora maluore gi nonro ni, ber kata rach, ma ine ni form mar rusa ni iwinjo maler. Ma biro konyi ngado bura makare kaluore gi yie kata dagi bedo achiel e nonro ni. Sama iseyie mondo ibedi e nonro ni ibiro miyi barua mar rusa ni mondo ikan kama ikane gikeni.

Momiyo mar Nonro

Nonro ni en kuom bedo gi winjo maber kuom bacteria gi gik ma kelo nyuol ka pok kinde mar dweche ochiko ochopo. Kuom mano akwayi gi luoro mondo iyie mondo wati gi specimen ma wabiro golo e duong mari e nonro ni ma ibiro ti godo fwenyo bacteria ka itiyo gi yore makare mag nonro kendo jononro biro fwenyo bende gik ma kelo bacteria gi. Ling ling mamalo ibi tigo sama ipimi.

Chenromarnonro

E nonro ni, ibiro ti gi pamba e duong mari ka igolo godo specimen mar nonro. Nurse ma nitiere e hosiptal mar Jaramogi Oginga Odinga teaching and referral biro timo chenro ni bang ja nonro maduong ka oseyudo rusa kuom jatuo. Bang mano gima ochoki ibi keti e Amsel gi Nugent score criteria

Tich mar jatuo

Yudo sample ka oa e duong mar ngama ochiwo rusa

Aina mar gir nonro

Pamba adek ma oywe godo duong mar ngato ka ngato ma ochiwo rusa

Kama ibiro kan e gige nonro

Achiel kuom pamba adek ibi kan ne nonro mabuora

Kinde ma nonro biro kawo

Nonro owinjore mondo otimre kuom higa achiel. Kata kamano obiro bedo mana mar neno jatuo dichiel kende

Ber

Ber mar riwruok gi nonro ni en ni :

Nonro ni biro konyo e fwenyruok mar tuo mar bacterial vaginosis. Yore mag fwenyo tuo ni gin yore ma nigi teko mar fwenyo tuo e yo ma yot. Jokma ibiro yudi gi tuo ni ibiro nwang ne gi daktari ma thiedho tuoche mag mine mondo gi yud thieth. Kuom mago, duoko ibiro ti godo loso yore gi chenro mag konyo mine kik nyuol ka kinde mar dweche ochiko pok ochopo. Onge pesa ma ibiro dwar kuom jotuo

Chandruok kod winjo marach

Chandruok ma luore gi nonro ni tin.Winjo marach matin biro mana bedo sama ikawo specimen e duong.

Ling ling

Ndiko ma luore kodi kata jatuo ni e nonro ni biro dong ma ling ling. Ibiro miyi copy mokete sei mar barua mar rusa

Kaka ibiro ti gi duoko

Duoko ma oyudi ibiro nyisi e School of Medicine , department mar Microbiology, Jaramogi Oginga Odinga teaching and referral hospital kod University of Nairobi Tropical and infectious diseases. Moloyo duoko go ibiro keti e mbui kod kitepe mag sayans misomo e mbui .

Bedo e nonro kuom hero

Bedoni e nonro ni en kuom hero mari. Po mono ni iweyo bedo e nonro ni onge huduma mora mora ma ibi tuoni mane nyiche imiyi.

Contact information

Laini mar tudruok

Ka intiere gi penjo sani kata e ndalo mabiro kaluore gi haki mari e nonro ni, inyalo tudori gi jok ma ondik piny kae :

Jelioth Muthoni, japuonjre mar masters e University of Nairobi e +254792487066

Jakom KNHUONERC.- **Prof. Anastasia Guantai**

P.O BOX 20723-00200 Nairobi. Tel#: 726300-9, Fax 725272/

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Contact telephone numbers 2726300 ext. 44102 email, uonknh_erc@uonbi.ac.ke

Dr. Julius Oyugi Supervisor Tel +254713898564

Prof. Walter Jaoko Supervisor Tel +254727555254

(B) Form mar Rusa

Rusa ka oa kuom jatuo

Weche duto ma luore gi nonro ni ose ler na kendo ayie mondo oti gi samples ka ae duong mara e nonro ni. Angeyo ni an gi thuolo mar yie kata dagi mondo oti gi specimen ma oa e duong mara e timo nonro ni. Achiwo rusa mara mondo oti gi specimen ma wuok e duong na e fwenyruok mar bacterial vaginosis gi gik ma kelo nyuol ma pok ochopo dweche ochiko.

Sei mar jatuo ----------

Sei mar janonro maduong ----------

Tarik ----------

(C) QUESTIONNAIRE

PROJECT TITLE: Profiling of bacteria species in bacteria vaginosis associated with preterm births in Kisumu County

1. DATE: ……..

2. PATIENTS NAME: …….

3. PARTICIPANTS IDENTIFICATION NUMBER (PID): ……..

4. WARD NO……

5. WEIGHT FROM CHARTS: ……

6. HEIGHT FROM CHARTS: ……

Part one: demography

8. Age

<20 ☐

21-25 ☐

26-30 ☐

31-40 ☐

>41 ☐

9. Marital status

Single (Never married) ☐

Steady partner living together ☐

Steady partner not living together ☐

Married monogamous ☐ Married polygamous ☐

Any other ☐

If others, please specify……..

10. What is your occupation?

Housewife ☐

Small scale business (fishing) ☐

Farmer ☐

Civil servant ☐

Casual laborer ☐

Unemployed ☐

11. What is the main source of income in your house?

Self service ☐

Fishing ☐

Business ☐

Husband ☐

Remittance ☐

Others ☐

11a. If others, please specify……..

12. What is your highest education level?

Never attended ☐

Primary ☐

Secondary ☐

Tertiary ☐

13. What is your place of residence?

Urban ☐

Rural ☐

14. Age of sexual debut (first time you had sex)

Less than 18years ☐

More than 18 years ☐

15a. How many sexual partners do you have or had in the past 6 months ?

None ☐

1 ☐

More than 1 ☐

15b. If more than 1, for how long……..

16. What is the circumcision status of your sex partner(s)?

Circumcised ☐

Not circumcised ☐

Circumcised and Non-circumcised ☐

17. Do you have a history of pre-term delivery?(the participant)

Yes ☐

No ☐

If yes, how many?.....

18. What was the period between your last pregnancy?

Less than 1 year ☐

More than 1 year ☐

19. Do you experience emotional stress?

Good ☐

Poor ☐

20. Have you ever used a contraceptive either currently or in the past?

Yes ☐

No ☐

If yes,

21. Which one did you use?

Pills ☐

Intrauterine Contraceptive Device (A Pack of six) ☐

Norplant ☐

Other ☐

If other, please specify…….

22. Have you been under any infertility treatment?

Yes ☐

No ☐

23. Did you use condom during the last sexual act?

Yes ☐

No ☐

24a. Do you experience vaginal irritation?

Yes ☐

No ☐

b. If yes, when and how often? ……..

25. Do you experience lower abdominal pain?

Yes ☐

No ☐

26a. Have you ever been infected with any sexually transmitted disease

Yes ☐

No ☐

b. If yes, How many times…….

27b. Which one were you infected with ……..

28. Do you have any vaginal discharge?

Yes ☐

No ☐

If yes, please specify the nature…….

29a. Do you sometimes get or smell unpleasant odor that has a fishy smell from your vaginal?

Yes ☐

No ☐

b. If yes, when was the last time you experience? .....

30. How many times do you bathe in a week? (……..)

31. What do you use when bathing?

Water only ☐

Soap and water ☐

Water and other (specific detergent) (…….)

32. What do you use for scrubbing your body?

Hands ☐

Apiece of cloth ☐

Your inner pant ☐

Sponge ☐

Any other means (specify)……..

33. How much water do you use when bathing?

About ¼ basin ☐

About ½ basin ☐

About ¾ basin ☐

A full basin ☐

More than a full basin ☐

34. How much time (in minutes) do you use when bathing? (…….)

35. How many pairs of panty do you own? (……)

36. Which material are your panties?

Cotton ☐

Nylon ☐

Other (specify)……

37a. How many times in a week do you change your pants? (……..)

B. How many times in a week do you wash your pants? (……)

38a. Do you ever wash your genitals ("private parts") apart from when you are bathing?

Yes ☐

No ☐

b. If yes, how many time in a week (……..)

39. What do you use to wash your vagina?

Water only ☐

Soap and water ☐

Other detergents (specify)……..

40a. Have you ever washed your vaginal immediately after sex?

Yes ☐

No ☐

b. If yes, how often do you wash your vagina after sex?

Always ☐

More than half of the time ☐

Half of the time ☐

Less than half of the time ☐

Never ☐

MASWALI

Kichwa cha utafiti: wasifu aina ya bakteria katika bakteria utoko kuhusishwa na kuzaliwa njiti katika kisumu county

1. TAREHE……..
2. JINA LA WAGONJWA: …….
3. WAKAZI WA IDENTIFICATION NAMBARI: ……..
4. WARD NO……
5. UZITO KUTOKA KWA CHARTS: ……
6. UREFU KUTOKA KWA CHARTS: ……

Sehemu ya kwanza: demography

8. Umri

<20 ☐

21-25 ☐

26-30 ☐

31-40 ☐

>41 ☐

9. Hali ndoa

Hajawahi kuolewa ☐

Mshirika wa kudumu anaishi pamoja ☐

Mshirika wa kudumu hamuishi pamoja ☐

Mke aliyeolewaWanandoa walioolewa ☐

Nyingine yoyote ☐

Ikiwa wengine, tafadhali taja ……..

10. Nini ufunzo wako?

Mke wa nyumbani ☐

Biashara ndogo (uvuvi) ☐

Mkulima ☐

Mtumishi wa umma ☐

Kazi ya kawaida ☐

Hunakazi ☐

11. Mapato yako mengi yatoka wapi?

Huduma ya kujitegemea ☐

Uvuvi ☐

Biashara ☐

Mchumba ☐

Uondoaji ☐

Vingine ☐

11a. Ikiwa wengine, tafadhali taja ……..

12. Umesoma hadi kiwango kipi?

Sikusoma ☐

Shule ya msingi ☐

Secondari ☐

Msituni ☐

13. Makazi yako ni yapi?

Mjini ☐

Kijijini ☐

14. Ulikuwa na miaka mingapi ulipo jihusisha kwa ngono mara ya kwanza?

Chini ya miaka 18 ☐

Zaidi ya miaka 18 ☐

15a. Umekuwa na wachumba wangapi, miezi sita iliyopota ?

Hakuna ☐

Mmoja ☐

Zaidi ya mmoja ☐

15b. Kama zaidi ya mmoja, kwa mda wa kiasi gani……..

16. Hali ya tohara ya mchumaba ama wachumba wako ni ipi?

Tohara ☐

Hajatahiriwa ☐

Wengine wametahiriwa wengine hawajatahiriwa ☐

17. Je, una historia ya utoaji mimba kabla ya muda? (mshiriki)

Ndio ☐

La ☐

Kama ndio, mara ngapi? .....

18. Umekaa kwa mda upi baada ya mimba yako ya mwisho?

Chini ya mwaka mmoja ☐

Zaidi ya mwaka mmoja ☐

19. Je, wewe ni unasumbuka kimawazo?

La ☐

Ndio ☐

20.Je, umejihusisha na mbinu yeyote ya upangaji uza, kwa sasa ama hapo awali?

Ndio ☐

La ☐

Kama ndio,

21.Ulitumia ipi?

Pills ☐

Kifaa hiki cha uzazi wa mpango (pakiti ya sita) ☐

Kupandikiza ☐

Zingine ☐

Kama zingine, tafadhari taja…….

22. Je, umekuwa chini ya utasa matibabu**?**

Ndio ☐

La ☐

23. Je, unatumia kondomu unapofanya ngono?

Ndio ☐

La ☐

24a Je, unawashwa sehemu ya siri?

Ndio ☐

La ☐

b. Kama ndio, unawashwa lini na mara ngapi? ……..

25. Je, unakuwa na maumivu ya tumbo, upande wa chini?

Ndio ☐

La ☐

26a. Je, ushawahi ugua ugwanjwa wowote wa ngono?

Ndio ☐

La ☐

b. Kama ndio, mara ngapi…….

27b. Ni ungojwa upi uliuguwa? ……..

28. Je una usaha ukeni**?**

Ndio ☐

La ☐

Kama ndiyo, tafadhali taja asili …….

29a. Je, wakati mwingine wapata au kuwa na harufu mbaya harufu ya fishy kutoka ukeni yako?

Ndio ☐

La ☐

bKama ndio, ni lini ulikuwa na tukio hili mara ya mwisho?.....

30. Je, waoga mara ngapi kwa wiki? (……..)

31. Je, watumia nini kuga?

Maji tupu ☐

Maji na sabuni ☐

Maji na vifaa vingine (tafadhali taja ni zipi) (…….)

32. Je, watumia nini kusugua mwili?

Mikono ☐

Kitambaa ☐

Suruali ☐

Sifongo ☐

Kitu kingine (taja ni kipi)……..

33. Je, watumia maji kiasi gani unapooga?

Robo basini ☐

Nusu basini ☐

Robo tatu basini ☐

Basini nzima ☐

Zaidi ya basini nzima ☐

34. Je, watumia dakika ngapi unapo oga? (…….)

35. Je, unasuruali ngapi? (……)

36. Je, ngozi ya suaruali zako ni ipi?

Cottoni ☐

Nyloni ☐

Ngozi nyingine (taja)……

37a. Je, wabadirisha suruali mara ngapi kwa wiki? (……..)

B. Je, waosha suruali mara ngapi kwa wiki? (……)

38a. Je, unaosha sehemu ya uke, wakati mwingine ila unapooga?

Ndio ☐

La ☐

b. Kama ndio mara ngapi kwa wiki (……..)

39 je, unaosha sehemu ya uke na nini?

Maji tupu ☐

Sabuni na maji ☐

Other detergents (specify)……..

Vitu zingine(tafadhari taja ni zipi)…….

40a. Ushawahi osha sehemu ya uke, mara tu ulipofanya ngono?

Ndio ☐

La ☐

b. Kama ndio, unaosha sehemu ya siri mara ngapi baada ya ngono?

Wakati wote ☐

Zaidi ya mara nusu ya wakati ☐

Nusu ya wakati ☐

Chini ya nusu ya wakati ☐

Keto kendo ngiyo kit gi matelo tuo kendo nyuol ka pod ndalo

1. Taki…….
2. Nying manguon:…….

3. Nying no.: ……..

4. Wuod no……

5. Pek mar otas:……

6. Bor mar otas: ……

Part one: demography

8. Iga

<20 ☐

21-25 ☐

26-30 ☐

31-40 ☐

>41 ☐

9. Weche mag kendruok

Migogo ☐

Jomo okendore to gidak ☐

Okinde to okodak kanyakla ☐

Jathako achiel ☐ Jadoho ☐

Waya ☐

Ka nthe machielo……..

10. Tichi?

Chi oot ☐

Jalupo ☐

Japur ☐

Ondike gi sirikal ☐

Jatij lwedo ☐

Jao orak ☐

11. Ere kaka tiji konyi e odi?

Tija ☐

Lupo ☐

Oala ☐

Chuora ☐

Yuto matin matin ☐

Joma moko ☐

11a. Ka nthe machielo ……..

12. Sombi ochopu kanye?

Ok nadhi ☐

Primar ☐

Sekondar ☐

Jakom ☐

13. Idak kure?

Taon ☐

Dala ☐

14. Odiochieng mokuongo manikuongo riworie gi osiepni

Pin mar igni apar ga aboro ☐

Igni mokalo apao ga aboro ☐

15a. Jo era adi maise bedo go ei dueche auchi el mokalo ?

Onge ☐

Achiel ☐

Mokal achiel ☐

15b. To ka en mokalo achiel, ……..

16. Kuom ndalo mage?

Odhi nyange ☐

Odhi nyange ☐

Odhi nyange, Odhi nyange ☐

17. Bende isebedo kod konyruolk ka ndaloni pod

Kamano ☐

Ok kamano ☐

To kaenkano, adi?.....

18. Nyaka nene kyulo nyaka koro sani en ndalo maromo nadi?

Matin ne iga achiel ☐

Mangeny ne iga achiel ☐

19. Tobe isebeno gi paro moro amora?

Maber ☐

Ohier ☐

20. To bende isetioga gi yedhe mag kungo nyuol eiodiochienge mokalo?

Kamano ☐

Ol kamano ☐

To ka en kamano,…….

21. En kuom mage itiogo?

Yedhe ☐

Ma otwe diuchiel ☐

Yath ma ikeo e lwedo ☐

Machielo ☐

Ka nthe machielo…….

22. To beisebedo gi konyuoruk moroamora kaka thagruule kuom nyuol?

Kamano ☐

Ok kamano ☐

23. To beneitio gi rabuoyunga eseche mag riuruok?

Kamano ☐

Ok Kamano ☐

24a. Ise bedo gi guonyruok eduongni?

Kamano ☐

Ok kamano ☐

b. To kaen kamano osetimi kuom ndalo adi? ……..

25. To beisebedo gi ichkach mar piny ich?

Kamano ☐

Ok kamano ☐

26a. To bende isebedo gi tuoche mag era?

Kamano ☐

Ok kamano ☐

b. Tu ku en kamano, kuom kinde mage…….

27b. En tuo mane ……..

28. To be isebedo gi tuo mora mora ka oluore gi?

Kamano ☐

Ok kamano ☐

To ka en kamano,kuom odiochieng…….

29a.To bende isebedo gi tik marach e duongni?

Kamano ☐

Ok kamano ☐

b. To kaen kamano, odiochieng mogik en karango?.....

30. Iluokori didi kuom ngalo abich? (……..)

31. Ango ma itiyo go ka iluokori?

Pi lilo ☐

Sabun gi pi ☐

Gimaitigo,ainya (…….)

32. Itiyo gi ango kuom rudho dendi?

Luendo ☐

Nanga ☐

Suruachi ma ixe ☐

Lau ma yom ☐

Machiel mopogore gi mago……..

33. Itio gi pi maromo nade kailuokori?

Ario ☐

Achiel gi nus ☐

Adek gi nus ☐

Ka opong ☐

Mokalo besen ka opong ☐

34. Itieko seche adi kuom luokruok? (…….)

35. In gi sirueche adi ma ingo? (……)

36. To gi chal nade?

Koton ☐

Juala ☐

Ka nthe machielo…….

37a. Iloko lepi mag ixe didi e ndalo abich? (……..)

B. To i luoko gi didi e ndalo abich? (……)

38a. To beisebebedo ka iluoko duongni ma opogore gi luok?

Kamano ☐

Ok kamano ☐

b. To ka en kamano didi e ndalo abich (……..)

39. Ango ma itio go kuom luok duongni?

Pi lilo ☐

Sabun gi pi ☐

Mamoko……..

40a. To be iseluokoga duongni mapixo bang riuruok?

Kamano ☐

Ok kamano ☐

b. To ka en kamano, didi mailuoko duongni bang riuruok?

Osebedo ☐

Mangeny ne seche achiel ☐

Seche ma ok oromo achiel ☐

Matin ne seche achiel ☐

Nyaka chieng ☐

(D) LABORATORY FORM

Patients ID:	
Date of sample collection:	Date of analysis:
Read by	Reviewed by
1. **Amsel criteria**	
Nature of discharge	
pH reading	
Whiff test (positive, negative)	
Clue cells	
Conclusion:	BV positive ☐ BV negative ☐

2. **Nugent scoring criteria**

Epithelial cells at 100X all others at 1000X	**Morphotype**	**Point**	**Morphotype**	**Point**
<1 per field =1 +	4+ Lacto	0	1+ G.vag	1
1-5 per field = 2+	3+ Lacto	1	2+ G.vag.	2
5-30 per field=3+	2+ Lacto	2	3+ G.vag.	3
>30 per field=4+	1+ Lacto	3	4+ G.vag.	4
	0 + Lacto	4	1-2 mobl.	1
			3-4 mobl.	2

Gram stain reactions		First reading	Second reading
Epithelial cells		/10×	/10×
White Blood cells		/10×	/10×
Red blood cells		/100×	/100×
Pleomorphic Gram positive Rods		/100×	/100×
Gram positive cocci		/100×	/100×
Gram negative cocci		/100×	/100×
Gram negative rods	Prevotella spp.	/100×	/100×
	Fusobacteria spp.	/100×	/100×
	Coliforms	/100×	/100×
Diptheroids		/100×	/100×
Other non-specified bacteria		/100×	/100×
Yeast cells			
Stem cells			
Trichomonas vaginalis			
Others (general)			
Specific indicators			
Lactobacillus (intermediate, Large species)		/100×	/100×
G.vaginalis/Bacteroides		/100×	/100×
Mobiluncus spp.		/100×	/100×
BV score (0 to 10)			
Clue cells			
BV diagnosis			

(E) DUMMY TABLES

Specific objective 1

Table 14: Distribution of participant age (years)

Group	Mean/Median	SD/IQR	Minimum	Maximum
BV positive				
BV negative				
All participants				

Table 15: Socio-demographic characteristics of patients

Variable	Category	Frequency	Proportion
Marital status	Single		
	Married		
	Separated		
	Divorced		
Occupation	H/wife		
	Casual		
	Farmer		
	Small business		
	Office work		
	Not indicated		
Education level	None		
	Primary		
	Secondary		
	College/University		

Table 16: Obstetric characteristic of participants

Variable	Category	Frequency	Proportion
	1		
Parity	2		
	3		
	4		
	5		
	+6		
History of UTI	Yes		
	No		
Gestation week at labor	<32		
	33		
	34		
	35		
	36		

Specific objective 2

Table 17: Bacterial vaginosis infection

Variable	Category	Frequency	Proportion
BV status	Positive		
	Negative		
Bacteria isolated	Lactobacillus		
	Gardenerella & Bacteroides		
	Mobiluncus		
	Fusobacteria		

Severity of BV infection	**Mild**		
	Moderate		
	Severe		

Specific objective 3

Table 18: Socio demographic factors and BV status

Variable	BV positive	BV negative	Chi-square	P-value
Marital status				
Single				
Married				
Divorced/separated				
Education status				
None				
Primary				
Secondary				
College/University				

(F) MATERIALS

Gloves

pH tester (Merck pH, range 4.0 to 7.0)

Glass slide and cover slips

Sterile Dacron swabs

10% Potassium hydroxide

A dropper

Gram stain reagents

Oil

70% alcohol – sterilizer

Disposable towels

4 Slide boxes

Normal saline