

**GROUP B STREPTOCOCCUS ANOGENITAL COLONIZATION  
RATE AND ANTIBIOTIC SUSCEPTIBILITY AMONG ANTENATAL  
WOMEN AT MOI TEACHING AND REFERRAL HOSPITAL, KENYA.**

**BY**

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Group B Streptococcus: Anogenital Colonization Rate and Antibiotic Susceptibility among Antenatal Women at Moi Teaching and Referral Hospital, Kenya.

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## DECLARATION

### **Student Declaration**

I declare that this research thesis is my original work and has not been presented in any other university or institution for the award of the degree or any academic credit.

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**DEDICATION**

To my parents Dr. Salahuddin Farooqui and Mrs. Fatma Farooqui for their precious advice and constant support throughout my academic journey.

To my husband Mr. Ubada Hussain for his patience, love and understanding for all the time I put in to my research.

To the antenatal women without whom this study would have been impossible.

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### Abstract

**Background:** Worldwide, Group B streptococcus (GBS) infection has been shown to be one of the leading causes of sepsis, meningitis and pneumonia in neonates. With approximately 5-35% of pregnant women being colonized, 1% to 2% of their newborns develop early onset sepsis within 7 days of life. Intrapartum prophylactic treatment with penicillin significantly reduces this risk by up to 80%. Despite penicillin being readily available at MTRH, no protocol for prevention of GBS sepsis currently exists at MTRH.

**Objective:** To determine the anogenital colonization rate and antibiotic susceptibility patterns of Group B Streptococcus among women attending antenatal clinic.

**Methods:** This was a prospective cohort study conducted between May and December of 2015 that involved recto-vaginal swabbing, and the swab culture and sensitivity, of gravid women  $\geq 35$  but  $< 41$  weeks gestation by best estimate based on last menstrual period and/or obstetric ultrasound, attending antenatal clinic, at MTRH. Consecutive sampling was used. Structured questionnaires were administered to eligible participants. Data analysis was done using the software for statistical computing known as R (R Core Team, 2015).

**Results:** Three hundred and eighty six women (386) met the inclusion criteria and consented to partake in the study. Median age of participants was 26.7 years, majority were married (86.8%), gravida 1 to 2 (67.9%) and had at least secondary education (79.2%). Median gestation duration of participants was 37 weeks. All participants followed up (385) delivered in a hospital. Only eight (2.1%, 95% CI: 0.9% - 4%) of those who accepted to participate in the study were culture positive for GBS. Of the eight, one participant was lost to follow up and of the remaining seven, five of the GBS culture positive participants (71.4%) were treated with antibiotics. No association was established between GBS carrier status and demographic, and clinical, characteristics of the participants. All the isolates of GBS were sensitive to penicillin, ampicillin, vancomycin, erythromycin, and clindamycin.

**Conclusion:** The GBS colonization rate among women attending antenatal clinic at MTRH was low (2.1%) with the GBS isolates being susceptible to penicillin, ampicillin, vancomycin, erythromycin, and clindamycin.

**Recommendations:** Based on the low prevalence, we do not recommend routine antenatal screening of GBS. As this was a facility based study the results may not be reflective of the whole population, thus we recommend a population based study.

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## ABBREVIATIONS

<b>ACOG:</b>	American College of Obstetricians and Gynecologists
<b>ANC:</b>	Antenatal Clinic
<b>CDC:</b>	Centers for Disease Control and prevention
<b>EOD:</b>	Early Onset Disease
<b>GBS:</b>	Group B Streptococcus
<b>IREC:</b>	Institutional Research and Ethics Committee
<b>ISO:</b>	International Organization for Standardization
<b>LOD:</b>	Late Onset Disease
<b>MBChB:</b>	Bachelor of Medicine and Surgery
<b>MCH:</b>	Maternal and Child Health
<b>MMED:</b>	Masters of Medicine (Degree)
<b>MTRH:</b>	Moi Teaching and Referral Hospital
<b>NBU:</b>	New Born Unit
<b>PGRH:</b>	Post Graduate Reproductive Health
<b>RH:</b>	Reproductive Health
<b>RMBH:</b>	Riley Mother and Baby Hospital
<b>SANAS:</b>	South African National Accreditation System
<b>SOM:</b>	School of Medicine
<b>UN:</b>	United Nations
<b>WHO:</b>	World Health Organization

## OPERATIONALISED DEFINITION OF TERMS

**Early onset disease:** Infections in newborns occurring within the first week of life are designated early onset disease.

**Developing country:** A developing country, which may also be referred to as a less-developed country, is a nation with a lower standard of living, underdeveloped industrial base, and low Human Development Index (HDI) relative to other countries.

**Developed country:** A developed country, industrialized country, or "more economically developed country" (MEDC), is a sovereign state that has a highly developed economy and advanced technological infrastructure relative to other less industrialized nations.

**Human Development Index:** It is a composite statistic of life expectancy, education, and income indices used to rank countries into four tiers of human development.

**Late onset disease:** Late onset infections occur in infants aged >1 week, with most infections evident during the first 3 months of life.

## CHAPTER ONE

### INTRODUCTION

#### 1.1 Background

*Streptococcus agalactiae*, or the Lancefield group B streptococcus (GBS) is a leading cause of infection in newborns, pregnant women, and older persons with chronic medical illness(Edwards & Baker, 2005; Stevens & Kaplan, 2000). In the early 1970s, group B streptococcus was recognized as an important cause of neonatal morbidity and mortality and was determined to be responsible for meningitis and sepsis in newborns, both in its early form in the first seven days of life (early onset disease) and in its later form from the seventh to the ninetieth day of life (late onset disease) (Platt & O'Brien, 2003). Due to the high risk of death, preventive measures against GBS are necessary.

Based on studies performed in developed countries, vaginal colonization with GBS occurs in 5–35% of women(Centers for Disease Control and Prevention, 2010; L. Lin et al., 1999; Meyn & Hillier, 1997; Pearlman et al, 1998; S J Schrag et al., 2000). Maternal colonization with GBS in the genitourinary or gastrointestinal tracts has been found to be the primary risk factor for EOD (Centers for Disease Control and Prevention, 2010). A prospective cohort study conducted in the 1980s revealed that pregnant women with GBS colonization were >25 times more likely than pregnant women with negative antenatal cultures to deliver infants who developed early-onset GBS disease(Boyer & Gotoff, 1985).

The only intervention which has been demonstrated to impact rates of GBS disease in neonates is the use of selective intrapartum antibiotic prophylaxis (IAP) to interrupt vertical transmission from GBS-colonized mothers. A study in 2002 showed that as overall use of antepartum antibiotics has increased over time, the attack rate for early-onset group B *Streptococcus* has significantly decreased by 75%(Towers & Briggs, 2002).Recent

figures are even more encouraging. In 2010, the Centers for Disease Control and Prevention in the United States reported a decrease in incidence of early onset GBS disease by up to 80% from the early 1990's which they attribute to use of intrapartum antibiotics. Studies done on susceptibility patterns show that penicillin is still the antibiotic of choice with ampicillin as an alternative. GBS has also been shown to be susceptible to vancomycin and first generation cephalosporins but resistance to erythromycin and clindamycin has been noted (Al-Sweih et al, 2005; Centers for Disease Control and Prevention, 2010; Committee opinion, 2013; Shabayek et al, 2009). No information is available on the susceptibility profile in our population.

In developed countries, group B streptococcus is the leading cause of neonatal sepsis and meningitis with a case fatality rate of 40 to 80%(Kulkarni et al, 2001). The best evidence available shows that a program of Screen and Treat is most effective in identifying carriers of GBS and preventing vertical transmission. Alternative protocols have attempted risk-based system, but this has shown to be inferior to screen and treat due to missing carriers and over treating(Taminato et al., 2011). In developing countries like Kenya, the prevalence, antibiotic susceptibility, scope of disease, and feasibility of a preventive program have not been adequately studied. Therefore, it is difficult to generalize previous results to our setting where antenatal care visits are few and often fragmented across multiple providers

## **1.2 Problem Statement**

Locally, in the New Born Unit of Moi teaching and Referral Hospital, we had over 200 suspected cases and over 30 confirmed cases of EOD accounting for almost 10% of admissions per year (RMBH records for the year 2014). GBS sepsis is a leading cause of maternal and perinatal morbidity and mortality and has been shown to be one of the most common causes of early onset neonatal sepsis throughout the world (Gil et al., 1999). The maternal colonization rate in our population is not known and no protocol exists for early onset neonatal GBS infection prevention. Recent studies have shown that there is a growing resistance to certain antibiotics used for prevention of EOD. However antibiotic susceptibility profile to GBS had not been studied in our setting and may have an effect on rational prescription of antibiotics.

## **1.3 Justification**

There is paucity of information about epidemiology and antibiotic susceptibility associated with GBS in our setting and no such study has ever been done in MTRH. No study on the feasibility of implementing a protocol of Screen and Treat is available for sub-Saharan Africa. Knowing the burden of the disease could inform health planning and allow us to ascertain if we could institute a standard of care that has thus far been lacking in our setting. Understanding the susceptibility profile would inform protocol development for rational prescription of antibiotics and for appropriate antibiotic prophylaxis selection especially for penicillin-allergic women who are at high risk for anaphylaxis. This study will serve as a baseline study for other related studies.



#### **1.4 Research Questions**

1. What is the colonization rate of GBS among pregnant women, between 35-41 weeks gestational age, seeking antenatal care in MCH at MTRH?
2. What is the antibiotic susceptibility profile in women who test positive for GBS?

#### **1.5 Objectives**

1. To determine the colonization rate of GBS among pregnant women, between 35-41 weeks gestational age by best estimate based on LMP and/or obstetric ultrasound, seeking antenatal care in MCH at MTRH.
2. To determine the antibiotic susceptibility profile in women who test positive for GBS.

#### **1.6 Scope and Limitations of the Study**

All the mothers who presented to antenatal clinic at MCH at  $\geq 35$  up to 41 weeks gestation by dates were eligible for this study. The following antibiotics were measured: penicillin, ampicillin, erythromycin, clindamycin & vancomycin. One limitation is that this was a facility based study and therefore the study findings may not be generalizable to the entire population of women who do not receive antenatal care and/or deliver at MTRH.

## CHAPTER TWO

### LITERATURE REVIEW

In the early 1900's *Streptococcus agalactiae* or group B *Streptococcus* (GBS) was known as the causal pathogen for bovine mastitis and held importance in veterinary medicine. However, in 1938, GBS was identified as a human pathogen when it was found to be related to three fatal cases of puerperal sepsis (Fry, 1938). In 1964, the first study of perinatal GBS infection was published documenting its relationship with negative maternal and neonatal outcomes (Eickhoff et al, 1964). This information led to massive effort to study GBS. Early findings noted that use of antibiotics, sulfa drugs initially and then penicillin, markedly decreased mortality from puerperal sepsis. Association between GBS infection in mothers and their newborn infants, however, was not made until the early 1960's (Koenig & Keenan, 2009).

In the mid-1980s clinical trials and well-designed observational studies first demonstrated that administration of intravenous antibiotics during labor to women at risk for transmitting GBS to their newborns could prevent invasive disease in the first week of life (i.e., early onset disease), early onset neonatal sepsis with case-fatality ratios up to 50% were reported in initial case series (Centers for Disease Control and Prevention, 2010). National guidelines were developed and implemented in the United States in the early 1990's and that has since led to approximately 80% reduction in the incidence of early onset neonatal sepsis caused by group B streptococcus (Jordan et al., 2008).

Maternal colonization with GBS in the genitourinary or gastrointestinal tracts has been found to be the primary risk factor for EOD due to GBS.

Worldwide, vaginal colonization with GBS occurs in 5–35% of women though patterns of prevalence of GBS colonization in pregnant women seems to vary throughout the world

(Centers for Disease Control and Prevention, 2010; L. Lin et al., 1999; Meyn & Hillier, 1997; Pearlman et al., 1998; S J Schrag et al., 2000). Country to country the colonization rate can vary greatly being as low as 2.3% in Pondicherry, South India(Sharmila et al, 2011) and as high as 54.9% in Mexico (Hernandez T. & Soriano B., 2006).

Within sub-Saharan Africa, the prevalence varies even city to city. For example in 2012 a colonization of 20.9% was demonstrated in Hawassa, Ethiopia, whereas in 2014, a colonization of 7.2% was demonstrated in Addis Ababa, Ethiopia. (Mohammed et al, 2012; Woldu et al, 2014). In Tanzania, a facility- based study showed GBS colonization in 23% of pregnant women (Joachim et al, 2009).

Even in Kenya, from the raw data, varying prevalence rates are seen from region to region. A study based in Nairobi, Kenya showed a GBS colonization rate of 25% (Mohamed, 2009) and yet another study done in Kisii, Kenya(Kaminja, 2015) demonstrated a colonization rate of 3%. A local study done in MTRH, presented in 2006, showed a vaginal colonization rate of 20.2% and an anorectal colonization rate of 34.1%(Were et al, 2006).

GBS is a gram positive coccus that, frequently, is part of the normal human genital and gastrointestinal flora and in many cases it does no harm. In pregnant women it has been shown to be associated with asymptomatic bacteriuria, urinary tract infection, chorioamnionitis (15%), postpartum endometritis (16%), pneumonia, puerperal sepsis. Group B streptococci are isolated in 2–15% of infected abdominal wounds after cesarean delivery and bacteremia without a focus in pregnant (15%) and postpartum women. Rarely, it may cause focal infections such as pneumonia, meningitis, and endocarditis(Gibbs et al, 2004).

Established risk factors for GBS colonization are age less than 20 years, married versus single, women with three pregnancies or less, use of intrauterine device, or recent antibiotic use (Collins et al, 1998).

It has also been suggested to be a causal factor of still birth in pregnant women and stillbirths related to GBS seems to have decreased in the United States, possibly due to screening programs (Gibbs et al., 2004). In developing countries data on this is scarce. Locally, in Kenya, a recent study demonstrated a high incidence of GBS-associated stillbirth (0.91 (0.25-2.3)/1,000 births (Seale et al., 2016).

EOD refers to infections in newborns that occur within the first 7 days of life. Early onset infections are acquired vertically through exposure to GBS during passage through the vagina of a colonized woman or via ascent of GBS from the vagina to the amniotic fluid after onset of labor or rupture of membranes, although GBS can also invade through intact membranes (Desa & Trevenen, 1984; Katz & Bowes, 1988). If GBS is aspirated into the fetal lungs, it can lead to bacteremia.

A prospective cohort study conducted during the 1980s revealed that pregnant women with GBS colonization were >25 times more likely than pregnant women with negative prenatal cultures to deliver infants with EOD (Boyer & Gotoff, 1985).

Studies show that at birth, 50-65% of infants born to colonized mothers are also colonized with GBS based on cultures drawn from mucus membranes and skin sites (external ear canal, oral and nasopharynx, umbilicus, anorectal). Out of this, approximately 98% of the colonized newborns remain healthy but 1-2% develop invasive GBS disease if no intervention is offered (Centers for Disease Control and Prevention, 2010; Kadanali et al, 2005; Mohammed et al., 2012).

Infants with early onset GBS disease may present with respiratory distress, apnea, or other signs of sepsis within the first 24–48 hours of life (Baker, 1978; Centers for Disease Control and Prevention, 2010). Common clinical syndromes of EOD are sepsis and pneumonia. Early onset infections may, less frequently, lead to meningitis. Due to better neonatal care the case-fatality ratio of early-onset disease has declined from as high as 50% in the 1970s (Baker & Barrett, 1974) to 4%–6% in recent years (Phares et al., 2008; S J Schrag et al., 2000). According to Phares et al (2008) and Schrag et al (2000) mortality is higher among preterm infants, with case-fatality rates of approximately 20% to 30% among those  $\leq 33$  weeks' gestation, compared with 2%–3% among full-term infants. In recent years, approximately 70% of cases of EOD are among babies born at term ( $\geq 37$  weeks' gestation) (Phares et al., 2008)

In Kenya, there is some literature on GBS as a cause of EOD but the results are not consistent. While two different studies at Kilifi District Hospital, (Berkley et al., 2005; English et al., 2003), showed that 9% and 15%, respectively, of EOD is caused by GBS, another study done in 2011 in Aga Khan University Hospital, Nairobi, gave only 1 per cent of EOD attributable to GBS (Kohli-Kochhar et al, 2011). A recent study from the Kenyan coast found that GBS associated EOD was at 0.76 (0.25-1.77)/1,000 live births (Seale et al., 2016).

Many studies have demonstrated the importance of adequate maternal diagnosis and treatment for the reduction of the vertical transmission of GBS and early-onset neonatal disease (Gibbs et al., 2004). Studies on the use of intravenous intrapartum antibiotic prophylaxis to prevent early-onset GBS disease in the infant have been ongoing since the 1980s. Clinical trials and well-designed observational studies have shown that intrapartum

antibiotic prophylaxis reduces vertical transmission of GBS, measured by infant colonization or by reduction in early onset disease (Centers for Disease Control and Prevention, 2010). Although early trials suggested an efficacy of 100% for intrapartum antibiotic prophylaxis for prevention of early-onset disease among infants born to women with GBS colonization, more recent studies found the effectiveness to be 86%–89% (F. Y. Lin et al., 2001; Phares et al., 2008).

Other strategies to reduce maternal colonization and vertical transmission have been studied (example intramuscular intrapartum antibiotic prophylaxis, antenatal (oral or intramuscular) antibiotics, chlorhexidine vaginal wipes or douches) though, so far, no other method has proven to be as effective as intrapartum antibiotics at preventing early-onset disease (Centers for Disease Control and Prevention, 2010). A 2013 Cochrane review showed that intra partum antibiotic prophylaxis reduced the incidence of EOD but had no effect on incidence of late onset disease regardless of causative organisms (Ohlsson & Shah, 2013).

The latest Centers for Disease Control guidelines recommend antepartum antibiotic prophylaxis and find the “screen and treat” program superior to risk based treatment (Centers for Disease Control and Prevention, 2010). Screen and treat program involves taking a rectovaginal swab of all pregnant women between 35-37 weeks of gestation and culturing for GBS. Patients with positive cultures are then given intrapartum antibiotic prophylaxis. Risk based treatment is when intrapartum antibiotic prophylaxis is administered to patients at risk of early onset disease. The risks are: previous infant with invasive GBS disease, GBS bacteriuria during any trimester of the current pregnancy, delivery at less than 37 weeks of gestation, amniotic membrane rupture greater than or

equal to 18 hours, Intrapartum temperature greater than or equal to 100.4°F (greater than or equal to 38.0°C) (Centers for Disease Control and Prevention, 2010; Committe opinion, 2013)

In most facilities in the United States, rectovaginal swabs are obtained for culture at 35 to 37 weeks gestational age. This screening-based approach was found to be 50% more effective in preventing EOD than a strategy of identifying women for intrapartum antibiotic prophylaxis during labor based on the presence of risk factors for GBS maternal colonization (Stephanie J Schrag et al., 2002). Screen and treat is currently not a standard of care in Kenya.

Penicillin remains the antibiotic of choice for intrapartum prophylaxis with ampicillin as an alternative (Committe opinion, 2013). The efficacy of alternatives to penicillin or ampicillin that have been used to prevent early-onset GBS disease among infants born to penicillin-allergic mothers (including cefazolin, clindamycin, erythromycin, and vancomycin) has not been determined in controlled trials (Centers for Disease Control and Prevention, 2010). Guidelines for treatment of patients with a non-anaphylactic penicillin allergy recommend cefazolin and for those with an anaphylactic allergy vancomycin is the best choice. Erythromycin resistance has been noted to be increasing (up to 32%) so it is no longer recommended (Committe opinion, 2013). This data is mostly from developed countries and has not been tested in our setting.

There are some studies in Africa on antibiotic susceptibility. A study performed in Tanzania in 2009, showed that all isolates were sensitive to vancomycin & ampicillin and resistance to clindamycin, erythromycin & penicillin G was found in 17.6%, 13% and 9.4% of isolates, respectively (Joachim et al., 2009). Another study performed in Egypt in the same

year showed that all isolates were susceptible to penicillin G, ampicillin and vancomycin. Resistance to cefotaxime, erythromycin, clindamycin was found to be 7.89%, 13.15% and 23.68% respectively (Shabayek et al., 2009).

The dosages of penicillin and ampicillin used for intrapartum GBS prophylaxis have been aimed to achieve adequate levels in the fetal circulation and amniotic fluid rapidly and avoid potentially neurotoxic serum levels in the mother or fetus (Centers for Disease Control and Prevention, 2010). The exact duration of antibiotics needed to prevent vertical transmission of GBS is unclear but it has been noted that beta-lactam antibiotics for GBS prophylaxis administered for  $\geq 4$  hours before delivery have been found to be highly effective at preventing vertical transmission of GBS and early-onset GBS disease (de Cueto et al., 1998; F. Y. Lin et al., 2001).



## **CHAPTER THREE**

### **METHODOLOGY**

#### **3.1 Study Setting**

The study was carried out in the antenatal clinic and labor ward of Moi Teaching and Referral Hospital (MTRH) and Riley Mother Baby Hospital. MTRH is situated in Eldoret Municipality, in Uasin Gishu County, Kenya. It is the second largest teaching and referral hospital in Kenya. Being the main referral hospital in Western Kenya, it has a catchment population of 13 to 15 million people which comprises about 40 percent of the Kenyan population. The antenatal clinic is staffed by nurses and clinical officers.

At the time of the study MTRH lab did not have the capacity to culture GBS so Lancet Laboratories were used for culture and sensitivity analysis. Lancet Laboratories is one of the leading pathology laboratories operating in Africa. It is a SANAS (South African National Accreditation System) accredited laboratory adhering to international criteria set out according to ISO standard 15189. Cost of each test in Lancet laboratory was Ksh 2900.

#### **3.2 Study Design**

This was a prospective cohort study.

#### **3.3 Study Population**

The study population were women presenting to routine antenatal clinic at  $\geq 35$  and  $< 41$  weeks gestation by best estimate based on last menstrual period or ultrasound.

#### **3.4 Sample Size Determination**

We used a Tanzanian study as a bench mark to help in calculating sample size as no similar study has been done locally. This study reports that GBS prevalence was 23% (Joachim et al., 2009)

$$\begin{aligned}
 n &= \left[ \frac{Z_{1-\frac{\alpha}{2}}}{\delta} \right]^2 P(1 - P) \\
 &= \left( \frac{1.96}{0.05} \right)^2 \times 0.23 \times 0.77 \\
 &= 272
 \end{aligned}$$

Where P is the population prevalence of GBS.

$\delta$  is the margin of error equal to the 5% used in this case, and

$Z_{1-\frac{\alpha}{2}}$  is the  $(1 - \frac{\alpha}{2}) \times 100\%$  quantile of the standard normal distribution.

Estimating approximately 30% of patients will be lost to follow-up, we adjust our sample size as follows

$$272 + 82 = 354$$

$$= 354$$

This was the minimum sample size that can be done. Any number greater than this could be studied provided the participants are not being subjected to any invasive procedure.

### **3.5 Eligibility Criteria**

#### **3.5.1 Inclusion Criteria**

- $\geq 35$  but  $< 41$  weeks gestation by best estimate based on last menstrual period and/or ultrasound.

#### **3.5.2 Exclusion Criteria**

- $< 35$  or  $\geq 41$  weeks gestation by best estimate based on last menstrual period and/or ultrasound.

### **3.6 Enrollment of Participants**

After the Institutional Research and Ethics Committee (IREC) approved the study, data collection began. Patients attending antenatal clinic at MTRH were approached by medical personnel, informed about the nature and purpose of the study and consent was obtained. Patients at  $\geq 35$  but  $< 41$  week's gestation by best estimate based on last menstrual period and/or ultrasound were selected for the study. If there was a discrepancy between the dates and ultrasound I used the ultrasound to determine gestational age. The relevant clinical data was entered into the data collection form.

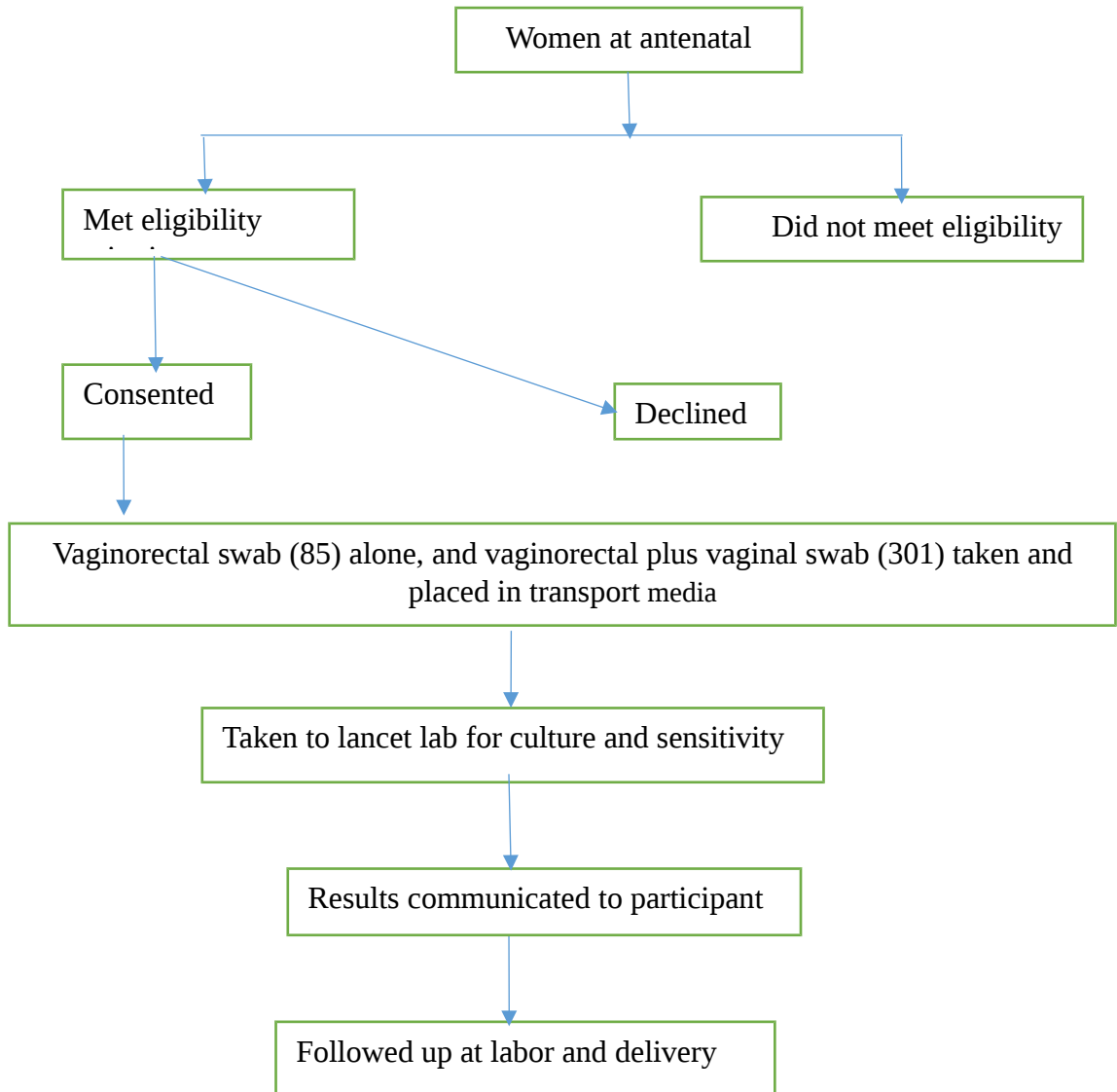
### **3.7 Methods and Materials**

Posters on GBS education and of the procedure were put up in the clinic. Women were explained in great detail on the risks and benefits of the test before asked to consent. A total of 386 (sample size calculated using the Cochrane, 1963, formula) pregnant women were enrolled between May 2015 and December 2015. The target population was women who presented to the antenatal clinic at Moi Teaching and Referral Hospital – the largest public referral hospital in western Kenya - at a gestational age of 35 to 41 weeks estimated from either last menstrual period or ultrasound. The delivery facility was not discussed or specified. As long as the antenatal clinic follow up was in MTRH the women were included in the study. If there was a discrepancy between the dates and ultrasound I used the ultrasound to determine gestational age [In the Kenyan public hospitals, first trimester ultrasounds are usually not available].

Informed consent was obtained, a structured data collection form used to collect demographics and a recto-vaginal swab was then performed by medical personnel. Women on antibiotics were not excluded from the study. Initially, the protocol involved taking a single swab. However, by participant number 85, none of the swabs had been positive for

Group B Streptococcus and, therefore, the methodology was re-examined. A decision was made to change the study protocol to include two swabs per patient, as a means of quality control. One swab was recto-vaginal and the other was a vaginal swab alone. The swabs were transported in Stuart non-nutritive transport media to the lab where they were then transferred to Columbia agar with CNA (colistin and nalidixic acid-antibiotics that inhibit growth of gram negative bacteria and support gram positive bacteria) for 18 to 24 hours. Columbia agar with CNA has 5% sheep's blood already added to it. Plates without colonies were sub cultured to a sheep blood agar plate with 5% sheep's blood overnight. GBS was confirmed using gram stain, hemolysis and catalase tests. GBS positive plates (as noted by color change on the plates) were tested for antibiotic sensitivity. The plates were read by 2 microbiologists and we also sent 2 plates to a sister lab in South Africa for inter lab quality control.

The patients were each given an antibiotic administration form (see appendix 7) to take with them. The culture results were conveyed to patients via a phone call and they were advised to tick the box for culture positive on the antibiotic administration form and instructed to present the form at the facility of delivery when they returned to deliver in order to be given intrapartum IV penicillin G. This was to be administered at an initial dose of 5 million units, followed by 2.5-3 million units every 4 hours until delivery, as per international recommendations (Committee opinion, 2013). All patients were followed up at delivery.

**Figure 1: Study procedure**

### **3.8 Sampling Technique**

Consecutive sampling was used to sample participants in clinic. Patients were counseled on the tests and its risk and benefits and an informed written consent taken by myself or research assistants.

### **3.9 Data Management**

#### **3.9.1 Data Collection**

A structured data collection form/structured questionnaire was used to collect demographic, obstetric and laboratory data extracted from the patient's file. The data to be collected included maternal age, gestational age at screening, history of antibiotic use in the past two weeks, level of education, occupation, previous obstetric history (parity, history of prior baby with known or suspected EOD), history of current pregnancy (previous infection in this pregnancy), marital status, ethnicity. All information collected was de-identified. Data collection took place until sample size was achieved

#### **3.9.2 Data quality assurance**

Study specific training was offered to the medical personnel on matters relating to data collection. Swabbing was carried out as per the American College of Obstetrics and Gynecology (ACOG) guidelines. Data was saved in lock and key data cabinets and data base was pass-word protected to secure access. Data was monitored on a weekly basis for its completeness and accuracy.

#### **3.9.3 Data Analysis and Presentation**

Data analysis was done using the software for statistical computing known as R (R Core Team, 2015). Categorical variables were summarized as frequencies and the corresponding percentages while continuous variables were summarized as median and the corresponding

inter quartile range (IQR). Gaussian assumptions were assessed using Shapiro Wilk test for normality. Age was categorized at ten year intervals. Association between the presence of GBS and categorical variables was assessed using Fisher's exact test. Association between the presence of GBS and age, and gestational duration was assessed two sample Wilcoxon rank-sum test and two sample t-test respectively. Results were presented using tables and graphs.

### **3.10 Ethical Considerations**

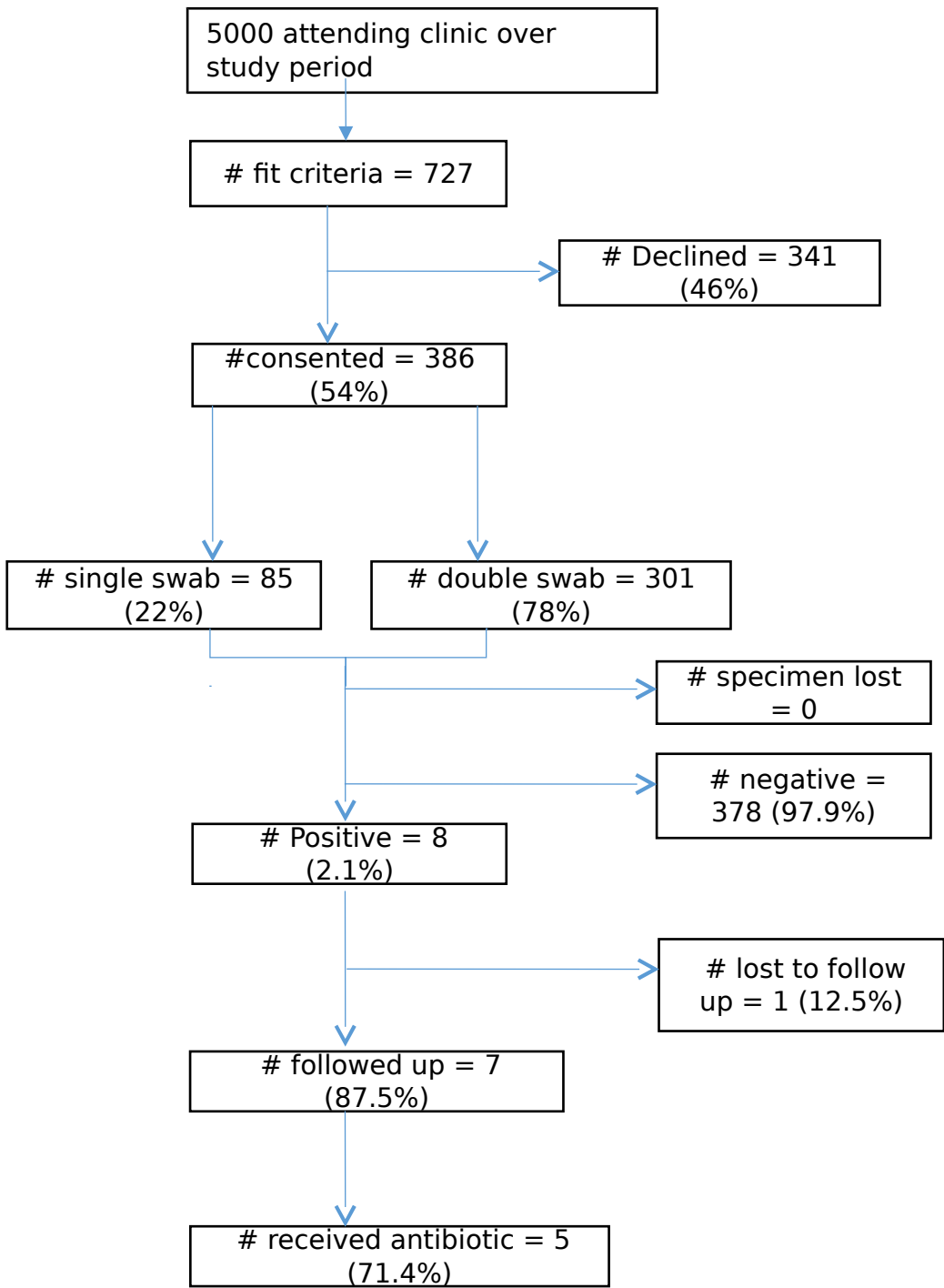
1. The IREC approval of the study was secured before the study began.
2. Permission to conduct the research was secured from the hospital management.
3. Informed consent was obtained from all participants before their enrollment into the study.
4. Education and counseling services was provided freely to all participants, including those who withdrew from the study. Patients were counselled on the test to be performed and on the possible outcomes. This information was also provided on the consent form. Counselling was done by the primary investigator or research assistant.
5. Confidentiality was maintained strictly by obtaining consent in a private room, storing the questionnaires and lab results in locked data cabinets, databases were protected with passwords known only to the research assistant and principal investigator.

## CHAPTER FOUR

### RESULTS

A total of 386 participants were sampled and their data subsequently analyzed.

Figure 2: Flow chart



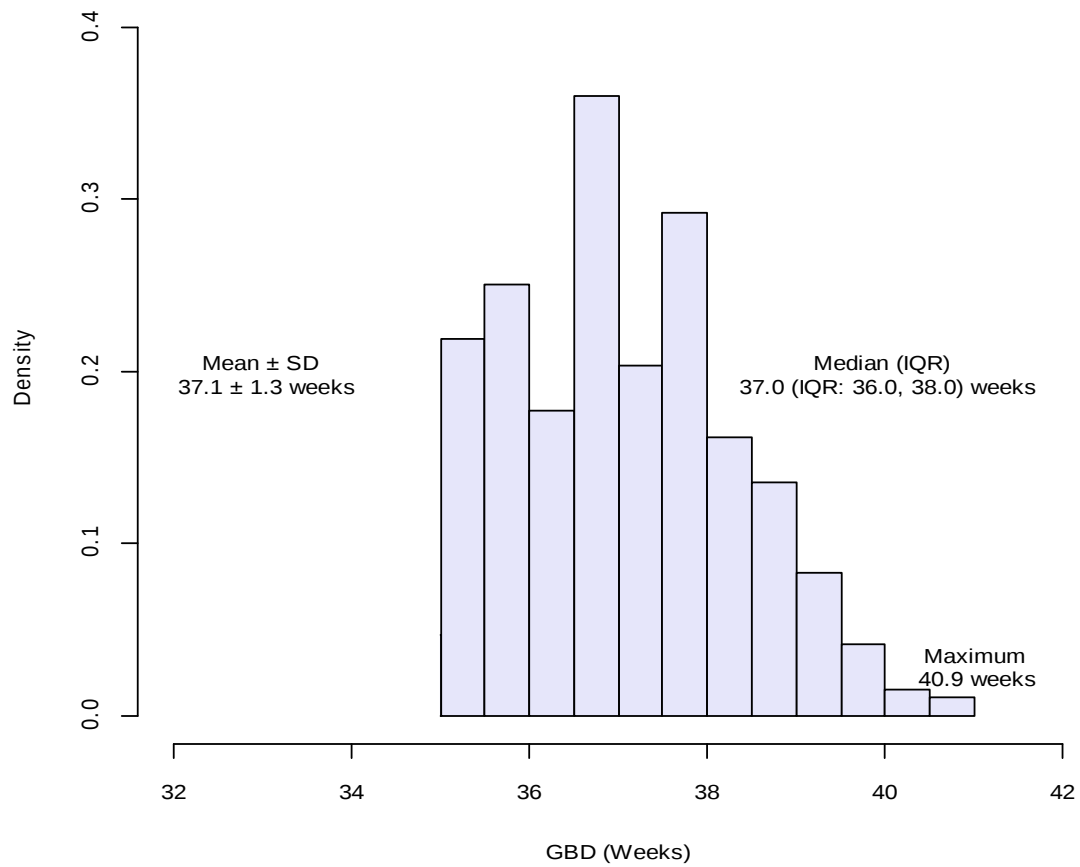


**Table 1: Demographic characteristics.** Sample size 386

<b>Variable</b>	<b>Median (IQR) or n (%)</b>
Age (years), Median (IQR)	26.7 (23.3, 30.7)
Range (Min., Max.)	17.1, 43.5
10 – 20	14 (3.6%)
20 – 30	261 (67.6%)
30 – 40	107 (27.7%)
>40	4 (1.0%)
Occupation	
None/student/other	77 (19.9%)
Employed /Self Employed	309 (80.1%)
Education level	
None	2 (0.5%)
Primary	78 (20.2%)
Secondary	148 (38.3%)
College/University	158 (40.9%)
Marital status	
Single	51 (13.2%)
Married	335 (86.8%)
Gravidity	
1-2	262 (67.9%)
3-5	115 (29.8%)
6+	9 (2.3%)

The median age of the participants was 26.7 (IQR: 23.3, 30.7) years. Two thirds of the participants (67.6%) were aged between 20-30 years. There were 14 (3.6%) and 4 (1.0%) who were aged 10-20 and 40-50 years respectively.

Up to 80% of the participants were either employed or self-employed. Only two participants had no formal education. Majority (40.9%) had college/University education, and one fifth of the participants had primary level of education. The rest had secondary education. Over 80% of the participants were married. Slightly more than a tenth were single. Two thirds of the participants (67.9%) of gravida 1-2, and 115 (29.8%) were in gravida 3-5.

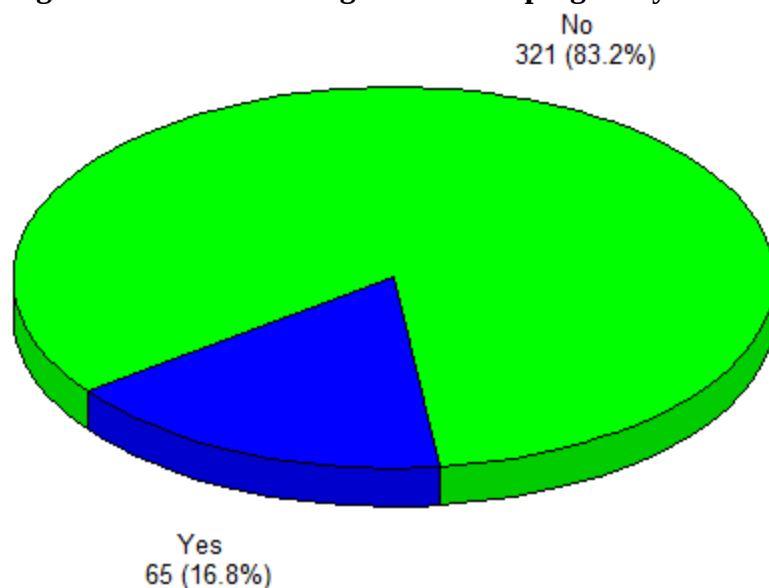
**Figure 3: Gestation duration**

The mean  $\pm$  SD was 37.1  $\pm$  1.3 weeks with no much difference from the median value. The minimum and the maximum were 35.0 and 40.9 weeks respectively.

**Table 2: Neonatal mortality and morbidity in previous pregnancy**

<b>Variable</b>	<b>n (%)</b>
Neonatal mortality (in previous pregnancy) during the first week of life. n is 239.	7 (2.9%)
Neonatal sepsis/infection (in previous pregnancy) during the first week of life. n is 239.	No 230 (96.2%)
	Yes 9 (3.8%)
Sepsis/infection. n is 9.	Confirmed 7 (77.8%)
	Suspected 2 (22.2%)

Multigravidas ( $\geq$  gravida 2) were 239 in total. Neonatal mortality rate in previous pregnancy was 2.9%, and sepsis/infection was 3.8% (by patients report). Of the neonates who had sepsis or infection, 77.8% were confirmed based on lab works, the rest of the cases of neonatal sepsis in previous pregnancy were suspected.

**Figure4: Infection during the current pregnancy**

The incidence of infection during the current pregnancy was 16.8%. Fifty four (83.1%; n as 65) of the participants with infection during the current pregnancy had urinary tract infection as per their urinalysis, and 2 (3.1%) had an infection with candidiasis. Each of the remaining nine participants had an infection with one of the following: amoebiasis, brucellosis, malaria, dysentery, upper respiratory tract infection, and typhoid.

These infections were diagnosed during the participants' prior antenatal clinic visits by clinical personnel. Antibiotics were administered to all the participants who developed infection during pregnancy.

**Table 3: Medical history. n is 386.**

Variable	n (%)
Medical conditions	
High blood pressure	2 (0.5%)
Asthma	4 (1.0%)
Other diagnoses	8 (2.1%)
Specific other diagnosis	47 (12.3%)
Does not know if allergic to penicillin	2 (0.5%)
Reactional allergy to penicillin	0 (0.0%)
Allergy to vancomycin	0 (0.0%)
Reactional allergy to vancomycin	0 (0.0%)
Allergy to ampicillin	0 (0.0%)
Reactional allergy ampicillin	0 (0.0%)
Allergy to other	12 (3.1%)

There were 2 (0.5%), and 4 (1.0%) who had history of high blood pressure and asthma respectively. Eight (2.1%) had history of other diagnoses (vulvitis, bacterial vaginosis, candidiasis).

Two participants did not know whether they were allergic to penicillin, and 12 (3.1%) were allergic to other drugs. Of those who allergy to other drugs, two thirds, 8 (66.7%), were allergic to Sulphur containing drugs, one was allergic to amoxicillin, one to aspirin.

**Table 4: Delivery related characteristics**

Variable	Sample size		n (%) or Mean±SD
Hospital delivery	386	Yes	385 (99.7%)
		No	0 (0.0%)
		Lost to follow up	1 (0.3%)
Hospital name	385	MTRH	374 (97.1%)
		Other	11 (2.9%)
Delivery outcome	385	Stillbirth	2 (0.5%)
		Live birth	383 (99.5%)

Admitted to NBU	383	1 (0.3%)
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Except one who was lost to follow up, all the participants delivered at the hospital. Of the 385 who delivered at the hospital, 374 (97.1%) delivered at MTRH. The rest delivered in the hospitals as shown in Table 4.

The participant who was lost to follow up was positive for culture. Therefore, of the remaining seven participants who were positive for culture, 5 (71.4%) received antibiotics. There were 2 (0.5%) stillbirths. Of the remaining 383, 1 (0.3%) neonate who was admitted to new born unit (NBU) at the age of one day. The two stillbirths were confirmed to have been born by mothers whose culture test results were negative. The child who was admitted to the newborn unit had a mother with negative culture test results.

**Table 5: GBS culture results**

<b>Variable</b>	<b>n (%)</b>
Culture Negative	378 (97.9%)
Positive	8 (2.1%)
Sensitive to penicillin	8 (100%)
Sensitive to ampicillin	8 (100%)
Sensitive to vancomycin	8 (100%)
Sensitive to erythromycin	8 (100%)
Sensitive to clindamycin	8 (100%)

Laboratory findings show that 8 (2.1%, 95% CI: 0.9% to 4.0%) cultured group B streptococcus, and all were sensitive to penicillin, ampicillin, vancomycin, erythromycin, and clindamycin.

**Table 6: Intrapartum antibiotics**

Variable	Sample size	N (%)
Lost to follow up from positive cultures	8	1(12.5%)
Intrapartum antibiotic prophylaxis	7	5 (71.4%)

Out of 386 participants only one participant was lost to follow up (unable to reach via phone to deliver results and get delivery details). This participant was part of the 8 that had a positive culture for GBS. Out of the 7 participants who had a positive culture for GBS and were followed up, 5 received timely intrapartum antibiotics (at least one dose of antibiotic 4 hours prior to delivery).

**Table 7: Association between positive culture and demographic, and clinical characteristics**

Variable		Negative (n=378)	Positive (n=8)	P
Age (years)		26.7 (23.3 – 30.7)	25.1 (23.8 to 29.4)	0.733 <sup>w</sup>
Age	<=30	269 (97.8%)	6 (2.2%)	1.000 <sup>f</sup>
	>30	109 (98.2%)	2 (1.8%)	
Occupation	None/student/other	87 (97.8%)	2 (2.2%)	1.000 <sup>f</sup>
	Employed /Self Employed	291 (98.0%)	6 (2.0%)	
Education	None	2 (100%)	0 (0.0%)	0.326 <sup>f</sup>
	Primary	78 (100%)	0 (0.0%)	
	Secondary	143 (96.6%)	5 (3.4%)	
	College/University	155 (98.1%)	3 (1.9%)	
Marital status	Single	49 (96.1%)	2 (3.9%)	0.286 <sup>f</sup>
	Married	329 (98.2%)	6 (1.8%)	
Gravidity	1-2	256 (97.7%)	6 (2.3%)	1.000 <sup>f</sup>
	3-5	113 (98.3%)	2 (1.7%)	

	6+	9 (100%)	0 (0.0%)	
GBD (Months)		37.0 ± 1.3	37.4 ± 1.4	0.461 <sup>t</sup>
Pregnancy infection	No	313 (97.5%)	8 (2.5%)	0.361 <sup>f</sup>
	Yes	65 (100%)	0 (0.0%)	
Neonatal mortality	No	372 (98.2%)	7 (1.8%)	0.137 <sup>f</sup>
	Yes	6 (85.7%)	1 (14.3%)	
Neonatal infection	No/unknown	370 (98.1%)	7 (1.9%)	0.173 <sup>f</sup>
	Yes	8 (88.9%)	1 (11.1%)	
<sup>†</sup> Delivery outcome	Still Birth	2 (100%)	0 (0.0%)	1.000 <sup>f</sup>
	Alive	376 (98.2%)	7 (1.8%)	

Employed / Self Employed: Customer service, Healthcare provider, Teacher, farmer, Housewife, Civil servant, Business person;

<sup>f</sup> – Fisher’s exact test;

<sup>t</sup> – two sample t-test;

<sup>w</sup> – two sample Wilcoxon rank-sum (aka Mann-Whitney U) test;

<sup>†</sup> - n=385

In this study, all of the women who were positive had either secondary or college level education. Six were married and two were single. One positive case had a previous neonatal death and another reported previous child sepsis. No patient who reported urinary tract infection in the current pregnancy had GBS. Neither of the women who experienced a stillbirth were GBS positive.



## **CHAPTER FIVE**

### **DISCUSSION**

This study, at the Moi Teaching and Referral Hospital in Eldoret, was conducted between May 2015 and December 2015. Over 5000 clients were approached identifying 727 that fit inclusion criteria of which 386 (53%) consented for the study. The low acceptance rate may imply that, currently, the idea of a rectovaginal swab is not acceptable. However, reasons for refusal were not explored. The refusal for consent may have created a bias in that majority of the participants who consented were young and educated.

The colonization rate of GBS was found to be only 2.07% in women at 35-41 weeks gestational age. Compared to data in other developing countries, this colonization rate is quite low. Local studies show varying rates. A study done by Were et al in 2002 in MTRH showed 34.1% in anorectal colonization and 20.2% vaginal colonization. This difference

may be attributable to increase in antepartum antibiotic access and use over the years; improvement in perineal hygiene over the years. A similar study in Nairobi demonstrated an anogenital colonization rate of 25% (Mohamed, 2009) and a more recent study done in 2015 in Kisii gave a colonization rate of 3%(Kaminja, 2015). The difference in colonization rates could be due to difference in urban versus rural regions. Examples of other regions with low prevalence include 2.5% in Lome, Togo (Mounerou et al., 2015); 3.8% in Denmark (Petersen et al., 2014); 2.3% in South India (Sharmila et al., 2011); 4.76% Abha, KSA (Al-sunaidi & Al-shahrani, 2011); 6% in Lima, Peru (Collins et al, 1998).

It has been noted that the rate varies in great proportions according to the study population and their demographics, hygiene practices of the study population, testing sites, the testing period, transport technique, type of culture and bacterial isolation technique used. Despite these differences, regional variations also exist as seen by the wide spectrum of rates all over the globe (Mounerou et al., 2015).

An explanation for the low colonization rate could be the demographics of our population. The mean age in this study population was 26.7 years whereas a study done in Saudi Arabia showed a higher colonization rate in women >40 years of age(Khan et al, 2015). Two thirds of the women in this cohort, 67.9%, were gravida 1-2 and a study in Tanzania showed increase in colonization in women with >5 deliveries although the difference was not statistically significant (Joachim et al., 2009). Another point to note was that the mean gestational age at swabbing was 37.1 weeks in this study whereas some studies show increase in colonization rates at gestation above 40 weeks(Joachim et al., 2009; Khan et al., 2015).Most of the women in this study, 79.2%, had at least a secondary education and only

0.5% had no formal education whereas the study Tanzania showed GBS was more prevalent in women with no formal education compared to women with primary, secondary or higher education (although it was not statistically significant) (Joachim et al., 2009).

There was no statistically significant association between age of the participants and culture positive result,  $p=0.733$ . It was, however, noted that 75% of the participants who cultured positive were less than 30 years of age. This was in keeping with what Joachim et al., 2009, found where 66.6% of their GBS positive participants were under 30 years of age.

Even though 75% of participants who cultured positive were either employed or self-employed, they had a lower chance of turning culture positive (2.0%) compared to those who were either not employed, a student or had other occupations (2.2). The difference, however, was not statistically significant.

Hundred percent of participants who were colonized with GBS had higher level of education (at least secondary education and more). There was, however, no statistically significant association between education and culture test results,  $p=0.326$ .

Even though 75% of those who turned positive for GBS were married, the difference in colonization between single and married women was not statistically significant ( $p=0.256$ ).

Gravidity was not associated with the presence of GBS,  $p=1.000$ , similarly, the gestational age was not associated with presence of GBS,  $p=0.461$ .

Infection during current pregnancy was not associated with the presence of GBS,  $p=0.361$ .

The results however point that those who never had infection tend to have GBS.

There was no statistically significant difference in demographic characteristics in women who tested positive for GBS versus those who tested negative. This was in keeping with

the studies done in Kenya by Mohamed and Were where they also found no significant association between sociodemographic and obstetric characteristics and GBS colonization (Mohamed, 2009; Were et al., 2006).

The GBS that was grown was susceptible to penicillin, vancomycin, ampicillin, clindamycin and erythromycin. This result was more encouraging than what was found in Tanzania where they found that all isolates were sensitive to vancomycin and ampicillin but resistance to clindamycin, erythromycin and penicillin G was found to be 17.6%, 13% and 9.4%, respectively (Joachim et al., 2009). The antimicrobial susceptibility finding in this study is particularly relevant in a Kenyan public hospital, where antibiotic shortages occur on a regular basis.

### **5.1 Study Limitations**

MTRH lab was unable to obtain enrichment media (LIM broth or TransVag broth) as recommended by the guidelines from CDC for GBS culture (Centers for Disease Control and Prevention, 2010). However, studies done comparing methods have not shown a meaningful difference when LIM broth was added to the culture protocol (El Aila et al., 2010).

Lack of early trimester obstetric ultrasound with most women was out of my control and meant that I could not establish an accurate gestational age.

The small number of positive samples (8) for susceptibility to antibiotics testing will not be reflective of the general population.

The refusal for consent may have created a bias in that majority of the participants who consented were young and educated.



## CHAPTER SIX

### CONCLUSION AND RECOMMENDATIONS

#### 6.1 Conclusion

The GBS colonization rate among women attending antenatal clinic at MTRH was low (2.1%).

All of the GBS isolates were susceptible to penicillin, ampicillin, vancomycin, erythromycin, and clindamycin.

#### 6.2 Recommendations

Based on the low prevalence, I do not recommend routine antenatal screening of GBS.

As this was a study in MTRH alone the results may not be reflective of the whole population, thus I recommend a multicenter study.

## REFERENCES

- Al-sunaidi, M., & Al-shahrani, M. (2011). Prevalence of Group B Streptococcus Colonization in Mothers and Babies at Abha General Hospital , Kingdom of Saudi Arabia, 79(2), 163–165.
- Al-Sweih, N., Jamal, M., Kurdia, M., Abduljabar, R., & Rotimi, V. (2005). Antibiotic susceptibility profile of group B streptococcus (*Streptococcus agalactiae*) at the Maternity Hospital, Kuwait. *Medical Principles and Practice: International Journal of the Kuwait University, Health Science Centre*, 14(4), 260–263.  
<http://doi.org/10.1159/000085746>
- Baker, C. J. (1978). Early onset group B streptococcal disease. *The Journal of Pediatrics*, 93(1), 124–125.
- Baker, C. J., & Barrett, F. F. (1974). Group B streptococcal infections in infants. The importance of the various serotypes. *Journal of American Medical Association*, 230(8), 1158–1160.
- Berkley, J. A., Lowe, B. S., Mwangi, I., Williams, T., Bauni, E., Mwarumba, S., ... Scott, J. A. G. (2005). Bacteremia among children admitted to a rural hospital in Kenya. *The New England Journal of Medicine*, 352(1), 39–47.  
<http://doi.org/10.1056/NEJMoa040275>
- Boyer, K. M., & Gotoff, S. P. (1985). Strategies for chemoprophylaxis of GBS early-onset infections. *Antibiotics and Chemotherapy*, 35, 267–280.
- Centers for Disease Control and Prevention. (2010). Prevention of Perinatal Group B Streptococcal Disease. *Mmwr*, 59(No. RR-10), 1–32.  
<http://doi.org/10.1097/01.EDE.0000032431.83648.8D>

Cochran, W. G. (1963). *Sampling Techniques* (2nd ed.). New York, NY, USA: John Wiley and Sons Inc.

Collins, T. S., Calderon, M., Gilman, R. H., Vivar, A., & Charache, P. (1998). GROUP B STREPTOCOCCAL COLONIZATION IN A DEVELOPING COUNTRY: ITS ASSOCIATION WITH SEXUALLY TRANSMITTED DISEASE AND SOCIOECONOMIC FACTORS, *59*(4), 633–636.

Committee opinion. (2013). Prevention of Early-Onset Group B Streptococcal Disease in Newborns. Committee Opinion, Number 485, April 2011 (Replaces No. 279, December 2002)(Reaffirmed 2013) - co485.pdf. *ACOG American College of Obstetricians and Gynecologists. Obstet Gynecol 2011;117:1019–27, 102(2), 1019–27.* <http://doi.org/10.1097/AOG.0b013e318219229b>

de Cueto, M., Sanchez, M. J., Sampedro, A., Miranda, J. A., Herruzo, A. J., & Rosa-Fraile, M. (1998). Timing of intrapartum ampicillin and prevention of vertical transmission of group B streptococcus. *Obstetrics and Gynecology, 91*(1), 112–114.

Desa, D. J., & Trevenen, C. L. (1984). Intrauterine infections with group B beta-haemolytic streptococci. *British Journal of Obstetrics and Gynaecology, 91*(3), 237–239.

Edwards, M. S., & Baker, C. J. (2005). Group B Streptococcal Infections in Elderly Adults, *41*, 839–847.

Eickhoff, Klein, Daly, Ingall, F. (1964). NEONATAL SEPSIS AND OTHER INFECTIONS DUE TO GROUP B BETA-HEMOLYTIC STREPTOCOCCI. *The New England Journal of Medicine, 271*, 1221–1228.  
<http://doi.org/10.1056/NEJM196412102712401>

El Aila, N. a, Tency, I., Claeys, G., Saerens, B., Cools, P., Verstraelen, H., ... Vaneechoutte,



- M. (2010). Comparison of different sampling techniques and of different culture methods for detection of group B streptococcus carriage in pregnant women. *BMC Infectious Diseases*, *10*, 285. <http://doi.org/10.1186/1471-2334-10-285>
- English, M., Ngama, M., Musumba, C., Wamola, B., Bwika, J., Mohammed, S., ...  
Newton, C. (2003). Causes and outcome of young infant admissions to a Kenyan district hospital, 438–443.
- Fry, R. M. (1938). PREVENTION AND CONTROL OF PUERPERAL SEPSIS: BACTERIOLOGICAL ASPECTS. *British Medical Journal*, *2*(4049), 340–342.
- Gibbs, R. S., Schrag, S., & Schuchat, A. (2004). Perinatal Infections Due to Group B Streptococci, *104*(5), 1062–1076.  
<http://doi.org/10.1097/01.AOG.0000144128.03913.c2>
- Gil, E. G., Rodriguez, M. C., Bartolome, R., Berjano, B., Cabero, L., & Andreu, A. (1999). Evaluation of the Granada agar plate for detection of vaginal and rectal group B streptococci in pregnant women. *Journal of Clinical Microbiology*, *37*(8), 2648–2651.
- Hernandez Trejo, M., & Soriano Becerril, D. (2006). [High prevalence of group B Streptococcus colonization in Mexican pregnant women]. *Ginecologia y obstetricia de Mexico*, *74*(3), 139–143.
- Joachim, A., Matee, M. I., Massawe, F. A., & Lyamuya, E. F. (2009). Maternal and neonatal colonisation of group B streptococcus at Muhimbili National Hospital in Dar es Salaam, Tanzania: prevalence, risk factors and antimicrobial resistance. *BMC Public Health*, *9*, 437. <http://doi.org/10.1186/1471-2458-9-437>
- Jordan, H. T., Farley, M. M., Craig, A., Mohle-Boetani, J., Harrison, L. H., Petit, S., ...  
Schrag, S. J. (2008). Revisiting the need for vaccine prevention of late-onset neonatal

group B streptococcal disease: a multistate, population-based analysis. *The Pediatric Infectious Disease Journal*, 27(12), 1057–1064.

<http://doi.org/10.1097/INF.0b013e318180b3b9>

Kadanali, A., Altoparlak, U., & Kadanali, S. (2005). Maternal carriage and neonatal colonisation of group B streptococcus in eastern Turkey: prevalence, risk factors and antimicrobial resistance. *International Journal of Clinical Practice*, 59(4), 437–440.

<http://doi.org/10.1111/j.1368-5031.2005.00395.x>

Kaminja. (2015). *Maternal group b streptococcus colonization and the associated early maternal and neonatal outcomes at the Kisii Teaching and Referral Hospital.*

University of Nairobi. <http://doi.org/2315>

Katz, V., & Bowes, W. A. J. (1988). Perinatal group B streptococcal infections across intact amniotic membranes. *The Journal of Reproductive Medicine*, 33(5), 445–449.

Khan, M. A., Faiz, A., & Ashshi, A. M. (2015). Maternal colonization of group B streptococcus: Prevalence, associated factors and antimicrobial resistance. *Annals of Saudi Medicine*, 35(6), 423–427. <http://doi.org/10.5144/0256-4947.2015.423>

Koenig, J. M., & Keenan, W. J. (2009). Group B streptococcus and early-onset sepsis in the era of maternal prophylaxis. *Pediatric Clinics of North America*, 56(3), 689–708,

Table of Contents. <http://doi.org/10.1016/j.pcl.2009.04.003>

Kohli-Kochhar, R., Omuse, G., & Revathi, G. (2011). A ten-year review of neonatal bloodstream infections in a tertiary private hospital in Kenya. *Journal of Infection in Developing Countries*, 5(11), 799–803. <http://doi.org/10.3855/jidc.1674>

Kulkarni, A. A., Pawar, S. G., Dharmadhikari, C. A., & Kulkarni, R. D. (2001).

Colonization of pregnant women and their newborn infants with group-B streptococci.

*Indian Journal of Medical Microbiology*, 19(2), 1–4.

Lin, F. Y., Brenner, R. A., Johnson, Y. R., Azimi, P. H., Philips, J. B. 3rd, Regan, J. A., ...

Clemens, J. D. (2001). The effectiveness of risk-based intrapartum chemoprophylaxis for the prevention of early-onset neonatal group B streptococcal disease. *American Journal of Obstetrics and Gynecology*, 184(6), 1204–1210.

<http://doi.org/10.1067/mob.2001.113875>

Lin, L., Song, J., Kimber, N., Shott, S., Tangora, J., Aroutcheva, A., ... Faro, S. (1999). The

role of bacterial vaginosis in infection after major gynecologic surgery. *Infectious Diseases in Obstetrics and Gynecology*, 7(3), 169–174.

<http://doi.org/10.1155/S1064744999000277>

Meyn, L. A., & Hillier, S. L. (1997). Ampicillin susceptibilities of vaginal and placental

isolates of group B streptococcus and *Escherichia coli* obtained between 1992 and 1994. *Antimicrobial Agents and Chemotherapy*, 41(5), 1173–1174.

Mohamed. (2009). *Prevalence of group B Streptococcus (GBS) Colonization in antenatal*

*Women at Kenyatta national Hospital (KNH)*. University of Nairobi.

<http://doi.org/2315>

Mohammed, M., Asrat, D., & Woldeamanuel, Y. (2012). Original article Prevalence of

group B Streptococcus colonization among pregnant women attending antenatal clinic of Hawassa. *Ethiopian Journal of Health Development*.

Mounerou, S., Y. D. A., Hondégla, A., Biova, A., Koumavi, E., & Sika, D. (2015). Group B

Streptococcal Carriage Rate in Vagina of Pregnant Women in Third Trimester in Lomé, Togo, 3(1), 7–10. <http://doi.org/10.12691/jpm-3-1-2>

Ohlsson, A., & Shah, V. S. (2013). Intrapartum antibiotics for known maternal Group B

streptococcal colonization. *The Cochrane Database of Systematic Reviews*, (1), CD007467. <http://doi.org/10.1002/14651858.CD007467.pub3>

Pearlman, M. D., Pierson, C. L., & Faix, R. G. (1998). Frequent resistance of clinical group B streptococci isolates to clindamycin and erythromycin. *Obstetrics and Gynecology*, 92(2), 258–261.

Petersen, K. B., Johansen, H. K., Rosthøj, S., Krebs, L., Pinborg, A., & Hedegaard, M. (2014). Increasing prevalence of group B streptococcal infection among pregnant women, (September), 5–9.

Phares, C. R., Lynfield, R., Farley, M. M., Mohle-Boetani, J., Harrison, L. H., Petit, S., ... Schrag, S. J. (2008). Epidemiology of invasive group B streptococcal disease in the United States, 1999-2005. *JAMA*, 299(17), 2056–2065. <http://doi.org/10.1001/jama.299.17.2056>

Platt, J. S., & O'Brien, W. F. (2003). Group B streptococcus: prevention of early-onset neonatal sepsis. *Obstetrical & Gynecological Survey*, 58(3), 191–196. <http://doi.org/10.1097/01.OGX.0000050764.67701.8C>

Schrag, S. J., Zell, E. R., Lynfield, R., Roome, A., Arnold, K. E., Craig, A. S., ... Schuchat, A. (2002). A population-based comparison of strategies to prevent early-onset group B streptococcal disease in neonates. *The New England Journal of Medicine*, 347(4), 233–239. <http://doi.org/10.1056/NEJMoa020205>



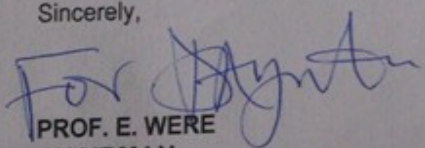
Schrag, S. J., Zywicki, S., Farley, M. M., Reingold, A. L., Harrison, L. H., Lefkowitz, L. B., ... Schuchat, A. (2000). Group B streptococcal disease in the era of intrapartum antibiotic prophylaxis. *The New England Journal of Medicine*, 342(1), 15–20. <http://doi.org/10.1056/NEJM200001063420103>

- Seale, A. C., Koech, A. C., Sheppard, A. E., Barsosio, H. C., Langat, J., Anyango, E., ... Berkley, J. A. (2016). Maternal colonization with *Streptococcus agalactiae* and associated stillbirth and neonatal disease in coastal Kenya. *Nature Microbiology*, *1*(7), 16067. <http://doi.org/10.1038/nmicrobiol.2016.67>
- Shabayek, S. A. A. E.-K., Abdalla, S. M., & Abouzeid, A. M. H. (2009). Vaginal carriage and antibiotic susceptibility profile of group B *Streptococcus* during late pregnancy in Ismailia, Egypt. *Journal of Infection and Public Health*, *2*(2), 86–90. <http://doi.org/10.1016/j.jiph.2009.03.004>
- Sharmila, V., Joseph, N. M., Arun Babu, T., Chaturvedula, L., & Sistla, S. (2011). Genital tract group B streptococcal colonization in pregnant women: a South Indian perspective. *The Journal of Infection in Developing Countries*, *5*(8), 592–5.
- Stevens, D. L., & Kaplan, E. L. (2000). *Streptococcal infections: clinical aspects, microbiology, and molecular pathogenesis*. New York: Oxford University Press.
- Taminato, M., Fram, D., Torloni, M. R., Belasco, A. G. S., Saconato, H., & Barbosa, D. A. (2011). Screening for group B *Streptococcus* in pregnant women: a systematic review and meta-analysis. *Revista Latino-Americana de Enfermagem*, *19*(6), 1470–1478.
- Towers, C. V., & Briggs, G. G. (2002). Antepartum use of antibiotics and early-onset neonatal sepsis: the next 4 years. *American Journal of Obstetrics and Gynecology*, *187*(2), 495–500.
- Were, E., Esamai, F., Liechty, E., Lemons, J., & Fuller, D. (2006). The vaginal and anorectal colonization by group B *Streptococcus* among term pregnant women in Moi Teaching and Referral Hospital. In *Annual Kenya Obstetrical and Gynaecological Society Meeting, Eldoret, Kenya*. (pp. 52–53). Eldoret.

Woldu, Z. L., Teklehaimanot, T. G., Waji, S. T., & Gebremariam, M. Y. (2014). The prevalence of Group B Streptococcus recto-vaginal colonization and antimicrobial susceptibility pattern in pregnant mothers at two hospitals of Addis Ababa , Ethiopia, *11*(1), 1–4. <http://doi.org/10.1186/1742-4755-11-80>

## APPENDICES

## Appendix 1: IREC Approval

 <b>MOI TEACHING AND REFERRAL HOSPITAL</b> P.O. BOX 3 ELDORET Tel: 334711/2/3 Reference: IREC/2014/261 <b>Approval Number: 0001351</b>	 <b>MOI UNIVERSITY</b> SCHOOL OF MEDICINE P.O. BOX 4606 ELDORET 12 <sup>th</sup> February, 2015								
Dr. Saudah Farooqui, Moi University, School of Medicine, P.O. Box 4606-30100, <u>ELDORET-KENYA.</u>	<div style="border: 1px solid blue; padding: 5px; width: fit-content; margin: 0 auto;"> <p style="margin: 0; font-size: small;">INSTITUTIONAL RESEARCH &amp; ETHICS COMMITTEE</p> <p style="margin: 0; font-size: x-large; color: red;">12 FEB 2015</p> <p style="margin: 0; font-weight: bold; color: blue;">APPROVED</p> <p style="margin: 0; font-size: x-small;">P. O. Box 4606-30100 ELDORET</p> </div>								
Dear Dr. Saudah,									
<b><u>RE: FORMAL APPROVAL</u></b>									
The Institutional Research and Ethics Committee has reviewed your research proposal titled:-									
<p style="margin: 0;"><b><i>"Group B Streptococcus Colonization among Antenatal Women: Prevalence and Antibiotic Susceptibility at Moi Teaching and Referral Hospital"</i></b></p>									
Your proposal has been granted a Formal Approval Number: <b>FAN: IREC 1351</b> on 12 <sup>th</sup> February, 2015. You are therefore permitted to begin your investigations.									
Note that this approval is for 1 year; it will thus expire on 11 <sup>th</sup> February, 2016. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date.									
You are required to submit progress report(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change (s) or amendment (s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.									
Sincerely,									
 <b>PROF. E. WERE</b> <b>CHAIRMAN</b> <b>INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE</b>									
<table border="0" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 20%;">cc</td> <td style="width: 20%;">Director - MTRH</td> <td style="width: 20%;">Dean - SOP</td> <td style="width: 20%;">Dean - SOM</td> </tr> <tr> <td></td> <td>Principal - CHS</td> <td>Dean - SON</td> <td>Dean - SOD</td> </tr> </table>		cc	Director - MTRH	Dean - SOP	Dean - SOM		Principal - CHS	Dean - SON	Dean - SOD
cc	Director - MTRH	Dean - SOP	Dean - SOM						
	Principal - CHS	Dean - SON	Dean - SOD						

**Appendix 2: Approval To Conduct Research At Mtrh****MOI TEACHING AND REFERRAL HOSPITAL**

Telephone: 2033471/2/3/4  
Fax: 61749  
Email: director@mtrh.or.ke  
**Ref:** ELD/MTRH/R.6/VOL.II/2008

P. O. Box 3  
ELDORET

12<sup>th</sup> February, 2015

Dr. Saudah Farooqui,  
Moi University,  
School of Medicine,  
P.O. Box 4606-30100,  
ELDORET-KENYA.

**RE: APPROVAL TO CONDUCT RESEARCH AT MTRH**

Upon obtaining approval from the Institutional Research and Ethics Committee (IREC) to conduct your research proposal titled:-

*"Group B Streptococcus Colonization among Antenatal Women: Prevalence and Antibiotic Susceptibility at Moi Teaching and Referral Hospital".*

You are hereby permitted to commence your investigation at Moi Teaching and Referral Hospital.

**DR. JOHN KIBOSIA**  
**DIRECTOR**  
**MOI TEACHING AND REFERRAL HOSPITAL**

CC - Deputy Director (CS)  
- Chief Nurse  
- HOD, HRISM



**Appendix 3: Consent Form**

My name is Saudah Farooqui and I am currently pursuing my Masters Degree at Moi University. A requirement of this course is to do a dissertation. I chose to study Group B streptococcus colonization among antenatal women: Prevalence and antibiotic susceptibility in Moi Teaching and Referral Hospital. I will ask you some questions about your socio demographic characteristics. You are free to respond or choose not to respond to some of the questions that you may find inconvenient to you.

A swab will be taken from your vagina and rectum. It will not cause any harm to you or the baby. If the culture turns positive you will receive treatment while in active labor. The drug used for treatment is not harmful to the baby but it may give you an allergic reaction (risk of allergic reaction is 0.7%-4% and risk of anaphylaxis is estimated to be 4/10,000-4/100,000). The importance of this treatment is to the baby as Group B Streptococcus colonization in you may lead to infection in the baby in their first week of life.

Your participation in the study will in no way change the treatment plan that your doctors deem is fit for you, or in any other way prejudice either of you. This study will not put you at any risk; no immediate benefit will accrue to you.

Information gathered will be treated with utmost confidentiality; your identity will be protected (your name will not be used and you will be identified with a number, only known to me and my immediate assistant). The information obtained will be used to improve services in MTRH, to form protocols and may be published in medical journals and/or presented in scientific symposia (both local and international).

The Moi University Ethics and Research Committee has approved this study

For any question or clarification, please do not hesitate to contact me on 0736238241 or contact the chairperson of IREC, MOI TEACHING AND REFERRAL HOSPITAL P.O BOX 3-30100 ELDORET

May I proceed with the questions? Yes/ No.

Respondent's signature..... Date .....

#### **Appendix 4: Taarifa Ya Ridhaa**

Jina langu ni Saudah Farooqui na nasomea shahada ya pili katika chuo kikuu cha Moi. Kinachohitajika katika somo hili ni kufanya utafiti. Basi nikaamua kufanya utafiti wangu kuhusu kundi B streptococcus miongoni mwa akina mama wajawazito: maambukizi na utumiaji wa antibiotic katika hospitali kuu ya rufaa ya Moi. Nitakuuliza maswali machache kukuhusu. Una hiari ya kujibu au kukataa kujibu maswali yoyote ambayo unahisi unasita kuyajibu.

Utapimwa kutumia vifutio damu kwenye sehemu yako ya siri na haitasababisha hatari yoyote kwako na pia mtoto wako. Ikiwa utapatikana kuwa na bacteria ya GBS, utapata matibabu utakapokua katika hali ya uchungu wa uzazi. Dawa inayotumika kwa matibabu haina hatari zozote kwa mtoto, ila itakupa mmenyuko(hatari za mmenyuko ni 0.7%-4% na inakadiriwa kuwa 4/10,000-4/100,000).

Matibabu haya yanamanufaa kwa mtoto kwa sababu GBS inaweza leta ugonjwa na maambukizi kwa mtoto anapokua katika wiki yake ya kwanza maishani.

Kushiriki kwako katika utafiti huu haitabadilisha mipango na matibabu ambayo daktari wako anapendekeza au kusababisha chuki kati yenu. Somo hili halitakuweka katika hatari yoyote; hakuna faida ya haraka utakayo ongezewa.

Ujumbe wako wowote utakayo peana itahifadhiwa kwa siri ya hali ya juu. Utambulisho wako itafichwa na pia jina lako halitatumika, utajulikana kupitia kwa nambari utakayopewa inayojulikana kwangu na msaidizi wangu pekee. Ujumbe tutakao pata itatumika kuendeleza huduma katika hospitali kuu ya rufaa ya Moi, kutengeneza itifaki zitakazo chapishwa katika majarida za matibabu au kuwasilishwa katika makongamano ya kisayansi nchini na kimataifa.

Idara ya maadili na utafiti cha chuo kikuu cha Moi kimeidhinisha na kupitisha utafiti huu.  
 Kwa maswali yoyote au ufafanuzi, tafadhali usisite kuuliza au kuzungumza nami kupitia  
 nambari yangu ya simu 0736238241 au kuzungumza na mwenye kiti wa Idara ya utafiti na  
 maadili ya hospitali kuu ya rufaa ya Moi, Sanduku la Posta 3-30100 ELDORET.

Naweza endelea kukuuliza maswali? Ndio

La

Jina ya mshiriki .....

Sahihi.....

Tarehe ...../...../2015.

Nambari ya

simu.....

Shahidi.....Sahihi.....Tarehe...../...../2015

Utambulisho wa Utafiti.....

**Appendix 5: Patient Enrollment Form**

Date.....study ID.....

Hospital Number

(IP/OP).....

**SECTION A. DEMOGRAPHIC CHARACTERISTICS**

Year of birth (YY/MM/DD) ...../...../.....

Occupation (a) Customer service (b) Health Care Provider (c) Teacher (d) Farmer (e)

Housewife

(f) Civil Servant (g) Business (h) Student (i) None (j)Other.....

Highest education level completed (a) None (b) Primary (c) Secondary (d)

College/university

**SECTION B: OBSTETRIC HISTORY**

Parity.....+..... Gravidity .....

LMP (YY/MM/DD) 2015/...../..... EDD (YY/MM/DD) 2015/...../.....

GBD...../40 + ...../7

Did any of your children die in the first week of life? (a) Yes (b) No (c) Unknown *If yes*

#.....

Did any of your children have Sepsis/Infection in first week of life? (a) Yes (b) No (c)

Unknown

If yes, (a) *confirmed* (b) *suspected*

Have you had any infection in this pregnancy (a) Yes (b) No (c) Unknown

If yes, at ..... Gestation by dates

Nature of infection..... (Diagnosis)

Antibiotics administered (a) Yes (b) No (c) Unknown

**SECTION C: FAMILY SOCIAL HISTORY**

Marital status (a) single (b) married (c) divorced (d) separated (e) widowed

Residence.....

**SECTION D: PREVIOUS MEDICAL HISTORY**

Have you ever had any of the following medical conditions? (Choose all that apply)

- (a) Diabetes
- (b) Heart disease
- (c) high blood pressure
- (d) HIV
- (e) Asthma

Have you had a Urinary Tract Infection (UTI) in this pregnancy? (a) Yes (b) No

Other or more information on above diagnoses; if yes,

specify.....

.....

.....

.....

.....

Do you have an allergy to: Penicillin (a) Yes (b) No (c) Unknown

If yes, reaction \_\_\_\_\_

Vancomycin (a) Yes (b) No (c) Unknown.

If yes, reaction \_\_\_\_\_

Ceftriaxone (a) Yes (b) No (c) Unknown.

If yes, reaction \_\_\_\_\_

Other (a) Yes (b) No (c) Unknown.

Specify .....

**\*\*Document all antibiotic allergies on Patient's Antibiotic Administration Form\*\*.**

**SECTION E: Lab results**

Culture (a) Positive (b) Negative

If positive, antibiotic susceptibility: Penicillin (a) Resistant (b) Sensitive

Ceftriaxone (a) Resistant (b) Sensitive

Vancomycin (a) Resistant (b) Sensitive

Erythromycin (a) Resistant (b) Sensitive

Clindamycin (a) Resistant (b) Sensitive

**SECTION E: Follow up**

Delivered in hospital (a) Yes (b) No (c) Lost to follow up

If yes – Hospital

name..... Date and

time of admission (YY/MM/DD) 2015/...../..... .....am/pm

Received antibiotics (a) Yes (b) No (c) Unknown

If yes - Date and time of 1<sup>st</sup> antibiotic administration (YY/MM/DD) 2015/...../.....

.....am/pm

Date and time of delivery (YY/MM/DD) 2015/...../.....

.....am/pm

Outcome (a) Live baby (b) Still birth

If (a) then- NBU admission (a) Yes (b) No.

If yes, at what age in days? .....

**Appendix 6: Antibiotic Administration Form**

Intrapartum antibiotic administration

STUDY ID: \_\_\_\_\_

Hospital number (IP/OP) \_\_\_\_\_

Maternal GBS Status:  Positive GBS       Negative GBS

Drug Allergies:  No known drug allergies     

Drug allergy \_\_\_\_\_

*Drug name + reaction type*

*For GBS positive patients with no known allergies to penicillin (please add to T sheet):*

Give: Penicillin G, 5 million units IV loading dose, then 3.0 million units IV every 4 hours  
until delivery

\_\_\_\_\_

*Name and Signature, Date and time*

*For GBS positive patients with allergy to penicillin, and no allergic contraindication to  
the following antibiotics (please add to T sheet):*

Give: Vancomycin 1 g IV every 12 hours until delivery

\_\_\_\_\_

*Name and Signature, Date and time*

*All patients receiving antibiotics should be monitored for adverse drug reactions such as  
Anaphylaxis (difficulty breathing/hypoxia/stridor, drop in blood pressure, lip swelling,  
tachycardia, seizure, decreased consciousness, dizziness, flushing,  
nausea/vomiting/diarrhea), Rash/Hives, itchiness, fever, new swelling, shortness of breath.*



*If any of these symptoms develop after starting antibiotics, MD should assess the patient immediately.*

*A) For Anaphylaxis:*

*i) Stop Antibiotic immediately*

*ii) Give: Epinephrine 0.3-0.5 mL of 1:1000 dilution (1mg/mL) IM or SC, every 5-20min;*

*Airway management: give oxygen, inhaled B2-agonists (salbutamol); Call ICU if airway compromised*

*Fluid resuscitation with IV normal saline or Ringer's lactate to support BP*

*iii) Antihistamines (ex. Diphenhydramine 50mg IV/IM) for symptomatic hives/itching*

*iv) Corticosteroids may prevent relapse (methylprednisolone 125mg IV q6h or prednisone 50mg po)*

*v) Glucagon (1-5mg IV over 5min) if inotropic or chronotropic support required*

*vi) Document allergy in patient's medical record and counsel patient*

*B) For Other Reactions:*


*i) Stop Antibiotic immediately*

*ii) Symptomatic Management*

*iii) Document allergy in patient's medical record and counsel patient*

## Appendix 7: Working instructions for microbiology lab- Lancet

Procedures controlled and /or distributed through Collaborator are allowed to be published for reference and training purposes. These published documents will not require any additional distribution control. Should a dispute arise as to the validity of any documents distributed through Collaborator the electronic version thereof on Collaborator shall override all published revisions that may be in circulation.

	<b>WORKING INSTRUCTIONS BACTERIOLOGY VAGINAL BENCH INSTRUCTIONS PROCESSING, MICROSCOPY CULTURES &amp; SENSITIVITIES</b>	
	<b>Doc Number:</b> QAAFRIW010	<b>Revision:</b> 001
	<b>Approved by:</b> Dr S Waner	<b>Issued by:</b> Q A Department
	<b>Date:</b> February 2014	<b>Date:</b> February 2014
	<b>Supersedes:</b> QAAFRIW010 (000)	<b>Author:</b> Suzette Van Driel

### Purpose

The purpose of this document is to have a combined manual of all the procedures undertaken on the Vaginal/STD Bench.

### Scope

This document describes the work flow and all procedures performed on the Vaginal/STD bench and applies to all technical Bacteriology staff.

### Responsibility

It is the responsibility of all staff performing the various procedures, to follow these instructions correctly.

### References

Each reference will be provided with the procedure where applicable.

<b>WORKING INSTRUCTIONS BACTERIOLOGY VAGINAL BENCH INSTRUCTIONS PROCESSING, MICROSCOPY CULTURES &amp; SENSITIVITIES</b>	
Doc Number: QAAFRIWI010	Revision: 001

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<b>WORKING INSTRUCTIONS BACTERIOLOGY VAGINAL BENCH INSTRUCTIONS PROCESSING, MICROSCOPY CULTURES &amp; SENSITIVITIES</b>	
Doc Number: QAAFR1W1010	Revision: 001

**Procedure****1. Specimen Types**

Swabs: Vaginal, Vulval, Cervical, Penile, Labial, Urethral, and Rectal for GC.

Fluids: Semen, Prostatic fluid.

**2. Processing****a. Administration**

1. Fill up & log media put in use on daily bench work-log.
2. Receive (from specimens) before processing.
3. Enter details of specimens processed on daily bench work-log.

**b. Plant the specimen on the following media as per table below:****Abbreviations**

Col BA = Colombia Blood agar

TM = Thayer Martin media

CHOC = Chocolate Agar

MAC = MacConkey

D+C = Dex and Chlor

Urea/Mycoplasma kit = Mycofast Evolution 3

Wet prep for Trichs = Wet Preparation for Trichomonas

Strep B plate = Blood agar

S.R = Special request

AC= Acinetobacter

PS= Pseudomonas

Order of processing:

1. Inoculate the media , avoiding contamination
2. Preparation and reading of the Gram stain
3. Preparation and reading wet Prep

**WORKING INSTRUCTIONS BACTERIOLOGY VAGINAL BENCH INSTRUCTIONS  
PROCESSING, MICROSCOPY CULTURES & SENSITIVITIES**

Doc Number: QAAFRIWI010

Revision: 001

**B1. Inoculate media as per specimen type**

	COL BA	TM	CHOC BACIT	MAC	D+C	Urea/ Myco plasma	Gram slide	Wet prep trichs
1. Vag/cervical labial vulval	X	X	-	-	X	S.R	X	X
2. Vaginal swab for Strep B	X							
3. Vaginal swab sent as a pus swab	X	X	-	X	X	S.R	X	X
4. Vaginal Swab – sent as a vaginal discharge	X	X		-	X	S.R	X	X
-----								
5. Penile Urethral	X	X	-	-	X	S.R	X	X
-----								
6. Urethritis screen:	X	X	-	-	X	S.R	X	X
plus chlamydia PCR, urine for trichs (spin & examine)								
-----								
	CO/BA	TM	CHOC BACIT	MAC	D/C	UREA/MY	GRAM	WET PREP
-----								
7. Child <13 yrs	X	X	X	-	X	S.R	X	X
-----								
		-	-	-	-	-	-	-
-----								
Rectal swab suspected GC	X	X	-	-	-	-	X	-
-----								
Semen Prostatic	X	X		X	X	S.R	X	X- plus neutrophil -per high power field (X40)

Chlamydia PCR only if specifically requested

**GENITAL ULCER:** should include:

Wet prep for dark ground exam for Spirochetes

Herpes cytology smear (fixed)

send to Cytology - Richmond

Herpes culture (specimen in transport medium)

send to Virology - Richmond

**Incubation**

BA, CHOC, TM plates : CO2

D+C, CHROMO, MAC : 02

<b>WORKING INSTRUCTIONS BACTERIOLOGY VAGINAL BENCH INSTRUCTIONS PROCESSING, MICROSCOPY CULTURES &amp; SENSITIVITIES</b>	
Doc Number: QAAFRIWI010	Revision: 001

**1. Vaginal/cervical/labial/vulval**

Inoculate on the following media:

- Col BA = Colombia Blood agar
- TM = Thayer Martin media
- D+C = Dex and Chlor
- Special request = Inoculate into a Mycoplasma transport media and ref to Richmond
- Gram stain
- Wet prep for Trichs = Wet Preparation for Trichomonas

**2. Vaginal swab – for Strept group B**

Inoculate onto blood agar

**3. Vaginal swab – for vaginal discharge**

Inoculate on:

- Col BA = Colombia Blood agar
- TM = Thayer Martin media
- D+C = Dex and Chlor
- Special request = Inoculate into a Mycoplasma transport media and ref to Richmond
- Gram stain
- Wet prep for Trichs = Wet Preparation for Trichomonas

**4. Vaginal swab – sent as a pus swab**

Specimen should be processed as a pus swab and all bacteria should be identified

Inoculate on the following media:

- Col BA = Colombia Blood agar
- Mac Conkey
- TM = Thayer Martin media
- D+C = Dex and Chlor – Chromogenic media
- Special request = Inoculate into a Mycoplasma transport media and ref to Richmond
- Gram stain
- Wet prep for Trichs = Wet Preparation for Trichomonas

**5. Penile Urethral**

- Col BA = Colombia Blood agar
- TM = Thayer Martin media
- D+C = Dex and Chlor
- Special request = Inoculate into a Mycoplasma transport media and ref to Richmond
- Gram stain
- Wet prep for Trichs = Wet Preparation for Trichomonas

**6. Urethritis screen**

- Col BA = Colombia Blood agar
- TM = Thayer Martin media
- D+C = Dex and Chlor
- Special request = Inoculate into a Mycoplasma transport media and ref to Richmond
- Gram stain
- Wet prep for Trichs = Wet Preparation for Trichomonas

Plus:

- Chlamydia PCR- ref to PCR Richmond
- A urine specimen for Trichomonas which is centrifuged and examined

<b>WORKING INSTRUCTIONS BACTERIOLOGY VAGINAL BENCH INSTRUCTIONS PROCESSING, MICROSCOPY CULTURES &amp; SENSITIVITIES</b>
<b>Doc Number:</b> QAAFRIWI010 <b>Revision:</b> 001

**7. Child****<13 years**

- Col BA = Colombia Blood agar
- TM = Thayer Martin media
- CHOC = Chocolate Agar plus Bacitracin
- D+C = Dex and Chlor
- Special request = Inoculate into a Mycoplasma transport media and ref to Richmond
- Gram stain
- Wet prep for Trichs = Wet Preparation for Trichomonas

**8. Rectal swab for Gonococcus**

- Col BA = Colombia Blood agar
- TM = Thayer Martin media – whole plate
- Gram stain

**9. Semen/Prostatic fluid**

- Col BA = Colombia Blood agar
- TM = Thayer Martin media
- MAC = MacConkey
- D+C = Dex and Chlor – Chromogenic media
- Special request = Inoculate into a Mycoplasma transport media and ref to Richmond
- Gram stain
- Wet prep for Trichs = Wet Preparation for Trichomonas

**Microscopy****1. Gram stain****2. Wet Preparation****1. Gram Stain****a. Preparation**

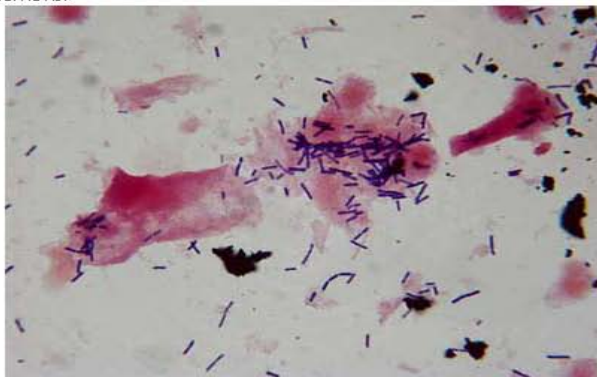
Make a smear for Gram stain, i.e. drop of fluid on slide and add a drop of saline or roll the swab onto the slide so that there is adequate material for assessment.

Heat-fix the smear before it is stained.

Method of Gram stain = refer to MBM051.

**b. Examine the smear after establishing the age and sex of the patient**

1. Using x10 magnification, scan the smear on low power (x10) so as to find areas with adequate material for assessment.



WORKING INSTRUCTIONS BACTERIOLOGY VAGINAL BENCH INSTRUCTIONS PROCESSING, MICROSCOPY CULTURES & SENSITIVITIES	
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Gram Stain – normal vaginal flora = epithelial cells with numerous gram positive bacilli = lactobacilli

2. Using  $\times 100$  (oil) magnifications, scan for the presence of:

- I. Fungi
- II. Clue cells and report The BV score
- III. WBC
- IV. RBC
- V. Neisseria bacteria

**I Fungi:**

Always report the absence or presence of budding yeast or yeasts and hyphae.

An interpretive comment will print if fungi are present.



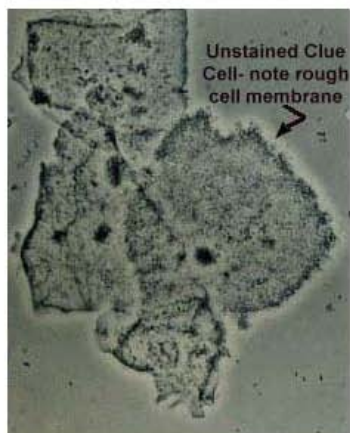
Gram stain – with yeast cells (purple) and pseudohyphae (long thread like structure)

II. **Clue cells** -squamous epithelial cells coated with small gram variable bacteria in females  $\geq 13$  yrs

Clue cells are considered significant and must be reported

Clue cells are only reported if there more than 20% of all squamous epithelial cells are "clue cells".

If clue cells are  $< 20\%$  of all squamous epithelial cells or if no clue cells are observed, report as "NONE".





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**Report The BV score**

Examine the slide at x100 magnification (oil immersion),

The following bacteria will be present in the presence of clue cells:

- Lactobacilli "Doderlein bacilli" (medium to large regular-shaped gram positive bacilli)
- Small gram variable bacilli (Gardnerella and Prevotella morphotypes)
- Curved gram negative bacilli (Mobiluncus morphotype)



Gram stain 1000X

Mobiluncus species – gram negative curve bacilli

The presence and quantity of these bacteria are used to compile BV Score which is reported as per the table below and that appears on the work card:

**N.B - The BV score is not reported on cervical swabs**

Lactobac	Score	Small GVB	Score	Curved GNB	Score	Quantitation
4+	0	4+	4	4+	2	4+=>30organisms
3+	1	3+	3	3+	2	3+=6 - 30 organisms
2+	2	2+	2	2+	1	2+ = 1 - 5 organisms
1+	3	1+	1	1+	1	1+ = <1 organism
0	4	0	0	0	0	0 = no organisms

**Method:**

Add Score \_\_\_\_\_ plus score \_\_\_\_\_ plus score \_\_\_\_\_ = \_\_\_\_\_ BV SCORE  
Lactobac Small GVB Curved GNB

Circle the appropriate "+" for the 3 different bacterial morphotypes.

Circle the corresponding score for each morphotype.

Write the score for each morphotype in the "add/plus score" space.

Add the three scores and put this sum in the "BV SCORE" space.

Circle the BV score value attained above on this table on the work card:

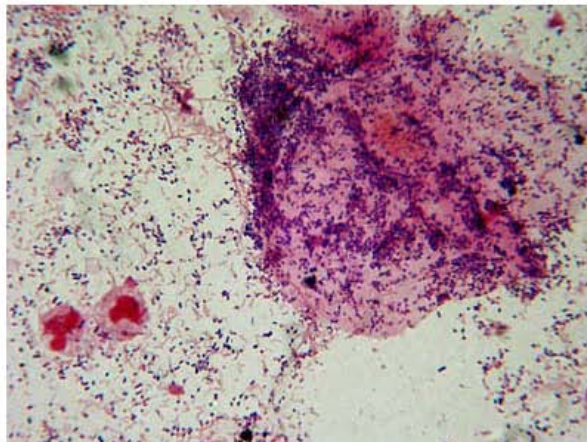
BV SCORE	0	1	2	3	4	5	6	7	8	9	10
	<- - - - Neg for BV - - - - ->				<- - Altered flora - - ->			<- - Suggestive - ->		<- - Diagnostic - ->	

An interpretive comment appears with the BV score.

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Gram stain (1000X) showing Clue cell = epithelial cells coated with gram variable bacilli

### III. Report the presence of WBC and RBC

Using  $\times 100$  magnification in areas of adequate material, scan at least 30 oil immersion fields (OIF) to decide:

#### i. Polymorphonuclear leucocytes (polys or pus cells):

(Semen and prostatic fluids=report from wet pep)

Reporting per HPF: 0 = NONE  
1 = Scanty (1-2)  
2 = Moderate (3-5)  
3 = Numerous  $\geq 6$

#### ii. Red blood cells:

Reporting per HPF: 0 = NONE  
1 = Scanty (1-2)  
2 = Moderate (3-5)  
3 = Numerous  $\geq 6$

### IV. Presence of Neisseria bacteria

**One must** report the absence of Gram negative diplococci.

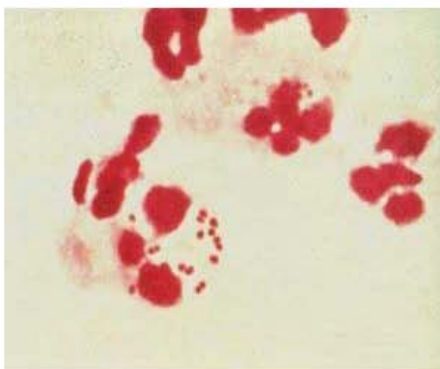
If present, report the bacteria as either:

- Intracellular (within the cytoplasm of the pus cell)
- Extracellular

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Gram stain - Polymorphonuclear leucocytes with gram negative diplococci within the cytoplasm =N. gonorrhoeae.

**In males**

The presence of Neisseria bacteria strongly indicates infection due to Neisseria gonorrhoeae.

**Important when reporting "GNDC" as Gram positive cocci may over-decolourise, giving the appearance of Gram negative cocci.**

**Ideally one should confirm via culture and identify with CTA sugars**

**In females**

An interpretive comment will print if Neisseria bacteria are "present".

**One must confirm that these bacteria are N.gonorrhoea via culture and CTA sugars as Neisseria species other than Gonococcus may be normal flora in the vagina**



Gram stain – vaginal smear with budding yeasts

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## 2. Wet Preparation

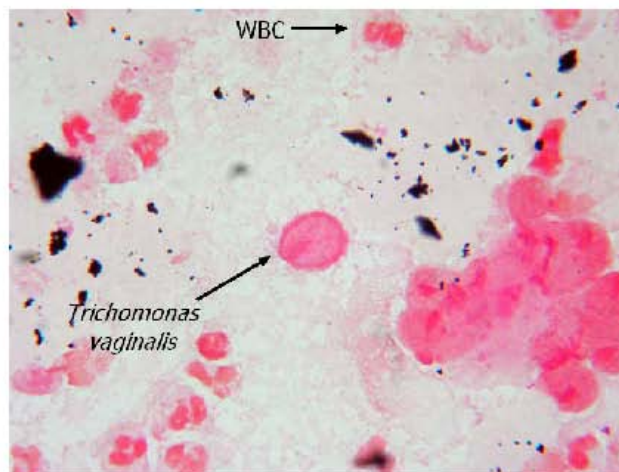
### Preparation and reading of the wet prep

#### Wet Preparation for Trichomonas and Polys in Fluids

1. Examine wet prep for Trichomonas vaginalis and WBC's in semen/prostatic fluids (HPF)
2. Enter results on the workcard & initial.
3. Correlate findings (polys, yeasts/hyphae) with gram stain.
4. If Trichomonas vaginalis present, result as organism mnemonic: P TV.
5. Phone the result if Trichomonas present.



Trichomonas on a wet prep



Gram stain – Trichomonas in a vaginal swab

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**Interpretive Comments****Neisseria bacteria**

Adult females and children have vaginal and cervical saprophytic flora that resemble Neisseria bacteria  
The direct Gramstain correlates, in only 50%-70% of cases, with the culture.

**Fungi:**

Yeasts & hyphae present: Diagnostic of Candidiasis  
Budding yeasts present: Highly suggestive of Candidiasis  
Candidiasis comment:  
Yeasts and hyphae indicate vaginal Candidiasis.

**BV score**

BV (Bacterial Vaginosis) or Nugent Score - - general comment:  
Since culture of Gardnerella vaginalis and other relevant pathogens is not always possible, the BV or Nugent Score, based on Gram stain is an aid in establishing the diagnosis of BV.

**Score of 0-3:**

Negative for Bacterial vaginosis

**Score of 4, 5, 6:**

Altered vaginal flora comment:  
Altered vaginal flora frequently represents a transitional state. If symptoms and signs persist, repeat testing may be warranted.

**Score of 7, 8, 9 or 10:**

Suggestive or Diagnostic of BV comment:  
Therapy for BV (if considered appropriate based on laboratory and clinical criteria) includes Clindamycin, Metronidazole, and Amoxicillin/Clavulanic acid. Male sexual contacts do not require therapy for initial episodes, unless balanitis is present. It is however prudent to treat sexual contacts of women with recurrent episodes of BV.

**Note:** >15 leucocytes /HPF are considered an abnormal number of leucocytes.

An interpretive comment will reflex: Bacteria present in the urethra is not always indicative of infection but can indicate the presence of commensals. (Refer: Mendel)

**Special requests****1. Mycoplasma/Ureaplasma (urogenital mycoplasmas)**

May be requested on the following specimens:

- Vaginal, labial and cervical
- Penile
- Specimens referred for Urethritis screen
- Specimens submitted in children < 13 years of age

Specimens should be inoculated into Mycoplasma/Ureaplasma transport media and referred to Richmond

**2. Chlamydia PCR**

This test is carried out in the PCR Laboratory. Place the swab or specimen if semen or prostatic fluid, into a blue topped PCR transport media.  
Send with a copy of the request note with the specimen to Richmond. The specimen should be sent on ice. (Dry swab or urine can be used for PCR)

**Note:** Chlamydia PCR, Mycofast or Viral transport media is an unsatisfactory specimen for bacterial MCS. If an MCS from these transport media is requested, the submitting doctor should be phoned and informed that the specimen is unsuitable.

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**Report**

Result the MCS as NOT DONE – UNSUITABLE SPECIMEN RECEIVED.

**3. Herpes or Viral Culture:**

These specimens must be sent on ice to virology at Richmond

They must be sent on Virology transport media and cannot be processed if not sent on virology transport media

**4. Human Papiloma Virus (HPV)**

Send specimen on Ice to PCR @ Richmond with a copy of the request form

**5. Fungi**

Send the specimen to Percardia with a copy of the request

**6. Spermatozoa**

- a. The presence of spermatozoa may be requested on a vaginal swab or indicated as - ?abuse/ penetration
- b. Make a cytological smear & fix using a Cytological fixative.

**To make a Cytology Smear:** Sterilize a glass slide. Allow to cool.

GENTLY rotate the swab in a drop of saline on the slide.

Send the slide to cytology- Richmond- for Papanicolaou staining.

Make a copy of the request form – note especially the specimen is from a case of suspected abuse

**Vaginal Bench Workflow****24 Hours**

1. Sign the bench log-sheet as the performing tech.
2. Examine all plates.
3. Perform identification and sensitivities, if indicated.
4. Note action and results on the work-card.
5. Send out preliminary reports.
6. Re-incubate all plates for up to 48 hours.

**Examine all plates and identify pathogens****a. Vaginal swabs referred for the presence of Strep group B**

- Read the blood agar
- If colonies suggestive of Streptococci are present perform a Lancefield grouping so as to diagnose Streptococcus group B. If Lancefield grouping is not available a CAMP test can be performed

**b. Examine the Dex and Chlor for the presence of yeasts**

If yeasts are present perform a GTT

If GTT is positive – Report as Candida albicans

If GTT is negative – Report as yeast –If the referring Doctor requests an identification the isolate should be referred to Percardia

**c. Vaginal Pus**

Vaginal swabs marked vaginal pus should be treated as a pus swab and all bacteria should be identified including anaerobes

**d. Guide to the reporting of Bacteria****i. The following bacteria are regarded as Non-Pathogenic****Vaginal/Cervical/Labial/Vulval**

1. Non-haemolytic streptococci
2. Strep viridans
3. Enterococci

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4. Coagulase negative Staphylococci
5. Lactobacilli (doderlein bacilli)
6. Corynebacteria
7. Any Gram negative bacilli unless +++ pure growth.

**Penile/Urethral/Seminal & Prostratic Fluid**

1. Strep viridans
2. Coagulase negative Staphylococci
3. Corynebacteria
4. Bacillus species

**ii. The following bacteria are regarded as Pathogenic****Vaginal/Cervical/Labial/Vulval**

1. Neisseria gonorrhoeae
2. Beta-haemolytic Streptococci, excluding Enterococcus
3. Group B streptococci
4. Staphylococcus aureus (+++pure growth)
5. Listeria monocytogenes
6. Gram Negative Bacilli (+++ pure growth)
7. Candida albicans or Yeasts.

**Penile/Urethral**

1. Neisseria gonorrhoeae
2. Staphylococcus aureus (+++ pure growth)
3. Gram Negative Bacilli (+++ pure growth)
4. Streptococci excluding Enterococcus
5. Candida albicans or Yeasts

**Seminal/Prostatic Fluids**

1. Neisseria gonorrhoeae
2. Staphylococcus aureus
3. Coagulase negative staphylococci are reported if:
  - >5 neutrophils per high power field are seen on microscopy
  - The bacteria is cultured as the only isolate and in +++ pure growth
4. Gram negative bacilli
5. Streptococci including Enterococci
6. Candida albicans or Yeasts

**Children (under 13 years):**

1. Neisseria gonorrhoeae
2. Staphylococcus aureus (+++ pure growth)
3. Streptococci (usually Strept pneumoniae or Strept pyogenes)
4. Gram Negative Bacilli (+++ pure growth)
5. Candida albicans or Yeasts
6. Haemophilus influenza (other species, discuss with pathologists).

**48 Hours**

Sign the bench logsheet as the performing tech.

1. Re-examine all plates.
2. Add a drop of oxidase reagent to all Columbia plates which have growth to detect the presence of N.gonorrhoea.
3. Record identification and sensitivity results on work-card.
4. Perform further identification and sensitivity tests, if indicated.
5. Note action and results on the work-card.

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6. Send out a further preliminary report if ID & sensitivity is not yet complete.
7. If complete, result the charge procedures and comments (if any).
8. Send out final report by signing off results & verifying (if indicated)
9. Keep culture plates for one week (pick off plates, TMs & sens plates)

**At least twice a week (Mon & Thurs)**

Pull outstanding reports & resolve.

**Identification of Pathogens & Sensitivity Testing**

1. Identification procedures refer to QAAFRIW1006
2. For CLSI interpretative zone sizes refer to MBWI110.

**Sensitivity tests are performed on all aerobic or facultative anaerobic bacteria as follows:**

**a. Gram negative sensitivity**

- Amikacin
- Ampicillin
- Augmentin
- Cefuroxime
- Cefepime
- Ceftazidime \*
- Ceftriaxone
- Ciprofloxacin
- Cotrimoxazole
- Doripenem – report the Meropenem result
- Ertapenem
- Gentamicin
- Imipenem
- Meropenem
- Piperacillin/Tozabactam combination
- Tigercycline
- Tobramycin\*
- Colistin – Tested if Carbopenems resistant bacteria are found

\* - Ceftazidime and Tobramycin do not get reported for GNB, only for AC and PS

**b. Acinetobacter / Pseudomonas / other non-fermenters including Aeromonas**

- Amikacin
- Cefepime
- Ceftazidime
- Ciprofloxacin
- Doripenem – report the Meropenem result
- Gentamicin
- Imipenem
- Meropenem
- Piperacillin/Tozabactam combination
- Tobramycin
- Colistin



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**c. Staphylococci**

Disc sensitivity is performed on Mueller Hinton with the following antibiotics:

**Test for:**

1. Cefoxitin - used as a surrogate marker for Oxacillin sensitivity

Using this result the following is reported:

- a) 1<sup>st</sup> generation cephalosporins
- b) Cloxacillin
- c) AUG: Amox-clav

**Other Antibiotics:**

- Clindamycin
- Cotrimoxazole
- Erythromycin
- Fusidic acid
- Gentamicin
- Levofloxacin – tested and reported for fluids
- Linezolid
- Mupirocin
- Penicillin
- Rifampicin
- Teicoplanin
- Telithromycin
- Tetracycline
- Vancomycin

**d. Streptococci**

- Ampicillin
- Clindamycin
- Erythromycin
- Levofloxacin
- Linezolid
- Penicillin
- Tetracycline
- Vancomycin

**e. Enterococcus**

Disc testing if performed on lacked blood agar for the following antimicrobials:

**Test for:**

- Ampicillin
- Augmentin
- Levofloxacin
- Linezolid
- Teicoplanin
- Vancomycin

**Gonococcus**

Cefixime  
Ceftriaxone  
Ciprofloxacin

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Penicillin-Ig Betalactamase is resistant and Penicillin is sensitive a Penicillin E test must be performed

Tetracycline

Beta lactamase

**Penicillin Breakpoint**

10. unit Disc

Zone size in mm

<u>Sensitive</u>	<u>Intermediate</u>	<u>Resistant</u>
Equal > 47	27-46	Equal < 26

MIC

<u>Sensitive</u>	<u>Intermediate</u>	<u>Resistant</u>
Equal < 0.06	0.12-1	Equal >2

**Resulting & Reporting****Microscopy Results**

Enter as soon as they are available.

**24 Hour Culture Results**

File as: Preliminary unverified or verified (PU or PV)

NG = No Growth after an initial 12 – 24 hours incubation.

NGNPB = No pathogenic bacteria at 24 hours.

NGPO = organism being investigated as a possible pathogen, report to follow

ID GNB = Gram Negative Bacillus ID to follow.

ID STREP = Streptococcus ID to follow.

Or

mnemonic of bacteria or yeast isolated and confirmed.

**NOTE:** Give the Clinician as much information as possible at 24 hours.**48 Hour (& Longer) Culture Results****Preliminary**

File as: Preliminary Unverified or Verified (PU or PV)

NGPO = organism being investigated as a possible pathogen

ID GNB = Gram Negative Bacillus ID to follow.

ID STREP = Streptococcus ID to follow.

Mnemonic of bacteria or yeast isolated and confirmed.

**Final (completed reports)**

File as: Final Verified (FV):

NG = No Growth after 48 hours incubation.

NGNPB = Normal bacterial flora.

Candida species, Neisseria gonorrhoeae,  
Gardnerella vaginalis and Streptococcus agalacticae (Group B) NOT isolated.

("C sps, N gonorrhoeae Strep agalacticae & Gardnerella vaginalis NOT isolated for adult females and  
"C sps + N. gon. NOT isolated" for males and children.

Or

Mnemonic of the organism isolated.

Report &amp; result the sensitivity, if not filed from Vitek.

S = Sensitive  
R = Resistant  
I = Moderate

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Cross out if the antibiotic was not tested.

Any additional antibiotics requested by doctor/pathologist not included in the panel should be handwritten on the workcard & results can be appended to the sensitivity results (enter/edit sensitivity interpretation).

#### Identification Charges

- |               |   |
|---------------|---|
| 1. SHORT      | Enter number of tests done (rapid indole or A/B plate only)   |
| 2. ID EXT/API | Enter number (maximum 3) of bacterial identifications done.   |
| 3. STAPH coag | Enter number of Pastorex done.                                |
| 4. GTT        | Enter number of GTT's done/ Chromo candida results            |
| 5. STREP type | Enter number of Streptococci identified = B, EfaI, A if typed |
| 6. OPT/BAC    | Enter number of Strep pneumo (opto), Strep A (bacit only).    |
| 7. FLU        | Enter number of Haemophilus identified.                       |
| 8. GC         | Enter number of Neisseria identified.                         |

#### Comments

The following should be added when relevant:

MESBL – Therapy with any cephalosporin and pip/tazobactam is clinically inappropriate.

Please contact the laboratory to discuss antibiotic use.

MRSA isolated:

NG comment: NGMRSA

Canned text comment: MRSA

Patients colonised/infected with methicillin-resistant (often multi-resistant) Staph aureus (MRSA) should be isolated until two consecutive negative cultures are obtained. Nasal carriers require treatment with topical mupirocin applied to anterior nares, chlorhexidine showers and hairwash. Family members may also be carriers. For further enquiries please contact a consultant microbiologist.

MPPNG:

Treatment regimens for patients with penicillin-resistant gonorrhoea and their contacts:

Spectinomycin	2.0gm	intra-muscular stat
Ceftriaxone	250mg	intra-muscular stat
Ciprofloxacin	500mg	orally stat
Cefixime	200mg	orally stat

Follow up swabs are recommended following completion of antibiotic therapy.

MVAG:

The microscopic features are consistent with bacterial vaginosis. This is due to a complex mixture of aerobes (Gardnerella) and anaerobes that typically produce a thin homogenous vaginal discharge which tends to coat the vaginal wall. Although "clue cells" are seen on microscopy, culture of Gardnerella is not always possible.

#### Phoning Of Reports

##### Urgent Reports

1. URGENT requests are to be communicated by telephone as soon as possible and then daily if there is further information for the doctor.
2. Urgent microscopies must be phoned on the same day- refer TAT.
3. Preliminary results should be entered & phoned as soon as the results are ready.
4. Final reports are to be phoned ASAP.

##### Other Reports to Be Phoned

1. Trichomonas vaginalis present on microscopy
2. Intracellular Gram negative diplococci (Neisserian organisms) present on Gram stain – after confirmation with a senior technologist or pathologist.

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3. Neisseria gonorrhoea isolates: include beta- lactamase result.
4. Strep pyogenes isolates.

**Refer To A Pathologist**

1. Neisseria gonorrhoeae isolates.
2. Any other results deemed appropriate by the technologist for review by the pathologist.

**Reference Texts**

Isenberg, Henry D., CLINICAL MICROBIOLOGY PROCEDURE HANDBOOK, American Society for Microbiology Microbiol, 1993.

Koneman, E.W. et al, COLOR ATLAS AND TEXTBOOK OF DIAGNOSTIC MICROBIOLOGY, 1997, 5th Edition.

Murray, P.R. et al, MANUAL OF CLINICAL MICROBIOLOGY, American Society for Microbiology, 1995, 6th Edition.

Connie R Mahon/George Manuselis, TEXTBOOK OF DIAGNOSTIC MICROBIOLOGY (page 407)

MYCOPLASMA/UREA PLASMA

Refer package insert.