PREVALENCE, ANTIMICROBIAL SUSCEPTIBILITY AND SEROTYPES OF GROUP B STREPTOCOCCUS RECTO-VAGINAL ISOLATES FROM PREGNANT WOMEN AT KENYATTA NATIONAL HOSPITAL

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DECLARATION

In submitting this dissertation, I certify that this is my original work and that it has not been submitted elsewhere for a degree. I understand the rules of plagiarism and that any work done by others should be properly cited. I declare that I am the sole author of this work and other people's work has been properly acknowledged in the text.

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CERTIFICATE OF APPROVAL

This is to certify that this research study has been conducted and written by **Mr. Jisuvei Clayton Salano**. I confirm that the candidate under my supervision carried out the work reported in this dissertation and is submitted with my approval for the Masters of Science degree in Tropical and Infectious Diseases.

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To Sarah, queen of my heart and mother of my children; and to all mothers.

ABSTRACT

Background: Estimates of group B streptococcus **(**GBS) disease burden, antimicrobial susceptibility, and serotypes in pregnant women are limited for many resource-limited countries including Kenya. These data are required to inform recommendations for prophylaxis and treatment of infections due to GBS.

Objective: We evaluated the recto-vaginal prevalence, antimicrobial susceptibility, serotypes and factors associated with rectovaginal GBS colonization among pregnant women at 12-40 weeks gestation receiving antenatal care at Kenyatta National Hospital (KNH) between August and November 2017.

Method: In this cross-sectional study, consenting pregnant women between 12 and 40 weeks of gestation were enrolled. Interview-administered questionnaires were used to assess risk factors associated with GBS colonization. An anorectal swab and a lower vaginal swab were collected and cultured on Granada agar for GBS isolation. Positive colonies were tested for antimicrobial susceptibility to penicillin G, ampicillin, vancomycin, and clindamycin using disk diffusion method. Serotyping was performed using Immulex Strep-B kit. Logistic regression was used to identify factors associated with GBS colonization.

Results: A total of 292 women were enrolled. Their median age was 30 years (interquartile range [IQR] 26-35) with a median gestational age of 35 weeks (IQR 30-37). Overall GBS was identified in 60/292 (20.5%) of participants. Among the positive isolates resistance was detected for penicillin G in 42/60 (72.4%) isolates, ampicillin in 32/60 (55.2%) isolates, clindamycin in 14/60 (30.4%) isolates, and vancomycin in 14 (24.1%) isolates. All ten GBS serotypes were isolated, and 37/53 (69.8%) of GBS positive participants had more than one serotype. GBS colonization was not significantly associated with maternal age (OR 1, CI 0.93-1.05; P 0.86), parity (OR 1.1, CI 0.77-1.51; P 0.65), gestation age (OR 1, CI 0.93-1.10; P 0.71), prior still births (OR 0.7, CI 0.45-1.16; P 0.18), history of pregnancy loss (OR 1.3,

CI 0.76-2.19; P 0.33), history of preterm birth in prior pregnancies (OR 1, CI 0.64-1.51; P 0.94), past histoty of neonatal death (OR 2.1, CI 0.80-5.60; P 0.13), history of neionatal infection (OR 0.5, CI 0.14-1.60; P 0.23), history of membrane rapture in prior pregnacy (OR 0.7, CI 0.30-1.60; P 0.39).

Conclusion: The prevalence of GBS colonization was high among mothers attending antenatal clinic at KNH. In addition, a high proportion of GBS isolates were resistant to commonly prescribed intrapartum antibiotics. Hence, other measures like GBS vaccination is a potentially useful approaches to GBS prevention and control in this population. Screening of pregnant mothers for GBS colonization should be introduced and antimicrobial susceptibility test performed on GBS positive samples to guide antibiotic prophylaxis.

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ACRONYMS AND ABBREVIATIONS

ACOG	-	American College of Obstetricians and Gynaecologists
AIDS	-	Acquired Immune Deficiency Syndrome
ANC	-	Antenatal Care
CAMP	-	Christie, Atkins and Munch-Petersen test
CTRL	-	Clinical Trials Research Laboratory
GBSDA	-	Group B Streptococcus Differential Agar
HIV	-	Human Immunodeficiency Virus
HUQAS	-	Human Quality Assessment Services
KNH	-	Kenyatta National Hospital
MDG	-	Millennium Development Goals
RCOG	-	Royal College of Obstetricians and Gynaecologists
SDG	-	Sustainable Development Goals
SPSS	-	Statistical Package for Scientific Study
TB	-	Tuberculosis
UN	-	United Nations
UNDP	-	United Nations Development Program
UoN	-	University of Nairobi
USA	-	United States of America

OPERATIONAL DEFINITIONS

Agreement - Harmony in the recovery of GBS from both the vaginal and rectal canal of the same participant

Anorectal - Around the anal area

Early neonatal period - Neonatal period from birth to 7 days

Early onset GBS neonatal sepsis -Sepsis due to GBS infection occurring in neonates between the 12 hours of birth and seven days of life

Group B Streptococcus - Gram-positive cocci bacteria colonizing the vagina or rectum of some healthy women. GBS colonization during pregnancy can and leads to early or late onset neonatal sepsis

Late onset GBS neonatal sepsis - Sepsis due to GBS infection manifesting in neonates between the ages of seven eight day and to ninety days of life

Neonate - Child in the first week after birth 28 days of life

Pregnant women participants - Women between the 12^{th} 35th and 40^{th} 37^{th} weeks of gestational.

Prevalence of GBS - Percentage of pregnant women participants having rectovaginal GBS colonization

Serotype - Subtypes of group B streptococcus including subtypes Ia, Ib, II, III, IV, V, VI, VII, VIII, IX

Susceptibility - Degree of sensitivity to antibioticsLikelihood of a bacterial growth specifically GBS in this study to be hindered by an antibiotic

Vagina - The female birth canal extending from the cervix to outside of the body

CHAPTER ONE

1.0 INTRODUCTION

Streptococcus agalactiae otherwise called group B streptococci (GBS), cause intrusive infections mainly in pregnant ladies and newborn children (mohMitima et al., 2014). Temporary GBS colonization of the female urogenital tract is a known causative factor of neonatal infections acquired during the childbirth process (Buchan, Olson, Mackey, & Ledeboer, 2014). Acquisition of GBS can be perinatal during labour or in utero through transmission of the bacteria from the maternal vaginal or anorectally colonized mucosa. Similarly, GBS is a risk factor for neonatal sepsis and mortality since it is higher in preterm than in term newborns (El Aila et al., 2010). Neonatal GBS infection can be early or late onset with early-onset disease (EOD) ensuing within the first 7 days of life and presents as pneumonia, meningitis, and sepsis that carries a mortality rate of up to 20% (Buchan et al., 2014).

In the 1970s GBS arose to be the leading infectious cause of early neonatal disease and death in the Western world (Narava, Rajaram, Ramadevi, Prakash, & Mackenzie, 2014) which led to an extensive use of antibiotics as preventive treatment during labour by these countries in the 1990s (Gilbert, 2004). This is because it was found that approximately 50-60% of infants born to GBS colonized mothers possessed positive GBS cultures from skin and mucous membranes with 1-2% of these newborns developing invasive GBS diseases (Sharmila, Joseph, Arun Babu, Chaturvedula, & Sistla, 2011). These preventive strategies followed recognition that maternal vaginal colonisation with GBS was the principal agent in the occurrence of GBS-related neonatal disease and death (Kobayashi et al., 2016), hence efforts

to identify and treat pregnant women who were colonised by GBS or were most in danger of passing the bacteria to their newborns (Narava et al., 2014).

Global rates of GBS colonization vary widely (Gilbert, 2004) with high prevalence rates having been reported in the United States (15-25%), Jordan (30%) and Gambia (33.7%) (Doare et al., 2016) while low prevalence rates have been reported in Italy (6.6%),Turkey (8.7%) and Iran (4%)(Namavar Jahromi, Poorarian, & Poorbarfehee, 2008). In sub-Sahara Africa, reported GBS colonization rates are high for example, 23% and 16.5% in Tanzania and Malawi respectively (Z. L. Woldu, T. G. Teklehaimanot, S. T. Waji, & M. Y. Gebremariam, 2014). On the other hand, the rates of GBS neonatal invasive disease both EOD and LOD range from 1.7 to 3.3 per 1000 live births in the USA and between 0.2 to 0.6 per 1000 live births in Europe, Canada and Israel (Altoparlak, Kadanali, & Kadanali, 2004). Studies in the USA have found case fatality rates for EOD due to GBS to range between 5 and 20% with infants born to pregnant women who are severely recto-vaginally colonized with GBS to be at an increased risk of neonatal sepsis (Doare et al., 2016; Sadaka, Aly, Meheissen, Orief, & Arafa, 2018; Seale et al., 2016).

Though excellent data on GBS exists among western countries, there is paucity of information on the recto-vaginal colonization and associated maternal and neonatal complications due to GBS among developing countries (Z. L. Woldu et al., 2014). Similarly, there is limited information on GBS serotypes that colonize mothers and infants in African countries, even so, some data suggest that serotype V may be important as both a colonizing and invasive disease serotype just as it was in the USA in the 1990s (Doare et al., 2016). In the most recent study in Kenya, Seale et al. (2016) reported a prevalence of 12% among expectant mothers in Kilifi county. In an earlier study, Salat *et al* found a high GBS prevalence of 25.2% among pregnant women at KNH in 2009 (Salat, 2009). However, these

two were limited to prevalence and risk factors of GBS but did investigate the antimicrobial susceptibility or serotypes of GBS isolated. Based on the year 2015 Kenyatta National Hospital statistics of 23,536 annual deliveries and an estimated 25.2% GBS prevalence, it is estimated that without screening and prophylaxis about 500 (2.12%) neonates die annually translating to approximately 2 deaths daily from early onset GBS disease. The GBS related infant mortality may even be higher in lower resource facilities.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Group B Streptococcus

Streptococcus is a Gram-positive bacteria belonging to the phylum Firmicutes. GBS are facultative, gram-positive diplococcic with approximately 99% of strains showing β haemolysis on blood agar plates. Lancefield classified two polysaccharide antigens from GBS: the group specific polysaccharide common to all strains, and the type specific polysaccharide that distinguishes serotypes. The predominant disease causing serotype are Ia, Ib, II, III and type V in the USA (Rench & Baker, 1993) and VIII in Japan and Denmark (Wagner, Murai, Wagner, Gunther, & Jelinkova, 1994) being clinically important.

2.2 Neonatal Disease

Prior to the introduction of protocols to screen and treat colonized pregnant women prior to delivery and prophylaxis at delivery, a lot of the perinatal morbidity and mortality was due to GBS, with as many as 1–3 in 1000 neonates affected (Chen, Avci, & Kasper, 2013). In the year 2016, the WHO identified GBS as an important pathogen responsible for causing large burden of disease among neonates and infants in low and middle-income countries (LMICs) (Kobayashi et al., 2016). Worldwide, neonatal infection with GBS remains the leading cause of sepsis and meningitis in the first 90 days of life (Kobayashi et al., 2016).

The most severe form of GBS in neonates is characterized by pneumonia, a fulminant sepsis and meningitis with a high rate of fatality and residual damage (Stephanie Schrag, Rachel Gorwitz, Kristi Fultz-Butts, & Anne Schuchat, 2002). Infection of the neonate can be intrauterine infection of the foetus, ascending vaginal infection from GBS colonized women who are typically asymptomatic; neonate contamination as it passes through the birth canal

and aspiration of infected amniotic fluid which can also lead to stillbirth (Stephanie Schrag et al., 2002).

Age of disease onset has been used in the classification of GBS infection into early onset disease (EOD, onset during the first 6 days of life) and late onset disease (LOD, onset between days 7–89 of life), with statistics indicating 60–90% of EOD to occur on the first day of life (Kobayashi et al., 2016). Early onset disease manifest as neonatal bacteraemia, septicaemia, pneumonia and meningitis. The clinical signs usually are apparent in the first 24 hours of life. Maternal colonization with GBS has been associated with stillbirths and preterm births (Goldenberg & Thompson, 2003). Given this pathogenic role and the relatively frequent vaginal colonization with GBS, several countries around the world have proposed screening strategies aimed at initiating prevention during the third trimester of pregnancy (M. Mahmoud et al., 2011) or intrapartum treatment to avoid mother-to-newborn transmission (Stade, Shah, & Ohlsson, 2004).

2.3 Risk factors for early onset disease

Among the recognised risk factors for EOD are, prolonged rupture of membranes, GBS bacteriuria during pregnancy, maternal GBS colonization, preterm delivery, birth of a previous infant with invasive GBS disease, young maternal age, maternal chorioamnionitis as evidenced by intrapartum fever, low levels of antibody to type specific capsular polysaccharides antigens, African-American race, and Hispanic ethnicity (Boyer et al., 1983). A single individual may be found to have all these factors, however, through multivariate analysis the following have been found to be independent predictors of EOD risk; being of Africa-America race, maternal age and gestational age (Boyer et al., 1983). Even so, there are conflicting reports on risk factors of GBS with both younger and older age

group, higher education, higher income, high sexual activity and obesity being reported as risk factors (Seale et al., 2016).

Similarly, maternal carriage of GBS is a major risk factor for late onset neonatal GBS disease (Lin, Weisman, Troendle, & Adams, 2003) with meningitis found to occur in up to one third of LOD cases among whom the risk of long-term neurological sequel has been found to be higher compared with survivors of EOD (Lin et al., 2003).

2.4 GBS Epidemiology

In the western hemisphere, the leading infectious cause of infant morbidity and mortality is GBS (Gilbert, 2004) with 10-35% of pregnant women in the United States being asymptomatic carriers of this bacteria in their genital and gastrointestinal tract at time of delivery (Gilbert, 2004). Positive GBS cultures are recovered from mucus membranes and skin (external ear canal, throat, umbilicus and anorectal sites) of 50-65% of infants born to GBS colonised mothers of whom 1-2% develop invasive disease (Narava et al., 2014). Prior to the introduction of GBS treatment of colonised women using intra-partum prophylaxis in the United States, the incidence of neonatal GBS infection was 1.7 cases per 1000 live births, however, following introduction of preventive measures a sharp decline in the incidence to 0.34-0.37 cases per 1000 live births was recorded between the year 2006-2008 (Narava et al., 2014).

There is a variation in the rate of vaginal GBS carriage among women with an American study by Mitima et al., (2014) conducted among pregnant women at 23-26 gestational weeks reporting a GBS carriage of 15.6% (Mitima et al., 2014). This carriage rate has been found to vary between different studies reporting on people sharing similar socio-economic conditions in countries with geographic proximity in the European continent (e.g., rates of 10% in

France vs. 23.7% in Belgium) (Stoll & Schuchat, 1998). On the other hand, the prevalence and rectovaginal GBS carriage rate among developing countries has been reported to vary between 1% and > 30% (Stoll & Schuchat, 1998); for instance Salat (2009) reported a prevalence of 25.5% while Seale et al. (2016) reported a prevalence of 12% in two different geographical location of the same country. On the other hand, Sadaka et al. (2018) reported a prevalence of 26.5% in Egypt while a multi country cross sectional study conducted in Kenya, South Africa and Rwanda reported a GBS prevalence of 20.2%, 23.1% and 37.3% respectively (Cools et al., 2016). Studies done in Ethiopia reported a GBS prevalence among pregnant women of 7.2 % (Z. L. Woldu et al., 2014)

2.5 Virulence factor and vaccine development

Group B streptococcus produce many extracellular substances some of which have a role either in virulence or as protective antigens (Edward MS, 2006). The best characterized are the capsular polysaccharides (CPS), which confer serotype specificity to GBS (Jennings, Katzenellenbogen, Lugowski, & Kasper, 1983). The capsule confers virulence to the organism at least in part by barring the installation of complements on the surface of the organism in the absence of serotype specific antibody. Protective immunity is related to antibodies directed at these serotype-specific capsular polysaccharide structure (Baker C J & S., 2000).

The rationale for development of a maternal vaccine against GBS followed observations made in 1976 which showed antibodies to type III CPS were transplacentaly transfer offering protection to infants against CPS type III GBS invasive disease, this finding was generalized to other GBS serotypes (Kobayashi et al., 2016). Since then, candidate GBS-CPS protein conjugate vaccine against serotypes Ia, Ib, and III have been developed and safety studies

conducted in healthy adults. Similarly, conjugate vaccine targeting serotype II and V have been developed and successfully passed phase 1 clinical trials (Baker et al., 1999).

Currently, GlaxoSmithKline is developing vaccines against GBS to be administered during pregnancy for subsequent prevention of invasive GBS disease in neonates and young infants. These vaccines include the trivalent (Ia, Ib, III) CPS-CRM197 GBS conjugate vaccine that has undergone phase I and II clinical trials and the pentavalent (Ia, Ib, II, III, V) CPS-CRM197 vaccine that is currently under pre-clinical studies (Kobayashi et al., 2016).

2.6 Antimicrobial susceptibility

S. agalactiae is uniformly sensitive to penicillin, however, since 2008 reports of strains with diminished susceptibility have been received (Nagano et al., 2012). For women with allergy to penicillin; those with suspected therapeutic failure or among those with high risk of anaphylactic, GBS intrapartum antibiotic treatment (IAP) is done using clindamycin or erythromycin (Dutra et al., 2014). Even so, there are reports from different regions of the world of increasing rates of resistance to these antibiotics including Europe (Gonzalez & Andreu, 2005), Asia, North America, and South America (Pinto et al., 2013).

Penicillin is the drug of choice for intrapartum treatment of GBS with the royal college of obstetrics and gynaecologists (RCOG) recommending 3g benzyl penicillin be administered intravenous as soon as possible after the onset of labour and 1.5 g every 4th hourly until delivery (CDC, 2010; RCOG green top guideline no 36, 2 nd edition). For patients with allergy to penicillin, 900 mg of intravenous clindamycin is recommended every 8th hourly. The current resistance of rate of GBS to clindamycin in England and Wales is 10% (CDC, 2010) hence there is a possibility of clindamycin being ineffective. The CDC and American college of obstetrics and gynaecologists (ACOG) have recommended penicillin, ampicillin, or cefazolin prophylaxis to be administered more than 4 hours before delivery to women

delivering at <37 weeks' gestation, however, effectiveness of these medication was found to be 78% at preventing early-onset GBS disease (Stephanie Schrag et al., 2002) (CDC, 2010).

Clinical trials conducted in the 1980s demonstrated that intravenous administration of penicillin or ampicillin to mother at risk of transmitting GBS to their unborn children was highly effective at preventing EOD due to GBS (Schrag & Verani, 2013).

Despite the adoption of intrapartum antibiotic prophylaxis in the Americas, Europe and Australia, GBS still is the main cause of infant morbidity and mortality in these countries (Gilbert, 2004). However, the mortality and morbidity is negligible compared to countries, Kenya included, where data on estimates of disease burden and prevalence of GBS is unavailable.

2.7 Serotype

The virulence of GBS is complex and multifactorial involving a number of virulence determinants that assist in the adhesion to and invasion of host cells besides evasion of the immune system. The surface component of GBS is made of a polysaccharide capsule that contains proteins such as the laminin binding protein (LMB) Cα, Cβ, and Rib, and produce a number of enzymes (like the C5a peptidase) and toxins/cytolysins associated with GBS virulence (Maisey, Doran, & Nizet, 2008). The capsular polysaccharides of the GBS species also contain antigenic and chemical variations that have made it possible to subdivide GBS to ten serotypes denoted as Ia,Ib, II-IX; similarly, the capsule are key virulence factors and targets for development of vaccine strategies (Dutra et al., 2014)

Distribution of GBS serotypes vary geographically with serotypes Ia, Ib, III and V reported as the most frequent among studies conducted in European countries, United States and in Latin America (Palmeiro et al., 2010). A study in Brazil reported serotypes Ia, Ib, II and V to

be the most common though their occurance varied from region to region (Dutra et al., 2014). In the study, serotypes VI to VIII were not isolated (Ekelund, Slotved, Nielsen, Kaltoft, & Konradsen, 2003). However, Lu et al. (2014) in a study among pregnant women in Beijing, China isolated nearly all GBS serotypes with serotypes VII and IX being the only ones not isolated (Lu et al., 2014).

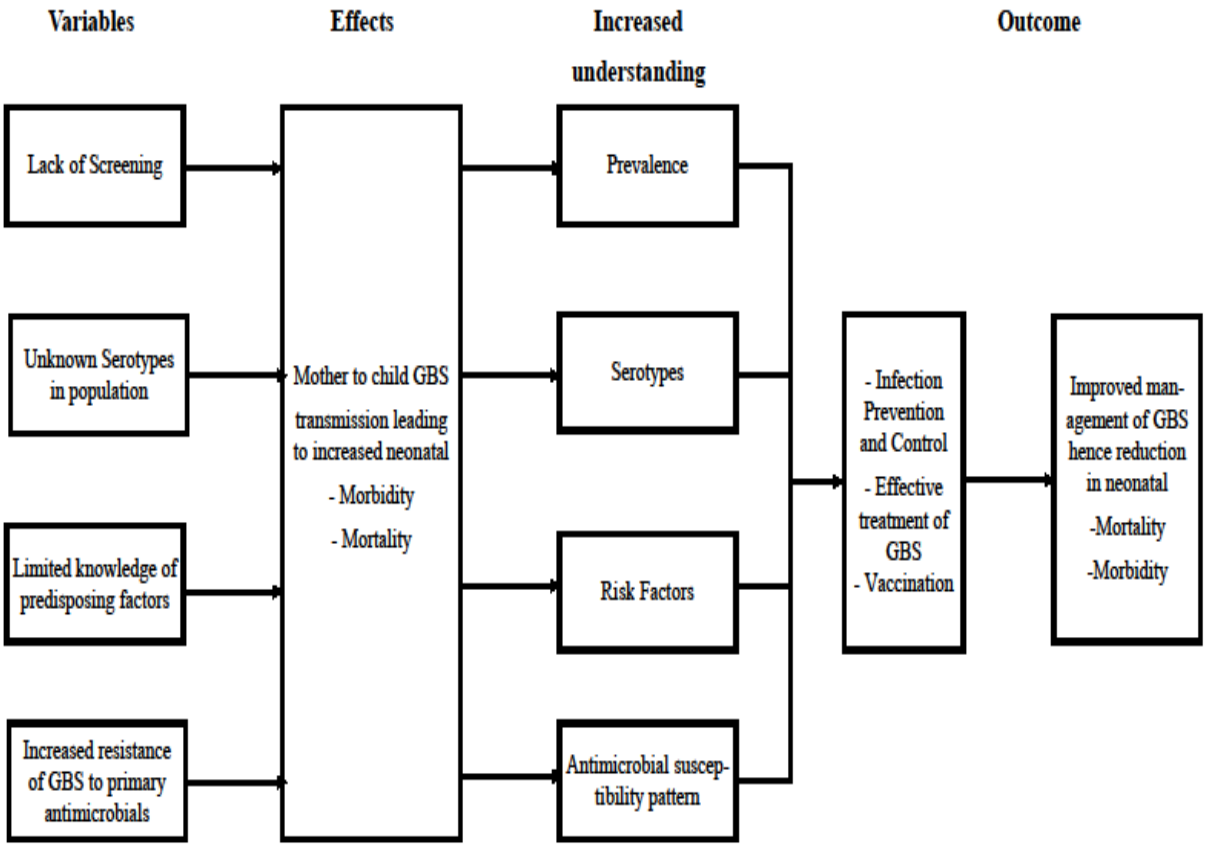
Other studies in Brazil (Palmeiro et al., 2010) have isolated serotypes Ia, II, III and V from 68.2% of vaginal specimens of asymptomatic pregnant women. In contrast, serotype IV has been isolated from 13.1% of infectious cases in Curitiba city, Paraná state, Brazil, (Palmeiro et al., 2010), while serotypes Ib (34.9%) and Ia (25.6%) were the most occurring in cohort of HIV-infected pregnant women in Rio de Janeiro city (Dutra et al., 2014).

In Kenya, a study by Gunturu et al (2011) isolated *Streptococcus agalactiae* serotypes Ia, Ib, II, III, IV, and V from different anatomical locations of adult patients (both male and female) with serotype III (30.8%) being the most commonly occurring followed by serotype Ia (30.2%), serotype V (17.2%), serotype Ib (14.2%), and serotype II (7.1%). This study however, focused on isolating GBS from blood, pus, urine and other body samples of both male and female patients but did not focus on serotypes occurring in anal-rectal GBS from pregnant women. Studies conducted among pregnant women have reported different serotypes to be occurring, for instance Cools et al. (2016) in a multisite study, reported serotypes Ia, Ib, II, III, IV and V among South African cohort while serotypes Ia, III, V, VI, VII and VIII were isolated in Kenyan women (Cools et al., 2016). In Europe and USA, serotypes Ia, Ib, II, III, IV, V, VI, VII and VIII have been isolated (Ippolito et al., 2010).

2.8 Theoretical framework

The theoretical framework on rectovaginal colonization of pregnant women with GBS has been developed from the literature review. From the review, it has been noted that a lack of

GBS screening of pregnant women, limited knowledge of GBS serotypes in population as well as a lack of knowledge of antimicrobial susceptibility patterns and risk factors predisposing women to GBS carriage could lead to an increase in GBS prevalence in the population. This will result in increased neonatal morbidity and mortality from GBS infections as a result of colonized women transmitting the bacteria to their children during child birth. However, an understanding of the prevalence, serotypes, risk factors and antimicrobial patters of the bacteria coupled with screening of the bacteria could lead to improvement in treatment and development of effective vaccines which when administered to pregnant women could lead to a reduction in the negative effects of GBS.



2.9 Problem statement

The 2014 Kenya demographics and health survey (KDHS) report indicated neonatal mortality rate (NMR) in Kenya had declined from 31 per 1,000 births to 22 per 1,000 live births between the years 2008-2014 (KNBS, 2015). Despite the decline, this rate is higher than the global average of 19 death per 1,000 live births. The infant mortality rate in the other hand stands at 39 deaths per 1,000 live births. It has been noted to reduce the high infant mortality rate, the NMR must be reduced (MoH, 2016).

Neonatal sepsis contributes to 7% of neonatal mortality deaths in Kenya (MoH, 2016) with *Escherichia coli* being the most associated causative agents (KNBS, 2015). On the other hand, the contribution of GBS on causation of neonatal sepsis in Kenya has received little focus; this is despite the overwhelming evidence from developed countries that reduction of GBS transmission from colonised mothers to their children during the birthing process led to reduction of neonatal sepsis and NMR (UNDP, 2016).

Part of the reason why developed countries were able to reduce the NMR was screening of all pregnant women between the 35^{th} and 37^{th} gestational week for GBS and giving intrapartum prophylaxis to those found colonized with the bacteria as well as administering vaccines against GBS to pregnant women in order to prevent transmission of GBS to their babies during child birth (UNDP, 2016). These interventions are lacking in Kenya.

The paucity of data on the prevalence, antimicrobial susceptibility pattern, serotypes and risk factors of GBS; as well as the little association it has been given with neonatal sepsis at KNH and Kenya as a whole may have contributed to the high neonatal mortality in the country as shown by KNH statistics where approximately 20% (469) of early-onset neonatal death are due to neonatal sepsis (KNH, 2015). This study seeks to contribute to this knowledge gab by

determining the prevalence, serotypes, antimicrobial susceptibility paters and risk factors to GBS rectovaginal colonization among pregnant women in this population. It is hoped that this knowledge will help in guiding treatment against GBS hence prevent neonatal infection and reduce neonatal mortality.

2.10 Justification

Health falls under the social pillar of Kenya's vision 2030, the second objective of health under the vision is to shift the emphasis of the national health bill from curative to preventive care. Special attention is focused at lowering the incidence of HIV/AIDS, malaria, TB, and infant mortality rates (M.P.N.D, 2007).

Hospital statistics from KNH for the period January 2015 to December 2015 rank neonatal sepsis as the second highest cause of neonatal death at the hospital with 20% of 1469 neonatal deaths being due to it. Similarly, there is a reported increase in antimicrobial resistance at the facility.

This research sought to determine the prevalence of GBS recto-vaginal colonization of pregnant women to inform health care stake holders on the need to screen for GBS rectovaginal colonization among pregnant women as well as prescribe antibiotics to which the bacteria is susceptible. On the other hand, knowledge of GBS serotypes in the Kenyan population may inform vaccines development by adopting vaccines to strains that are specific to GBS serotypes in this population. The study may also inform stake holders on the need of coming up with a policy on GBS screening during pregnancy which might lead to reduction in incidence of GBS related neonatal morbidity and mortality.

2.11 Research Question

What is the prevalence, antimicrobial susceptibility pattern, serotypes and risk factors for Group B Streptoccocus colonization among pregnant women receiving antenatal care at Kenyatta National Hospital?

2.12 Broad Objective

To determine the prevalence, antimicrobial susceptibility pattern, serotypes and risk factors for Group B Streptococcus colonization among pregnant women (12-40 weeks gestation) attending antenatal clinic at Kenyatta National Hospital.

2.12.1 Specific Objectives

1. To determine the prevalence of Group B Streptococcus among pregnant women receiving antenatal care at Kenyatta National Hospital.
2. To determine the antimicrobial susceptibility pattern of Group B Streptococcus isolates from pregnant women receiving antenatal care at Kenyatta National Hospital.
3. To determine the serotypes of isolated Group B Streptococcus bacteria from pregnant women receiving antenatal care at Kenyatta National Hospital.
4. To determine risk factors for Group B Streptococcus colonization among pregnant women receiving antenatal care at Kenyatta National Hospital.

CHAPTER THREE

3.0 METHODS

3.1 Study Design

This was a cross sectional descriptive study conducted between August and November 2017 among pregnant women at gestational ages 12 weeks to 40 weeks receiving antenatal care (ANC) at Kenyatta National Hospital.

3.2 Study area description

The study was conducted at KNH ANC clinic. KNH is the largest national reference centre in Kenya. The ANC clinic runs every morning from Tuesday to Thursday. These clinics are conducted by consultants and postgraduate doctors. The catchment area for the clinic includes residents of Nairobi, Kiambu, Thika and Machakos counties. The clinic attends to an average of 120 women (both old and new) clients each clinic day at different gestation ages (KNH, 2015).

3.3 Study Population

This study's population were pregnant women attending ANC clinics at KNH. The women had confirmed pregnancy above 12 weeks of gestation. Since there is no antenatal screening for GBS in Kenya, none of the pregnant women are aware of their carrier status.

3.4 Inclusion/Exclusion criteria

3.4.1 Inclusion criteria

- Pregnant women without pregnancy complications such as placenta previa and vaginal bleeding
- Ability to provide informed consent.
- Aged 18 years and older

3.4.2 Exclusion criteria

- Pregnant women with contraindications to anorectal/vaginal swab collection e.g. placental previa.Having been on antibiotics treatment within two weeks prior to the study.
- Current history of per-vaginal bleeding.
- Rapture of membranes
- Women in labour

3.5 Sample Size Calculation

The study population was determined using the Fishers formula (1998).

A previous study by Salat in 2009 (Salat, 2009) found the anorectal prevalence of GBS in KNH to be 25.2%

$$\mathbf{n = \frac{Z^2pq}{d^2}}$$

Where:

n = the desired sample size

Z = the standard normal deviate at the required confidence level (95% = 1.96).

p = the proportion in the target population estimated to have characteristics being measured (25.2% anorectal colonization)

q = 1-p

d = the level of statistical significance set (0.05)

$$\mathbf{= \frac{1.96^2 \times 0.252 \times (1-0.252)}{0.05^2}}$$

= 290

This study enrolled 292 pregnant women of gestation age 12 and 40 weeks

3.6 Sampling Technique

Consecutive simple random sampling was employed in selecting study participants. Early in the morning of every clinic day, a general talk on group B streptococcus, its effects in pregnancy and role in causation of neonatal sepsis was given to pregnant women visiting the clinic. Later, all pregnant women receiving ANC clinic services at the hospitals were approached and those who met the inclusion criteria and consent to participate in the study were enrolled.

3.7 Data Collection Instruments

Questionnaires and laboratory tests were used in collecting data to answer study questions. A coded questionnaire was administered to participants to obtain sociodemographic and obstetrics history relevant to the study. Vaginal and rectal culture and other laboratory test results were retrieved from laboratory results form.

3.7.1 Screening and recruitment

A trained study nurse reviewed hospital files of antenatal clients as they come to the ANC clinic triage room. Those who met study inclusion criteria were informed of the study, among these, those who agreed to participate in the study were considered eligible.

Eligible clients were invited into the study clinician consultation room. The principal investigator (PI) took the participants through the information on the consent form regarding the purpose of the study, physical examination and specimen collection. Clients were allowed to ask any study related question and were answered to their satisfaction. Those who consented to participate signed the consent form. No study procedure was conducted prior to signing the consent form.

3.7.2 Clinical procedures

Interviewer-administered questionnaires were used to gather obstetric and other study relevant information from participants; this was followed by collection of study samples for laboratory analysis.

3.7.3 Laboratory Specimens

Two swabs were collected, one from the lower vaginal and the other from the anorectal area using sterile Dacron swab.

3.7.4 Procedure for specimen collection

Prior to sample collection, study participants received counselling to ensure they were comfortable with the procedure. Those not comfortable with the PI collecting the samples were allowed to self-collect; however, this followed a short demonstration on how to self-collect the sample.

Prior to sample collection, a Group B Streptococcus Differential Agar (GBSA) (Granada agar, Hardy Diagnostics) plate was retrieved from a cooler box and labelled with the participant's study number and date of sample collection. The plate was divided into two halves; one half was labelled vaginal swab and the other anal swab. The PI requested the study participant to lie in a semi-lithotomy position on the examination couch and then hand gloved a pair of sterile latex gloves. While at the foot of the bed, the woman's external genitalia was examined and using the left hand, he gently separated the labia to access the vaginal introits. Without speculum placement, a sterile swab was used to swab the vagina and the swab immediately inoculated on the corresponding side of the GBSA plate. A second sterile swab was used to swab the anorectal area and the swab immediately inoculated on the corresponding side of the GBSA plate. The plate was then replaced in the cooler box.

After sample collection, the participant dressed up and was allowed to leave the study consultation room. A coloured label with study initials was stuck on participants ANC file to avoid double recruitment.

The specimens were transported to the clinical trials and research laboratory (CTRL) within 6 hours of sample collection. This lab occupies laboratory 2 and 3 of the ground floor of the Department of Obstetrics and Gynaecology, College of Health Science, Faculty of Medicine of the University of Nairobi. The laboratory participates in external quality assurance with Human Quality Assessment Services (HUQAS) – Kenya as well as taking part in inter-lab quality assurance with the Kenya AIDs Vaccine Institute laboratory.

3.7.5 Laboratory bacterial isolation procedure

3.7.5.1 Procedure for processing clinical specimens for culture of GBS

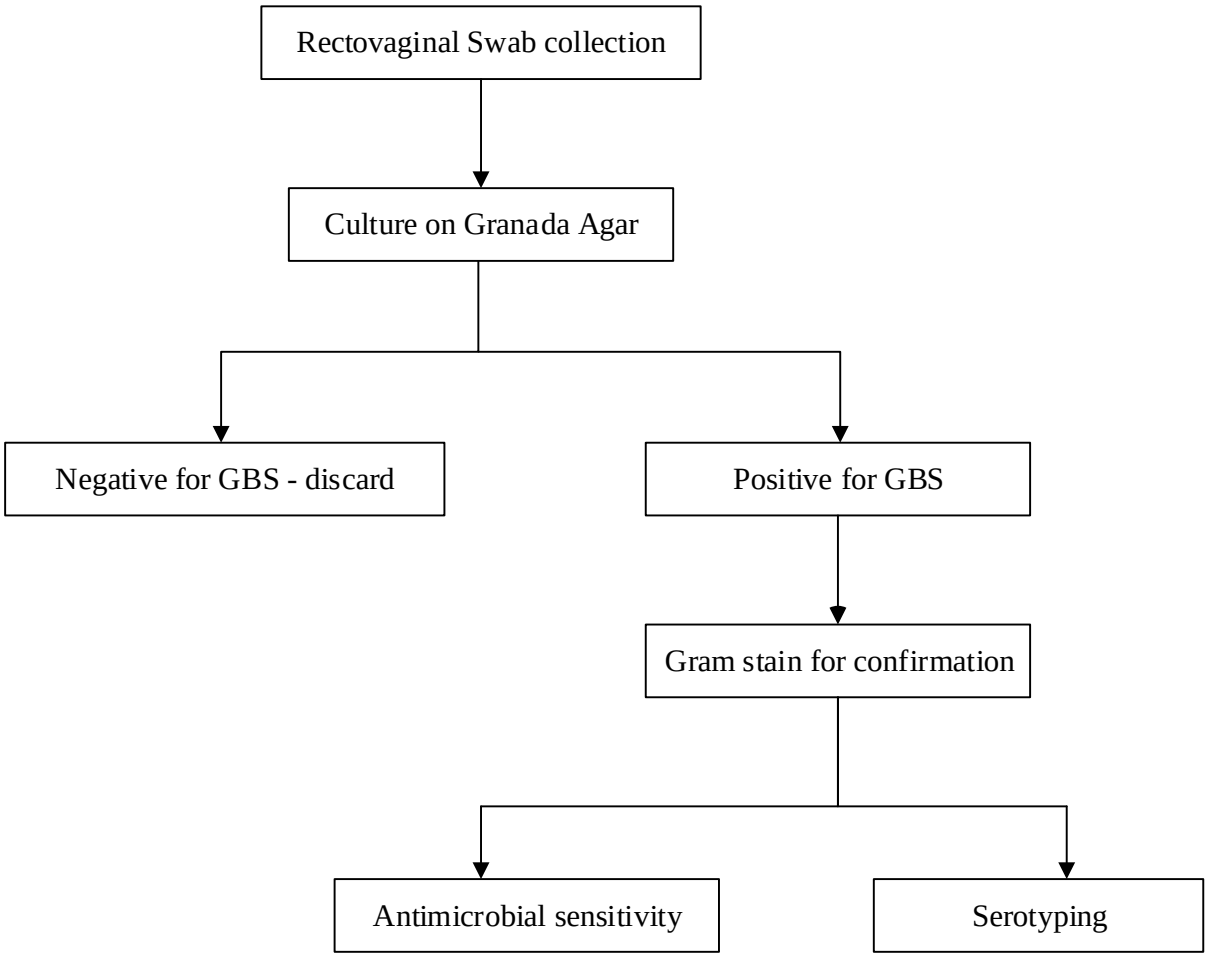
In the lab, culture plates were retrieved from the cooler box and registered in the study sample reception book. While following CTRL laboratory standard operation procedure on microbial culture, a sterile wire loop was used to streak the plates care being observed not to overlay contents of one half of the plate with the other.

The plates were incubated anaerobically in a candle jar at 35 ± 2^0C for 18-24 as per the instructions of the GBSA medium manufacture. After 18-24 hours, the plates were inspected for growth. GBS positive cultures were identified by the characteristic orange coloured colonies (Appendix VIII). GBSA being a selective media for *S. agalactiae*, only gram stain was used to ascertain the orange colonies as streptococcus. Culture results were recorded in the lab results worksheet indicating group B streptococcus culture results for both anal and vaginal swab.

For GBS positive cultures, using a sterile wire loop, a portion of the positive colonies was picked and inoculated into a well labelled (participant study number, collection date, inoculated microbe) 2ml vials containing skimmed milk. This was stored at -20^{0}C until testing for GBS serotypes.

Another portion of GBS positive colonies was picked using a sterile wire loop and emulsified in a clean test tube containing sterile normal saline for antimicrobial susceptibility testing.

Flow chart showing the procedure for processing clinical specimens for culture of GBS



Clients were informed of their culture results during their subsequent ANC visit and a copy filed in their ANC files. Clinicians attending to clients at the ANC clinic were informed of

the study to facilitate treatment of culture positive participants according to study treatment regimen. A study poster was pinned on the KNH labour ward notice board to inform staff of the study and need of intrapartum treatment of culture positive participants who delivered at the hospital. This information was conveyed to clinicians and nurses at the labour ward.

3.7.5.2 Antimicrobial susceptibility test

Disc diffusion test was used to determine antimicrobial susceptibility of isolates. Using a sterile wire loop, a portion of GBS positive colonies was picked and emulsified in a clean test tube containing sterile normal saline. The turbidity of the emulsified colonies was standardised to 0.5 McFarland standard then using a sterile cotton swab, inoculated on Muller Hinton Agar enriched with 5% sheep blood. The plates were allowed to dry before Penicillin G-10µg (Hardy diagnostic, Lot: 385950), ampicillin-10µg (Hardy diagnostic, Lot: 391375), clindamycin-2µg (Hardy diagnostic, Lot: 384766) and vancomycin-30µg (Hardy diagnostic, Lot: 388310) drug discs being placed on the agar equidistant from each other. The plates were incubated anaerobically in a candle jar at 35 ± 2^0 C for 18 – 24 hours. The zones of inhibition as set by Clinical and Laboratory Standards Institute (CLSI) 2011 (penicillin G-10units (S≥24 mm), ampicillin-10µg (S ≥24 mm), clindamycin-2µg (R≤15 mm, S≥19 mm) and vancomycin 30µg (S≥ 17 mm)) were used to determine the susceptibility of GBS to the drug under test at the end of the incubation.

These tests were done in conformity with CLSI 2011 recommendations (Franklin R. Cockerill et al., 2011). The antibiotics used in the susceptibility tests are those used for intrapartum management of GBS at KNH as per the hospital protocol (KNH, 2015).

3.7.5.4 Serotyping

Stored samples were retrieved, thawed to room temperature, vortexed and sub-cultured on Granada agar that had been appropriately labelled. The plates were incubated anaerobically

in a candle jar at 35 ± 2^0 C for 18 – 24 hours. The colonies were picked and emulsified in normal saline that had been placed in a correspondingly labelled culture tube.

The serotype of GBS was determined by latex agglutination method using Immulex Strep-B kit (Statens serum Institute-Denmark Article 54991/Lot: V3). This kit contains 10 vials of antisera each corresponding to one GBS serotype, control reagents are also supplied. For each GBS isolate, on a carbon test card with 10 circles labelled corresponding to the serotypes from Ia, Ib, II - IX, 10µl of GBS emulsified in normal saline was placed. A 10µl drop of the corresponding Immulex Strep B antisera was added to the suspension and mixed with a mixing stick before gently rocking while observing for agglutination. Agglutination that occurred within 5 seconds of mixing was interpreted as a positive reaction for that serotype. Lack of agglutination within 5 seconds or an agglutination that occurred more than 5 seconds after the mixing was interpreted as negative for that serotype. This test was as per the manufacturer's instruction. Results for each culture was recorded in the corresponding patient's lab results worksheet. An experienced lab technologist conducted all the laboratory tests. A pure culture of *Streptococcus pyogenes* obtained from the microbiology department of the University of Nairobi was used as the control microbe in isolation and serotyping of GBS.

3.8 Data Analysis

Study participant data was fed into an Ms Excel-2016. The excel file was exported into SPSS® version 25 for cleaning and coding. This was then saved as a .dta 13 SE file and exported to STATA® version 13 for data analysis. Logistic regression was used to check the association between tested risk factors and GBS colonization. Similarly, both univariate and multivariate analysis were used in testing the association between the risk factors and GBS colonization. P value of <0.05 was set for statistical significance.

3.9 Ethical Issues

Approval to conduct the study was given by the following bodies: University of Nairobi Institute of Tropical and Infectious Diseases (UNITID), the Board of Postgraduate Studies of the University of Nairobi, the Ethics and Review Board of Kenyatta National Hospital and University of Nairobi (KNH-UoN ERC) (Study registration number P521/07/2016). The KNH-UoN ERC study approval number was KNH-ERC/A/399. The Research and programs office of KNH and the Director of Reproductive services at KNH. .

Signed informed consent forms were obtained from all study participants before enrolling them into the study. Voluntary withdrawal of participants was allowed at any point of the study without any repercussion.

CHAPTER FOUR

4.0 RESULTS

Between August and November 2017, a total of 350 pregnant women were screened. In total, 292 eligible pregnant women at different gestation ages and receiving antenatal care at Kenyatta National Hospital consented to participate in this study. From these, 292 questionnaires were fully filled and 292 (100%) vaginal swab samples and 288 (98.6%) anorectal swab samples collected for GBS culture. Four participants declined to have anorectal swabs collected.

4.1. Sociodemographic and reproductive characteristics of pregnant women receiving antenatal care at Kenyatta National Hospital

The study participants had a median age of 30 years (IQR 26-35). About one in five (n=55, 18.8%) were aged bellow 25 years, majority (n=177, 60.6%) were aged between 26 to 35 years while another one in five were aged 36 years or older (n=60, 20.5%). The median gestation age of study participants was 35 weeks (IQR 30 - 37). Less than half (n=132, 45.2%) of the participants were of gestation age 34 weeks and below, 39.7% (n=116) between 35 to 37 gestation weeks and 15.1% (n=44) were term i.e. greater than 37 weeks.

Half of the participants (n=156, 53.4 %) were multiparous. The median parity was three pregnancies (IQR 2-4). Only 53 (18.2%) of the pregnant women were primigravida. Approximately three quarters of participants (n=210, 71.9%) had one or more live births, however, 29 (9.9%) of participants though having been pregnant before, had never delivered a live birth.

Table 1: Sociodemographic characteristics of study participants

Participant characteristics		Total (N=292) (100%)
Age (Years)	≤ 25	55 (18.8%)
	26 -35	177 (60.6%)
	≥ 36	60 (20.5%)
Gestation groups	< 35 Weeks	132 (45.2%)
	35-37 Weeks	166 (39.7%)
	> 37 weeks	44 (15.1%)
Parity	Null para	53 (18.2%)
	One	83 (28.4%)
	Multiparous	156 (53.4%)

Approximately one third of participants (n=93, 31.8%) had a history of stillbirth, one in ten (n= 32, 11.0%) had a history of abortion or ectopic pregnancies while approximately one fifth (n=57, 19.5%) had a history of preterm birth. One in five participants (n=58, 19.9%) had a history of membranes rupture, more than 18 hours before labour. Approximately one tenth of participants (n=32, 11%) had a history of neonatal death within the first week of childbirth. Children born to one in ten of participants (n=30, 10.3%) in previous pregnancies had an infection within the first week of childbirth. Allergy to penicillin was reported among 5.1% (n=15) participants. HIV infection was reported among 5.1% (n=15) of participants. More than half of participants (n=164, 56.2%) had white coloured vaginal discharge, approximately one in five (n=56, 19.2%) had clear coloured discharge, brown coloured discharge was produced by 40 (13.7%) of participants while approximately one in ten (n=32, 11%) had yellow coloured vaginal discharge.

Table 2: Reproductive characteristics of pregnant women receiving antenatal care at Kenyatta National Hospital.

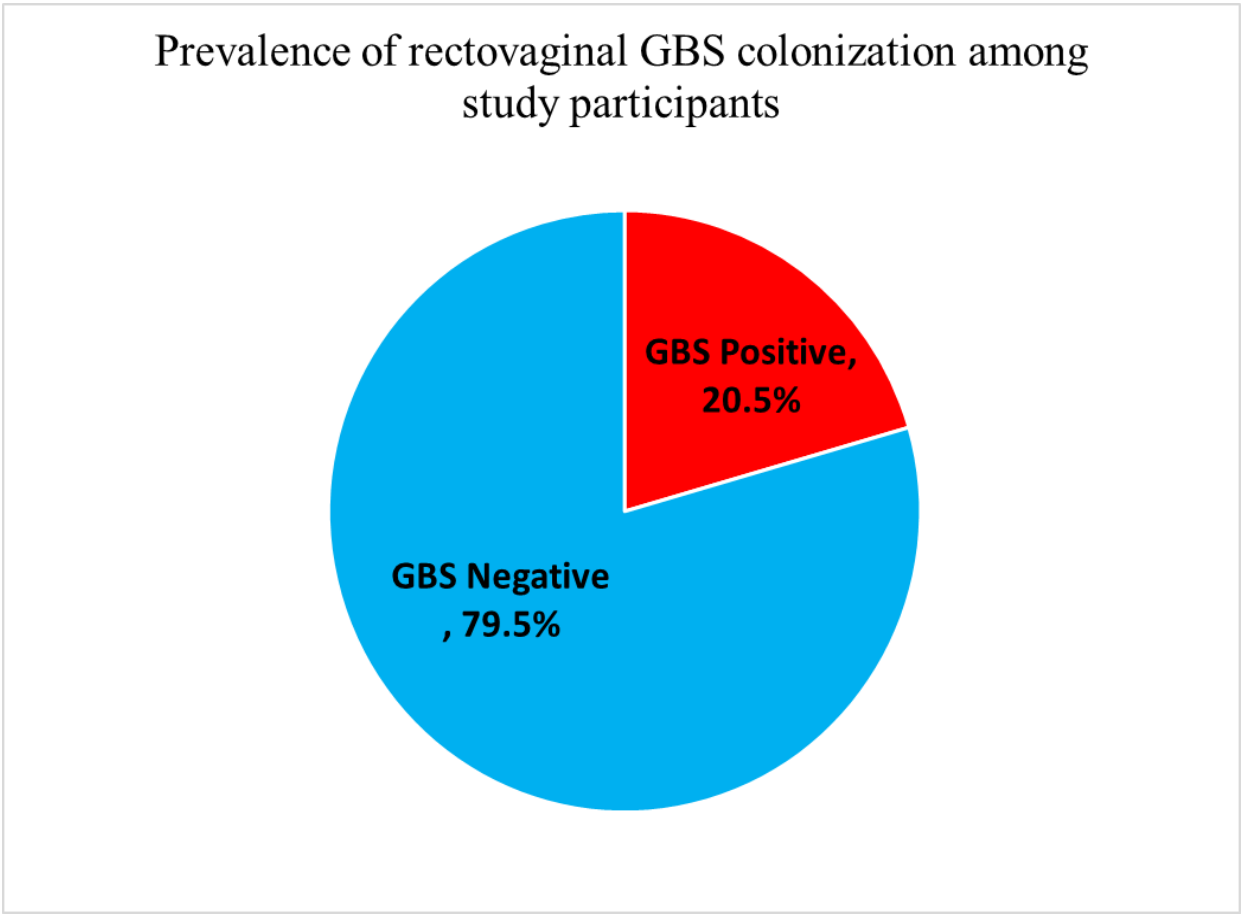
Demographics		Total (N=292) N (%)
Number of prior stillbirth		93 (31.8%)
History of pregnancy loss in prior pregnancies (abortion or ectopic)		32 (11%)
History of preterm birth in prior pregnancy		57 (19.5%)
Rupture of membranes for >18 hours before labour in prior pregnancy		58 (19.9%)
Past history of neonatal death in first week of birth		32 (11%)
Past history of neonatal infection after birth		30 (10.3%)
Fore water break in past pregnancy Rupture of membranes?		7 (2.4%)
Allergy to penicillin	Yes	15 (5.1%)
	Don't Know	57 (19.5%)
HIV status	Positive	15 (5.1%)
Colour of vaginal discharge	Yellow	32 (11%)
	Brown	40 (13.7%)
	White	164 (56.2%)
	Clear	56 (19.2%)

4.2 Objective One: To determine the prevalence of Group B Streptococcus among pregnant women receiving antenatal care at Kenyatta National Hospital

4.2.1 Prevalence of GBS among pregnant women receiving antenatal care at Kenyatta National Hospital

Out of the 292 study participants 20.5% (n=60) tested positive to recto-vaginal colonization on both culture and gram stain.

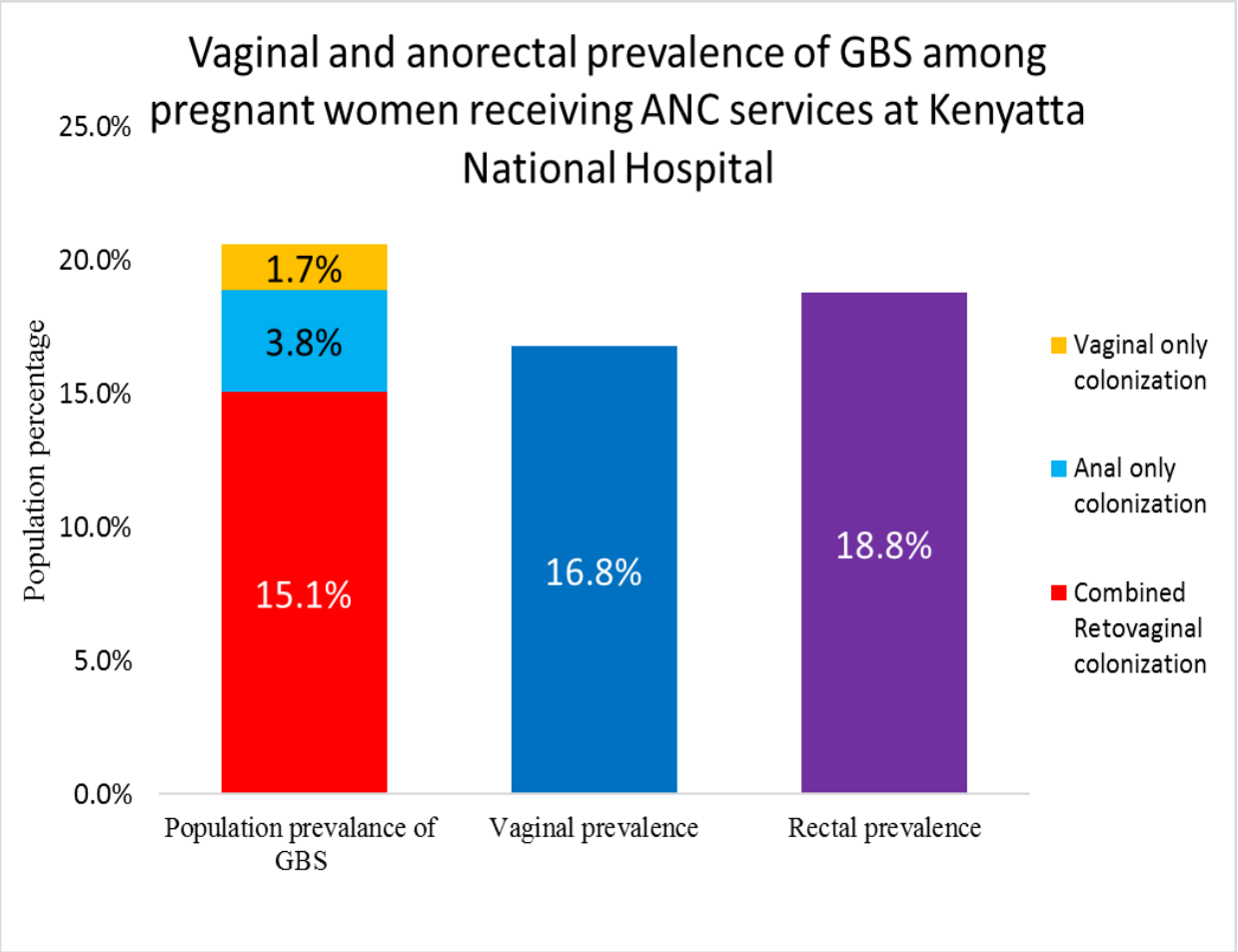
Figure 1: Prevalence of rectovaginal GBS colonization among pregnant women receiving antenatal care at Kenyatta National Hospital



4.2.2 Vaginal and anorectal prevalence of GBS among pregnant women receiving antenatal care at Kenyatta National Hospital

GBS was cultured from vaginal swabs of 16.8% (49/292) of participants and anorectal swabs of 18.8% (55/288) of participants. Combined, 15.1% (44/292) of participants had both anal and vaginal GBS colonization while 3.8% (11/288) had rectal only colonization with 1.7% (5/292) having vaginal only colonization..

Figure 2: Vaginal, Anorectal and Combined rectovaginal prevalence of GBS among study participants



4.2.3 Level of agreement between anal and rectal GBS colonization among study participants

There was high agreement between anal and vaginal colonization with GBS of 76.8% kappa (p-value <0.0001).

Table 3: Level of agreement between vaginal and rectal colonization

Symmetric Measures					
		Value	Asymptotic Standardized Error[a]	Approximate T[b]	Approximate Significance
Measure of Agreement	Kappa	0.768	0.047	13.872	0.000
N of Valid Cases					

a. Not assuming the null hypothesis.
b. Using the asymptotic standard error assuming the null hypothesis.

4.2.4 Sociodemographic and obstetric characteristics of GBS rectovaginal colonization among pregnant women receiving antenatal care at Kenyatta National Hospital

The prevalence of GBS was highest among participants within the 26-35 years age bracket at 13.0% (n=38), approximately one in twenty participants (n=12, 4.1%) aged above 36 years were GBS colonised while 10 (3.4%) participants aged below 25 years were GBS colonised. Participants within the 35-37 weeks gestation bracket had the highest prevalence to GBS (n=28, 9.6%) followed by participants of gestational age below 35 weeks (n=26, 8.9%). The prevalence of GBS was lowest among participants of gestational age above 37 weeks (n=8, 2.1%).

The prevalence of GBS among participants with a history of stillbirth was 6.5% (n=19), abortion or ectopic pregnancy was 3.1% (n=9), preterm was 4.1% (n=12); for participants with a history of fore water break, more than 18 hours before labour, the prevalence was 3.4% (n=10). The prevalence was 2.1% (n=6) among participants allergic to penicillin, and 1.0% (n=3) among HIV-positive participants; participants with white coloured vaginal discharge had the highest GBS colonization with a prevalence of 11.0% (n=32) followed by clear coloured vaginal discharge with a prevalence of 4.5% (n=13).

Table 4: Sociodemographic and reproductive characteristics among GBS colonized pregnant women receiving antenatal care at Kenyatta National Hospital.

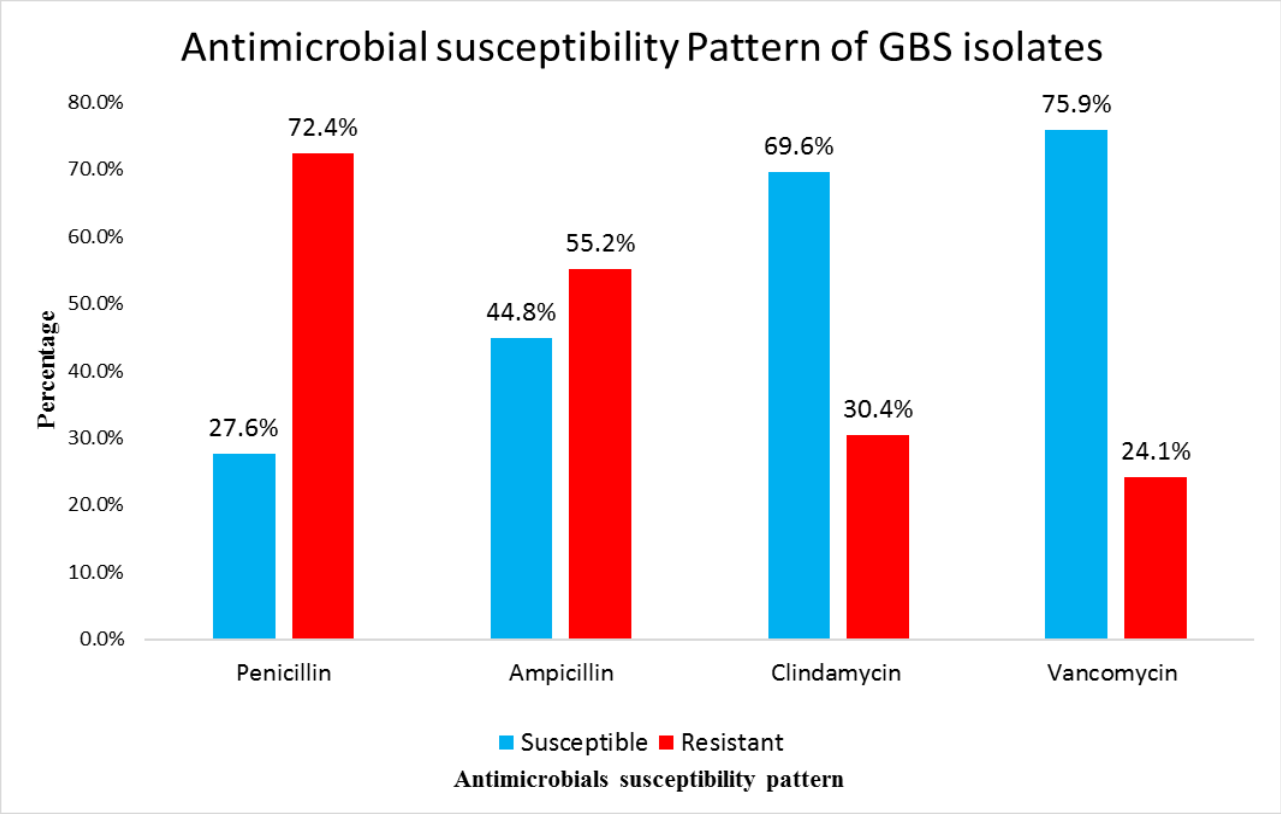
Sociodemographic and reproductive characteristics		GBS culture results				
		Negative (N=232)		Positive (N=60)		P
		n (%)	mean (SD)	n (%)	mean (SD)	
Maternal age (Years)	<26	45 (15.4%)	30.55 (5.53)	10 (3.4%)	30.72 (5.42)	0.864
	26 -35	139 (47.6%)		38 (13.0%)		
	>36	48 (16.4%)		12 (4.1%)		
Gestation age (weeks)	<35	106 (36.3%)	33.18 (5.31)	26 (8.9%)	33.02 (5.01)	0.323
	35-37	88 (30.1%)		28 (9.6%)		
	>37	38 (13.0%)		6 (2.1%)		
Parity	0	43 (14.7%)	1.33 (0.77)	10 (3.4%)	1.43 (0.77)	0.499
	1	69 (23.6%)		14 (4.8%)		
	>1	120 (41.1%)		36 (12.3%)		
Number of prior live births if parity >0	0	25 (8.6%)	1.36 (0.82)	4 (1.4%)	1.54 (1.05)	0.566
	>0	164 (56.2%)		46 (15.8%)		
Number of prior stillbirth	Yes	74 (25.3%)	0.63 (0.96)	19 (6.5%)	0.56 (0.84)	0.935
History of pregnancy loss in prior pregnancies (abortion or ectopic)	Yes	23 (7.9%)	0.15 (0.44)	9 (3.1%)	0.26 (0.7)	0.526
History of preterm birth in prior pregnancy	Yes	45 (15.4%)	0.37 (0.82)	12 (4.1%)	0.38 (0.83)	0.945
Rupture of membranes for >18 hours before labour in prior pregnancy	Yes	48 (16.4%)	0.58 (0.79)	10 (3.4%)	0.5 (0.77)	0.688
Past history of neonatal death in first week of birth	Yes	23 (7.9%)	0.47 (0.79)	9 (3.1%)	0.48 (0.77)	0.526
Past history of neonatal infection after birth	Yes	25 (8.6%)	0.48 (0.79)	5 (1.7%)	0.42 (0.76)	0.782
Fore water break in past pregnancy Rupture of membranes?	Yes	5 (1.7%)		2 (.7%)		0.636
Allergy to penicillin	Yes	9 (3.1%)	0.47 (0.83)	6 (2.1%)	0.33 (0.68)	0.051
	Don't Know	50 (17.1%)		7 (2.4%)		
HIV status	Positive	12 (4.1%)	0.05 (0.22)	3 (1.0%)	0.05 (0.22)	0.628
Colour of vaginal discharge	Yellow	25 (8.6%)	2.83 (0.85)	7 (2.4%)	2.85 (0.9)	0.942
	Brown	32 (11.0%)		8 (2.7%)		
	White	132 (45.2%)		32(11.0%)		
	Clear	43 (14.7%)		13 (4.5%)		

4.3 Objective Two: To determine the antimicrobial susceptibility pattern of Group B Streptococcus isolated from pregnant women receiving antenatal care at Kenyatta National Hospital

4.3.1 Antimicrobial susceptibility pattern of GBS rectovaginal isolates among pregnant women receiving antenatal care at Kenyatta National Hospital

Penicillin G, ampicillin and vancomycin susceptibility pattern was tested on 58 samples while clindamycin susceptibility was tested on 46* of the samples. GBS bacterium isolated in this population had the highest resistance to penicillin G (72.4%, n=42) and ampicillin (55.2%, n=32). Resistance to vancomycin and clindamycin was at 24.1% (n=14) and 30.4% (n=14) respectively.

Figure 3: Antimicrobial susceptibility pattern of GBS isolates among pregnant women receiving antenatal care at Kenyatta National Hospital



*Clindamycinn susceptibility was performed on only 46 colonies due to depletion of cultures prior to condictinng the test.

4.3.2 Comparison of antimicrobial susceptibility pattern of GBS isolated from the vaginal and anorectal canals among pregnant women receiving antenatal care at Kenyatta National Hospital.

Comparison of antimicrobial susceptibility pattern between anal and vaginal GBS isolates found 69.4% of vaginal isolates to be resistant to penicillin G compared to 75.5% of anal isolates. On the other hand, 51.0% of vaginal isolates were resistant to ampicillin compared to 56.6% of anal isolates, 20.4% of vaginal isolates were resistant to vancomycin compared to 24.5% of anal isolates while 30.0% of vaginal isolates were resistant to clindamycin compared to 31.0% of anal isolates.

Table 5: Comparison of GBS antimicrobial susceptibility between vaginal and anal isolates

	Antimicrobial	**Susceptibility pattern**	
		Susceptible (n,%)	**Resistant (n,%)**
GBS Vaginal colonization	Penicillin	15 (30.6%)	**34 (69.4%)**
	Ampicillin	24 (49.0%)	**25 (51.0%)**
	Clindamycin	39 (79.6%)	**10 (20.4%)**
	Vancomycin	28 (70%)	**12 (30%)**
GBS Anal colonization	Penicillin	13 (24.5%)	**40 (75.5%)**
	Ampicillin	23 (43.4%)	**30 (56.6%)**
	Clindamycin	40 (75.5%)	**13 (4.5%)**
	Vancomycin	29 (69.0%)	**13 (31.0%)**

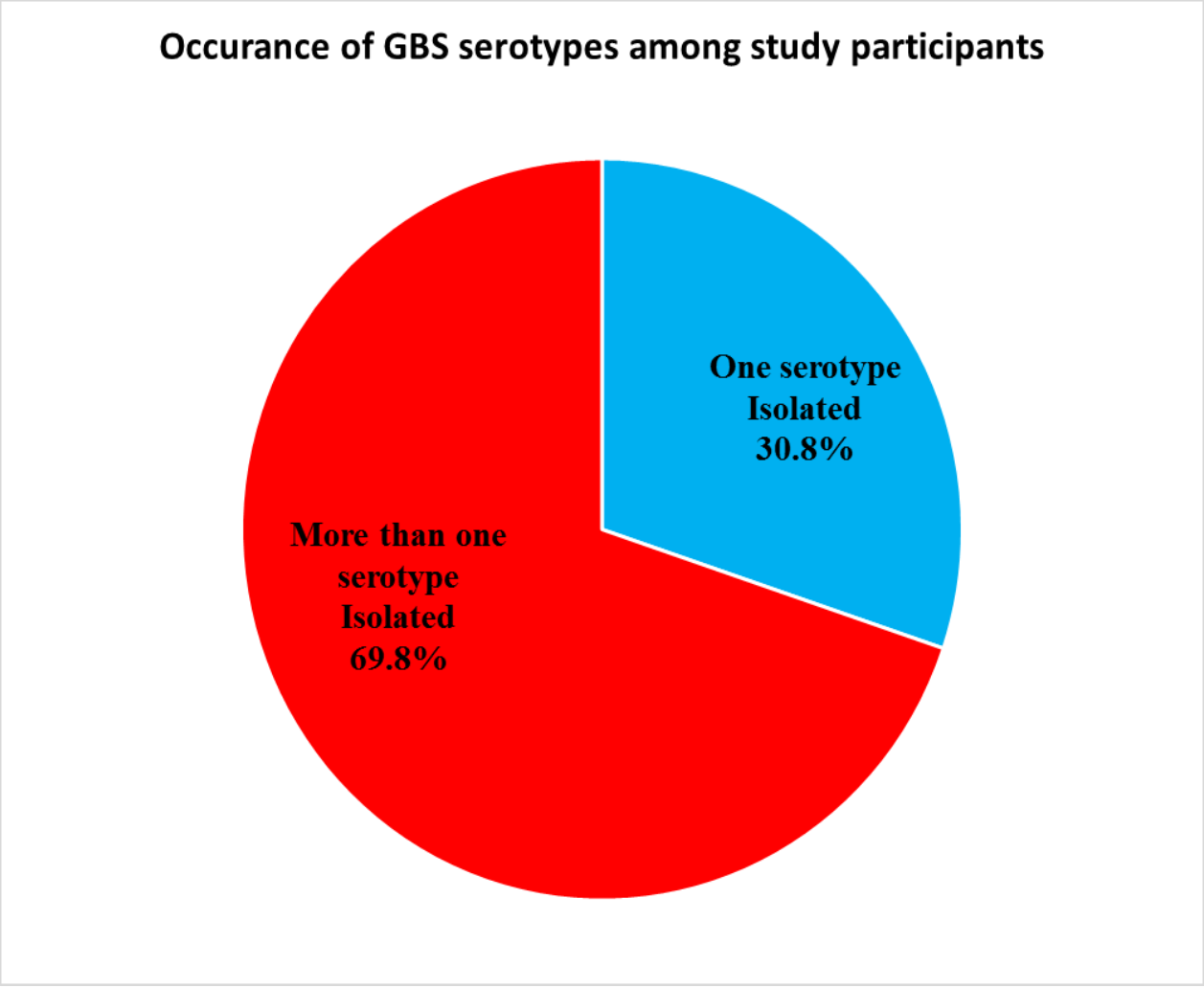
*

4.4 Objective Three: To determine the serotypes of Group B Streptococcus bacteria isolated from pregnant women receiving antenatal care at Kenyatta National Hospital

4.4.1 Occurrence of GBS serotypes among study participants

Out of the 60 GBS positive samples, serotype testing was conducted on 53 samples; seven samples did not grow after repeated sub-culturing. In this study, 69.8% (37/53) of participants were colonised by more than one GBS serotype.

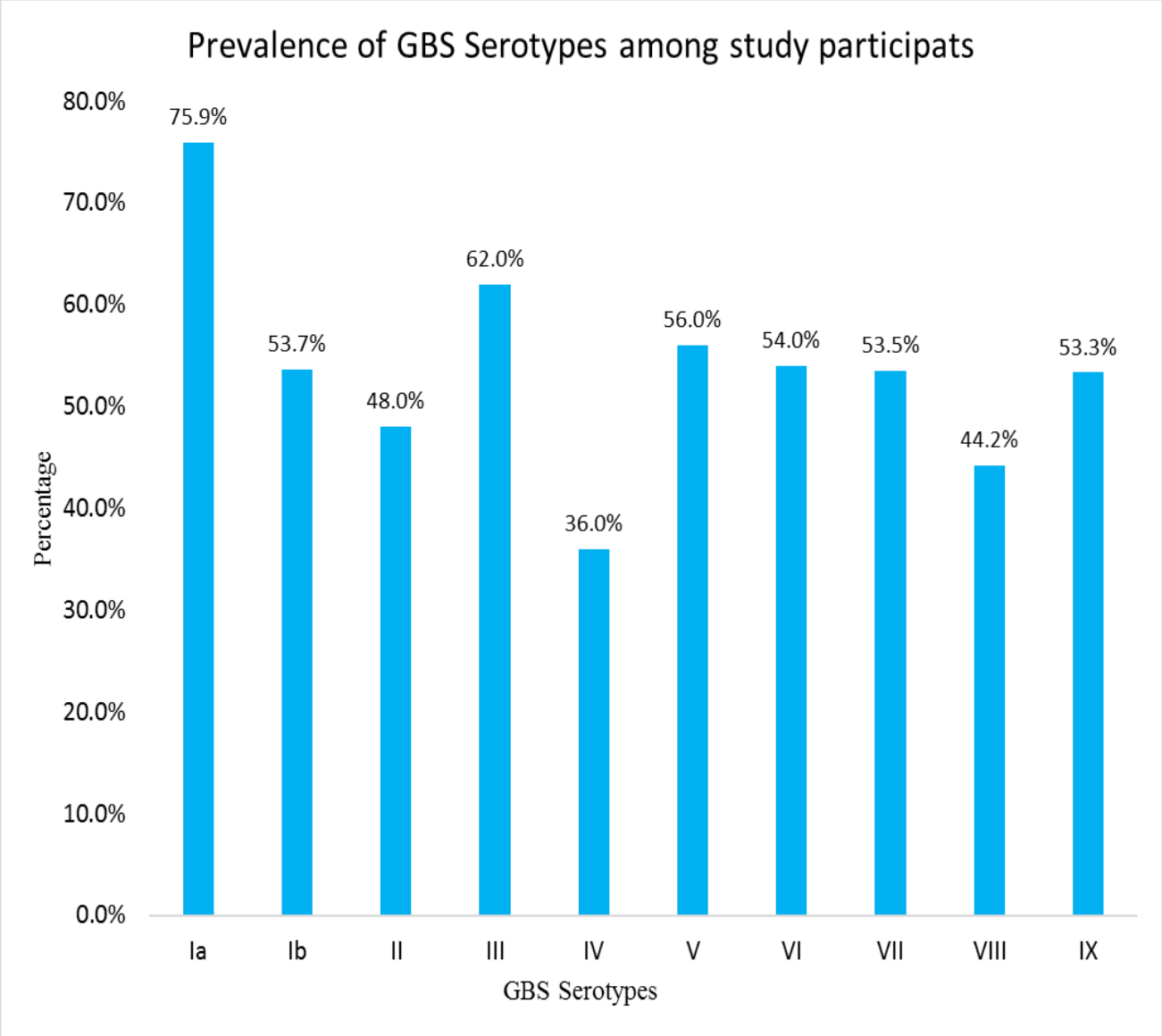
Figure 4: Occurrence of GBS serotypes among participants



4.4.2 Serotypes of isolated Group B Streptococcus bacteria

All ten known GBS serotypes occurred in this population. Serotype Ia was the most common serotype isolated from 75.9% of tested cultures followed by serotype III at 62.0%. Serotype IV was the least common serotype isolated from 36.0% of tested cultures.

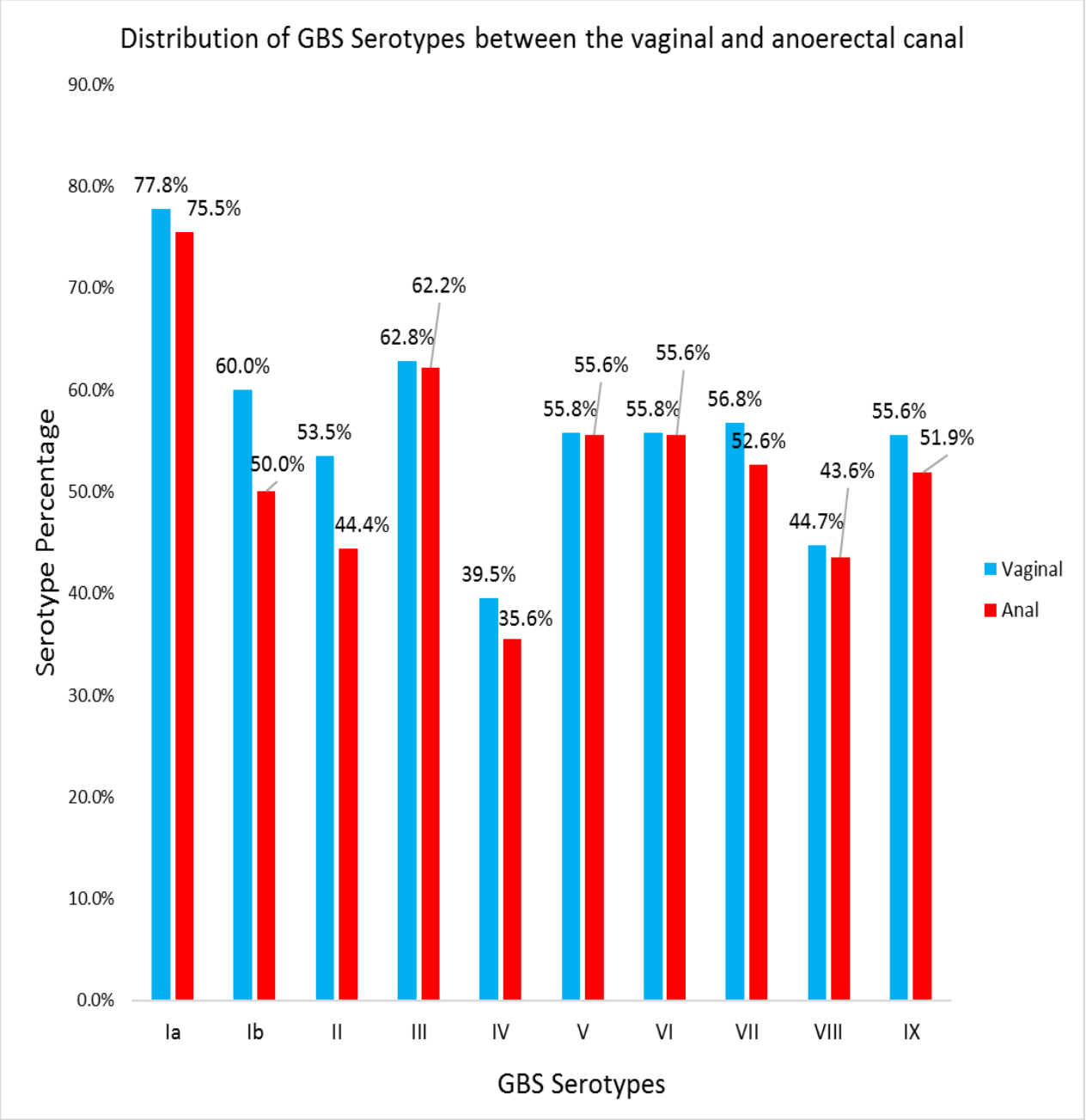
Figure 5: Prevalence of GBS serotypes among study participants



4.4.3 Prevalence of serotypes between vaginal and anorectal isolates

The percentage occurrence of isolated GBS serotypes was found to be higher in the vaginal canal compared to the anorectal canal as indicated in figure 7.

Figure 6: Percentage occurrence of GBS serotypes in vaginal and rectal canals



4.4.4 Antimicrobial susceptibility pattern of GBS serotypes isolated from the rectovaginal canals of pregnant women receiving antenatal care at Kenyatta National Hospital

As summarised in table 5, there was high resistance to penicillin G and ampicillin by all isolated GBS serotypes that ranged from 68% to 84% and 57% to 78% respectively. In comparison, resistance to vancomycin and clindamycin was low at 16% to 26% and 15% to 33% respectively.

Serotypes IV isolates were the most resistant to penicillin G and ampicillin at 83.3% and 77.8% respectively while serotype Ia had the highest resistance to clindamycin at 33.3%; serotype VI was the most resistant to vancomycin at 25.9%. Overall, serotype IV was the most resistant to all tested antimicrobials while serotype II was the least resistance to tested antimicrobials.

Table 6: Antimicrobial susceptibility pattern of GBS serotypes isolated among pregnant women receiving antenatal care at Kenyatta National Hospital

GBS Serotype		Penicillin G susceptibility	Ampicillin susceptibility	Vancomycin susceptibility	Clindamycin susceptibility
		Resistant	**Resistant**	**Resistant**	**Resistant**
Ia	41 (n, %)	33 (80.5%)	25 (61.0%)	8 (19.5%)	**11 (33.3%)**
Ib	32 (n, %)	15 (68.2%)	14 (63.6%)	5 (22.7%)	6 (31.6%)
II	24 (n, %)	17 (70.8%)	16 (66.7%)	4 (16.7%)	3 (15%)
III	21 (n, %)	23 (74.2%)	22 (71%)	**8 (25.8%)**	6 (27.3%)
IV	18 (n, %)	**15 (83.3%)**	**14 (77.8%)**	4 (22.2%)	3 (21.4%)
V	28 (n, %)	20 (71.4%)	16 (57.1%)	7 (25%)	5 (22.7%)
VI	27 (n, %)	22 (81.5%)	19 (70.4%)	7 (25.9%)	6 (28.6%)
VII	23 (n, %)	17 (73.9%)	15 (65.2%)	5 (21.7%)	3 (15.9%)
VIII	19 (n, %)	15 (78.9%)	14 (73.7%)	4 (21.1%)	3 (21.4%)
IX	16 (n, %)	12 (75%)	10 (62.5%)	3 (18.7%)	3 (23.1%)

4.4.5 Association between GBS serotype and antimicrobial susceptibility among pregnant women receiving antenatal care at Kenyatta National Hospital.

Test of association between GBS serotype and antimicrobial susceptibility as summarized in table 10, showed serotype Ia to be associated with penicillin resistance (OR 4.8; CI: 1.265-18.311, p=0.021). Serotypes III (OR 6.84; CI:1.899-24.672, p=0.003), IV (OR 5.12; CI:1.372-19.077, p=0.015), VI (OR 4.45; CI:1.353-14.653, p=0.014), and VIII (OR 4.67; CI:1.255-17.358, p=0.022) were associated with ampicillin resistance. None of the isolates was associated with either vancomycin or clindamycin resistance.

Table 7: Association between GBS serotype and antimicrobial pattern indicating OR

Serotype	Penicillin G				Ampicillin				Vancomycin				Clindamycin			
	OR	95% CI		P	OR	95% CI		P	OR	95% CI		P	OR	95% CI		P
Ia	**4.81**	**1.265**	**18.311**	**0.021**	3.52	0.926	13.353	0.065	0.81	0.18	3.635	0.781	1.33	0.709	0.294	0.71
Ib	1.25	0.343	4.558	0.735	2.41	0.684	8.471	0.171	0.82	0.198	3.432	0.79	1.38	0.668	0.312	0.67
II	1.08	0.321	3.626	0.902	2.73	0.862	8.625	0.088	0.54	0.137	2.157	0.385	0.33	0.154	0.071	0.15
III	1.68	0.49	5.745	0.41	**6.84**	**1.899**	**24.672**	**0.003**	1.86	0.426	8.087	0.411	1.31	0.714	0.307	0.71
IV	3	0.717	12.553	0.132	**5.12**	**1.372**	**19.077**	**0.015**	1.02	0.254	4.104	0.977	0.74	0.702	0.158	0.7
V	1.17	0.346	3.933	0.804	1.33	0.434	4.094	0.615	1.5	0.377	5.965	0.565	0.76	0.714	0.182	0.71
VI	3.38	0.947	12.098	0.061	**4.45**	**1.353**	**14.653**	**0.014**	1.66	0.418	6.606	0.47	1.5	0.585	0.351	0.59
VII	1.89	0.519	6.868	0.334	3.48	0.99	12.242	0.052	0.83	0.202	3.435	0.801	0.29	0.124	0.062	0.12
VIII	2.25	0.567	8.927	0.249	**4.67**	**1.255**	**17.358**	**0.022**	1.33	0.286	6.214	0.714	0.76	0.746	0.149	0.75
IX	2.25	0.478	10.595	0.305	3	0.676	13.309	0.148	0.85	0.141	5.07	0.855	0.48	0.399	0.087	0.4

4.5 Objective Four: To determine risk factors for Group B Streptococcus colonization among pregnant women receiving antenatal care at Kenyatta National Hospital.

Logistic regression was conducted to determine predictors of positive GBS colonization. The variables were analysed both individually and in combination to ascertain their ability to predict positive GBS colonization. None of the variables in this study was a predictor of positive GBS colonization as indicated by the greater than 0.05 p value in table 10.

Table 8: Logistic regression indicating to determine risk factors

GBS Results	Odds Ratio	95% Conf. Interval		P
Maternal age (Years)	1	0.935306 -	1.05806	0.868
Parity	1.1	0.77031 -	1.513787	0.656
Parity group	2.3	0.874628 -	5.994125	0.092
Gestation Age	1	0.934227 -	1.105132	0.71
Gestation groups	0.8	0.445559 -	1.513384	0.528
Number of prior live births	1.1	0.769202 -	1.505438	0.669
Still births	0.7	0.458743 -	1.16479	0.187
History of pregnancy loss in prior pregnancies (abortion or ectopic)	1.3	0.767093 -	2.195339	0.331
History of preterm birth in prior pregnancy	1	0.643328 -	1.510532	0.948
Rupture of membranes for >18 hours before labour in prior pregnancy	0.7	0.303695 -	1.601734	0.396
Past history of neonatal death in first week of birth	2.1	0.801911 -	5.60262	0.13
Neonatal infection	0.5	0.149276 -	1.605294	0.238
Fore water break in past pregnancy/Rupture of membranes	1.4	0.227579 -	8.407042	0.725

CHAPTER FIVE

5.0 DISCUSSION

At 20.5%, the rectovaginal prevalence of GBS among pregnant women receiving antenatal care at Kenyatta National Hospital was slightly higher than that reported by prior studies in this region or similar settings. In Mombasa, Cools et al. (2016) reported a GBS prevalence of 20.2% while the prevalence has been reported to vary between 10 and 35 % in the USA (Gilbert, 2004) and between 1 and 30% in other developing countries (Bolukaoto et al., 2015; Cools et al., 2016; Gilbert, 2004; Stoll & Schuchat, 1998). Even so, this prevalence is slightly lower than that reported by Salat *et al*, in a study conducted in the same population in the year 2009 (Salat et al, 2009). Difference of the prevalence in the two studies could be explained by the difference in gestational ages of women who formed study participants of the two studies. Salat restricted his participants to women of gestational age 35 to 37 weeks while this study had participants of gestational ages 12 to 40 weeks. In the current study, grouping of participants based on gestational age, found those of gestational age between 35 to 37 weeks to have higher prevalence (9.6%) of GBS in comparison to gestational groups <35 weeks (8.9%) and >37 weeks (2.1%). Previous studies (Boyer et al., 1983) have reported gestational age of women as a risk factor to GBS with the prevalence being higher among women of gestational age between 35 and 37 weeks (Boyer et al., 1983; Doare et al., 2016).

Similarly, the prevalence reported in the current study is higher than that reported by Lu et al. (2014) in Beijing, China (7%), Woldu et al, (2014) in Ethiopia (7.2%) and Seale et al. (2016) in Kilifi, Kenya (12%) (Lu et al., 2014; Seale et al., 2016; Z. L. Woldu et al., 2014). The variations in the prevalence reported in the current study, and that reported by Cools et al. (2016) and Seale et al. (2016) agrees with findings by Stoll and Schuchat (1998) of

occurrence of regional variation in GBS prevalence even among people sharing geographical boundaries and with similar socio-economic conditions (Cools et al., 2016; Seale et al., 2016; Stoll & Schuchat, 1998). On the other hand, difference in the prevalence could be due to the culture method used, this study used Granada agar which has higher sensitivity and specificity to GBS compared to blood agar plates used by Lu et al. (2014) (Lu et al., 2014). It has been noted that variation in GBS prevalence could be as a result of culture methods and type of medium used (Zufan Lakew Woldu et al., 2014).

The anorectal GBS carriage (18.8%) in this study was slightly higher than the vaginal carriage (16.8%) though it is thought the anorectal colonization could be higher than reported as some participants who tested positive to vaginal colonization with GBS declined to give anal swab. Nevertheless, the vaginal GBS carriage reported in this study is higher than that reported by Salat (2009) of 14%, however, the anorectal GBS colonization in this study is lower than that reported by Salat (2009) of 21.2%. The differences in anal GBS prevalence in the two studies could be due to some women in our study declining rectal swab collection while the high vaginal colonization could be due to the superior Granada culture media used by this study as opposed to blood agar plates used by Salat in GBS isolation. Even so, both studies reported high agreement between anorectal and vaginal GBS recovery of 76.8% ($p<0.0001$) in the current study and 84.7% ($p<0.0001$) by Salat (Salat, 2009).

The antimicrobial pattern of isolated GBS was tested against penicillin G, ampicillin, vancomycin and clindamycin which are the drugs used in intrapartum treatment of women suspected to be GBS colonised at KNH (KNH, 2015). The bacteria showed a high resistance to penicillin G and ampicillin of 72.4% and 54.2% respectively. There was however low resistance to clindamycin and vancomycin at 30.4% and 24.1% respectively. Previous studies have reported GBS to be susceptible to penicillin, ampicillin and cephalosporins (Bolukaoto et al., 2015; Verani, McGee, & Schrag, 2010) even though bacteria with increasing minimum

inhibitory concentration to penicillin and ampicillin have been reported (Bolukaoto et al., 2015; Verani et al., 2010). The high resistance of GBS to penicillin G reported in our study mirrors that reported by Mengist, Zewdie, Belew, and Dabsu (2017) of 77.3% (Mengist et al., 2017). Findings of the current study partly agrees with those of Lu et al. (2014), Yoon et al. (2015) and Mengist et al. (2017) who reported a resistance to clindamycin of 55.7%, 55.4% and 50% respectively (Lu et al., 2014; Mengist et al., 2017; Yoon et al., 2015). However, the resistance in the three studies is higher than that reported in the current study of 30.4%. Nevertheless, the isolates in Yoon et al. (2015) and Lu et al. (2014) studies retained 100% susceptibility to penicillin, ampicillin and vancomycin. On the other hand, the resistance of GBS to clindamycin reported in our study is higher than that reported by Bolukaoto et al. (2015) of 17.2%, however, Bolukaoto et al. (2015) isolates retained 100% susceptibility to penicillin, ampicillin, vancomycin and high level gentamycin even though they had high resistance to tetracycline of 86.7% (Bolukaoto et al., 2015).

To the best of our knowledge, no previous study has compared the antimicrobial susceptibility patterns between GBS bacteria isolated from the anorectal canal and those isolated from the vaginal canal as did this study. Our findings showed a higher resistance of anal isolates to tested antimicrobials in comparison to vaginal isolates. Increase in resistance of GBS to β-lactam antibiotics has been attributed to alterations in the penicillin binding protein 2x (Verani et al., 2010) while resistance to clindamycin has been attributed to presence of erm-methylase gene (Metcalf et al., 2017). In his study to determine antibiotic resistance of GBS among pregnant women in Garankuwa, South African, Bolukaoto et al. (2015) reported methylation of *ermB* genes to be the single most common mechanism of resistance employed by isolated bacteria. Other mechanisms identified were efflux pump mediated by *mefA* genes and *ermTR* genes. This high resistance limits antibiotic use and restricts treatment to clindamycin and vancomycin.

All the known ten serotypes of GBS were found to be occurring among study participants in the current study. The most occurring serotype was Ia (75.9%) followed by III (62%), V (56%), VI (54%), Ib (53.7%), VII (53.5%), IX (53.3%), II (48%) and VIII (44.2%). Serotype IV was the least occurring in this population at 36%. 66.7% of participants were found to harbour more than one serotype of GBS. These findings agree with Rench and Baker (1993) who reported serotypes Ia, Ib, II and III as the most occurring, similarly, they mirror Lu et al. (2014) who isolated eight GBS serotypes among pregnant women in his study with the exception of VII and IX. Nine GBS serotypes (Ia, Ib, II-VIII) have been reported to occur in Europe and USA (Ippolito et al., 2010). Previous studies conducted in Kenya have reported the occurrence of six GBS serotypes Ia, III, V, VI, VII and VIII in a Mombasa cohort (Cools et al., 2016). Our findings also agree with observations of Dutra et al. (2014) who reported variations in the regional distribution and occurrence of GBS serotypes as this study found serotype Ia to be the most occurring in this population while Cools et al reported serotype III as the most occurring among pregnant women in Mombasa (Cools et al., 2016; Dutra et al., 2014). It is however, important to note that serotype distribution in a population could change with time. Yoon et al. (2015) in a GBS study conducted over a 20 years period found the dominant serotypes to change with time (Yoon et al., 2015).

Serotype Ia (80.5%) and VI (81.5%) had significant resistance to penicillin G while serotypes Ia (61%), III (71%), IV (77.8%), VI (70.5), VII (65.25) and VIII (73.7%) registered significant resistant to ampicillin. Colonization with these serotypes was also a predictor of antimicrobial resistance with having serotype Ia predicting for penicillin G resistance (OR 4.8; CI: 1.265-18.311, p=0.021) as was VI (OR 3.38; CI:0.947-12.098, p=0.061). Being colonized with serotypes III (OR 6.84; CI:1.899-24.672, p=0.003), IV (OR 5.12; CI:1.372-19.077, p=0.015), VI (OR 4.45; CI:1.353-14.653, p=0.014), VII (OR 3.48; CI:0.99-12.242, p=0.052) and VIII (OR 4.67; CI:1.255-17.358, p=0.022) was a predictor for ampicillin

resistance. Our findings differ from those by Yoon et al. (2015), who in their study, found no strains to be resistant to penicillin (Yoon et al., 2015). They also reported a resistance of 93.8% to clindamycin by serotype V. This level of resistance to clindamycin by serotype V is higher than that reported in our study of 22.7%.

Though some of the participants in this study reported having a history of the risk factors associated with GBS such as stillbirths, abortions, ectopic pregnancy, history of preterm births, history of neonatal deaths, history of neonatal infection and history of fore water break more than 18 hours to labour, no statistically significant association was found between these factors and GBS colonization in this study. Similarly, there was no association between maternal age, gestational age, parity and number of live birth with GBS colonization. Other studies have reported an association between GBS colonization and some of these risk factors. For examples Salat (2009) had previously reported GBS colonization to be associated with a history of stillbirth in the same population as did Seale et al. (2016) in Kilifi and Doare et al. (2016) in the Gambia who besides still birth, also noted GBS colonization to be associated with early onset neonatal disease and gestational age (Doare et al., 2016; Salat, 2009). Even though the current study did not find and association between GBS colonization and associated risk factors, the role of GBS in causing still births and neonatal sepsis cannot be ruled out given the relatively high GBS prevalence, still births and neonatal sepsis in this setting.

CHAPTER SIX

6.0 CONCLUSION

This study found the prevalence of group B streptococcus among pregnant women seeking antenatal services at KNH to be high at 20.5%. Anorectal GBS colonization was higher in comparison to vaginal colonization; even so, recovery of the bacteria from one canal had a high likelihood of recovering it from the other. No significant association was found between GBS and stillbirths, abortions or ectopic pregnancy, history of preterm births, history of neonatal deaths, history of neonatal infection and history of fore water break more than 18 hours to labour. Group B streptococcus isolated in this study were resistant to all tested antimicrobials with the highest resistance being to penicillin G while the least resistance was to vancomycin. All known ten GBS serotypes were found occurring in this population with serotype Ia being the most prevalent. All serotypes had strains that were resistant to all the four antimicrobials used for susceptibility testing in this study. Recovery of serotypes Ia and VI was associated with resistance to penicillin G while serotypes Ia, III, IV, VI, VII and VIII were associated with resistance to ampicillin. This association was statistically significant. None of the serotypes association with resistance to either clindamycin or vancomycin had statistical significant.

6.1 Study limitations

1. Not all GBS positive samples were analysed for clindamycin susceptibility pattern, hence the resistance of GBS in this setting might be higher than that indicated in this study.
2. Some participants declined anorectal swab collection, however, with the high probability of recovering GBS from either canals of colonized women (kappa 0.79, $p<0.001$), vaginal GBS culture results can be relied on.

CHAPTER SEVEN

7.0 RECOMMENDATIONS

In view of the findings of this study, the following recommendations are made;

- Due to the high GBS prevalence in this study, all pregnant women receiving antenatal services at KNH should undergo GBS screening. The gestational age 35-37 weeks will be ideal for this screening to be done.
- For pregnant women found to be rectovaginally colonized with GBS, an antimicrobial susceptibility test should be performed on the isolated GBS before intrapartum treatment to avoid administering antibiotics to which the bacteria are resistant.
- Continuous monitoring of GBS antimicrobial resistance should be done to document the emergence of antimicrobial resistant strains.
- Development of a vaccine against serotypes occurring in this population should be explored as a preventive strategy.

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APPENDICES

Appendix I: Patient consent form

PREVALENCE, ANTIMICROBIAL SUSCEPTIBILITY AND SEROTYPES OF GROUP B STREPTOCOCCUS RECTO-VAGINAL ISOLATES FROM PREGNANT WOMEN AT KENYATTA NATIONAL HOSPITAL

Investigators

Investigator	**Position**	**Institution**
Mr. Jisuvei C. Salano	Principal Investigator	UoN
Dr. Anne Maina	Supervisor	UoN
Dr. Alfred Osoti	Supervisor	UoN

Principal Investigators.	**KNH-UoN ERC Secretary Contact**
Contact: 0720-327025	2726300 ext 44102

Investigators Statement

We are kindly requesting you to be a participant in a research study. The aim of this consent form is to give you the information you require in helping you decide whether to or not to participate in this study. Please read this form carefully. You may ask questions on what we are asking of you, if there is any risk, your benefits, your rights as a volunteer or anything else about the research or that is stated on this form and is not clear. When all your questions have been answered, you can decide whether you want to participate in the study or not. This process is called informed consent.

Background information

Group B streptococcus is a bacterium that may be found in the vagina (birth canal). Women who have GBS in their vagina often do not show any signs or symptoms of disease. This bacterium is not a sexually transmitted infection. One third of all pregnant women have been found to carry these bacteria in their vagina during pregnancy. When present during pregnancy, GBS may increase the chance of a woman giving birth before the expected day of delivery. It also increases the chance of the water breaking before labour and the infection of the womb after birth. Half the babies born to women who have GBS in their vagina during pregnancy get infected. These infections include pneumonia and blood infections.

Purpose of the study.

This study will help us to find out how common GBS is among pregnant women attending clinic at the Kenyatta National Hospital. If we find that GBS is common amongst our pregnant mothers, we will advocate making GBS screening a routine a routine test for pregnant women at this hospital.

Study procedure

If you agree to participate in this study, a medical history will be taken and a physical examination done. This will entail inquiry about age, history of current pregnancy, previous miscarriages and still births.

In the presence of a female nurse (chaperon) the physician will collect two swab specimens from you (one from the lower vagina and one from the anorectal region). The swabs used in specimen collection are sterile and non- traumatic.

You will be informed about the results of the test during your next antenatal visit, this results will be shared with your primary care physician(s) for intervention where appropriate.

Confidentiality.

All information obtained will be strictly confidential and will not be released to other persons other than your primary care physician(s). Similarly, samples collected will only be used for purposes of this study and destroyed thereafter.

The quality of care given to you in this hospital will not be compromised by your refusal to participate in this study. Participation in this study is voluntary (at your own will). You have the right not to participate or withdraw at any time. About 300 women will take part in this study. One visit will be required for sample collection while a second visit will be for you to get your results. These results will be ready after one week.

Participant's benefits and Risks

Benefits

- You will be informed of your results during your next antenatal clinic visit.
- You will not pay any laboratory charges
- If it is found that you have positive tests for GBS, the results will be recorded in your file so that you are given treatment during delivery to prevent your baby from getting the infection during the birth process. This is the treatment recommended by the Centre for disease control and prevention (CDC), the Royal College of Obstetrics and Gynaecology and the America Congress of Obstetrics and Gynaecology. The United States of America. The treatment will involve: intravenous injection of Benzyl Penicillin (Penicillin G) 5 million units start as soon as possible after the onset of active labour and 2.5 units every 4 hours until delivery. Alternatively, intravenous ampicillin 2 grams start followed by 1 gram every 4 hours will be given. For women who are allergic to penicillin, 2 grams' intravenous cefazolin start followed by 1 gram every 8 hours until delivery alternatively, clindamycin 900

milligrams every 8 hours until delivery will be given. However, for isolates not susceptible to clindamycin vancomycin 1gram every 12 hours until delivery will be given.

- A separate prescription indicating the drug, dosage, frequency and route of administration will be given to you in case you deliver in different health facility.

Risks

- No risks to you or your baby are anticipated from participating in this study.

This study has been approved by the University of Nairobi Institute of Tropical and Infectious Diseases and the Ethics Review Board of the University of Nairobi and Kenyatta National Hospital.

If you have any question on your rights as a study participant, you can call Professor A.N Guantai secretary of UoN-KNH ethics review board, phone number 020-2726300, extension 44102 or 44103.

Signature

Investigator.....

Date.....

Subjects Statement

I……have been explained to and understood the studies purpose, procedures, risks and benefits and give my consent to participate in the study.

.....

Subjects Signature/Thumb print

Appendix II: Cheti cha ridhaa ya kushiriki katika utafiti

KIWANGO CHA MAAMBUKIZI, USUGUDHIDI YA DAWA NA AINA YA VIJIDUDU VYA GROUP B STREPTOCOCCUS VINAVYOPATIKANA KATIKA SEHEME YA UKE - TUU WA WANAWAKE WAJAWAZITO KATIKA HOSPITALI KUU YA KENYATTA

Watafiti

Mtafiti	**Cheo**	**Chuo**
Bw. Jisuvei C. Salano	Mpelelezi mkuu	UoN
Dkt. Anne Maina	Msimamizi	UoN
Dkt. Alfred Osoti	Msimamizi	UoN

Mtafiti mkuu	**Namba ya simu ya Katibu - KNH- UoN ERC**
Namba ya simu: 0720327025	2726300 ext 44102

<u>Taarifa ya watafiti</u>

Twakuomba kwa ukarimu uwe mhusika kakika utafiti tunaoufanya. Niya ya ridhaa hii ni kukupa taarifa unayohitaji kukusaidia kuamua ikiwa utashiriki kwenye utafiti huu au la. Tafadhali soma fomu hii kwa makini. Waweza kuuliza swali lolote kuhusu yale tunayo hitaji kutoka kwako, ikiwa kunahatari, faida kwako, haki zako kamamuhusika au chochote kinacho husika nahuu utafiti au kilicho kwenye hii fomu na hakieleweki. Maswali yako yote yakisha jibiwa, unauhuru wa kuamua ikiwa utashiriki huu utafiti au la. Harakati hii yajulikana kanama ridhaa (hiyari) yakushiriki utafiti baada ya kujulishwa.

Habari ya tanzia

Group B streptococcus (GBS) ni kijidudu ambacho chaweza kupatikana kwenye uke (njia ya uzazi). Wanawake walio na GBS kwenye uke wao mara nyingi huwa hawaonyeshi dalili zozote za ugonjwa. Kijidudu hiki hakiambukizwi kupitia ngono. Theluthi moja ya wanawake wajawazito wamepatikana kuwa na kijidudu hiki kwenye uke wao wakati waujauzito. GBS inapopatikana wakati wa ujauzito, inaweza kuongeza uwezekano wa mwanamke kuzaa kabla ya wakati rasmi wa kujifungua. Pia yaongeza uwezekano wa kupasua maji ya uzazi kabla ya uchungu wa kijifungua kuanza na kupata maradhi ya chumba cha uzazi baada ya kuzaa. Nusu ya watoto wanaozaliwa na wanawake walio na GBS kwenye uke wao hupata maambukizo na kupelekea maradhi ambayo yanaajumuisha homa ya mapafu na homa ya damu.

Madhumuni ya utafiti

Utafiti huu utatuwezesha kujua kiwango cha uwepo wa GBS kati ya wanawake wajawazito wanaohudhuria kliniki katika hospitali kuu ya Kenyatta. GBS ikipatikana kati ya wanawake wajawazito wengi, tutashinikiza kufanywa kwa uchunguzi wa GBS kati ya wanawake wajawazito kwenye hospitali hii.

Taratibu za utafiti

Ukikubali kushiriki huu utafiti. historia yako ya afya itachukuliwa na ufanyiwe ukaguzi wa kimatibabu. Hii itahusisha kuulizwa umri wako, historia ya ujauzito wa sasa, kuharibika kwa mimba za awali na kuzaa kwa mtoto aliyefia tumboni.

Kwa uwepo wa muuguzi wa kike, daktari atakusanya vifuto viwili vya kutoka kwako, cha kwanza kutoka sehemu ya uke na cha pili kutoka kwenye tuu. Kifaa kinachotumiwa kuchukua vifuto hivi ni safi wala hakisababishi maunivu. Utafahamishwa matokeo ya vipimo

hivi katika ziara yako ya kliniki itakayofuata, matokeo haya yatajuzwa muuguzi wako pekee kwaajali ya matibabu panapofaa.

Usiri

Matokeo yatakayo patikana yatakua ya siri wala hayatatolewa kwa mtu yeyote ila muuguzi wako. Pia, vipimo vitakaavyochukuliwa vitatumika kwa minajili ya utafiti huu pekee na baadaye kuharibiwa baada ya kukamilika kwa utafiti.

Ubora wa huduma unayopokea kwenye hospitali hii hautapunguzwa kwa kukataa kwako kushuriki huu utafiti. Ushiriki kwenye huu utafiti niwa hiyari. Una haki yakutoshiriki au kujitoa wakati wowote. Takriban wanawake mia tatu (300) watashiriki hii huu utafiti. Utahitaji ziara mbili kwa utafiti huu, yakwanza ya kuchukua vipimo na yapili kukupa majibu ya vipimo. Majibu haya yatakua tayari baada ya juma moja.

Manufaa na hatari

Manufaa

- Utafahamishwa matokeo yako katika ziara ya kliniki itakayofuata
- Hutalipishwa pesa zozote kwa vipimo vya maabara
- Iwapo utapatikana na maambukizi ya GBS, matokeo yatanakuliwa kwenye faili yako iliupewe matibabu wakati wa kujifungua kwaminajili ya kuzuia mwanao kuambukizwa. Haya ndio matibabu yanayo pendekezwa na kituo cha kukinga na kuzuia maradhi (cdc) cha Merekani. Matibabu yatahusisha kupewa kipimo cha units millioni tano cha dawa ya Benzyl penicillin kupitia mshipa haraaka iwezekanavyo pindi uchungu wa kuzaa utakapoanza na kiwango cha 2.5 million units kilabaada ya masaa manee hadi utakapojifungua. Mbadala, utadungwa mshipani gramu mbili za dawa ya ampicillin maramoja na kufuatiwa na gramu moja kila baada ya masaa manne. Kwa wanawake waliona mzio (allergy) kwa dawa ya

penicillin, watadungwa milligram mbili za dawa ya cefazolin mshipani kisha gramu moja kila baada ya masaa manane hadi ujifungue au gramumia tisa za clindamycin kila baada ya masaa manane hadi ujifungue. Kwa wale walio na vijidudu sugu kwa dawa ya clindamycin wataapewa gramu moja ya vancomycin kila baada ya masaaa kumi na mawili hadi kujifungua

- Ikiwa unania ya kujifungua kwenye hospitaali mbadala, utaandikiwa nukuu itakayo onyesha dawa, kiwango na njia ya mapokelezi itakayotumiwa.

Hatari

- Hatari yoyote haitazamiwi kwako wala mwanao kutokanaa na kushiriki kwako kwa huu utafiti.

Utafiti huu umepatakibali kutoka kwa taasisi ya magonjwa ya tropiki yanayoambukizwa ya chuo kikuu cha Nairobi na amati inayosimamia utafiti ya chuo kikuu cha Nairobi na hospitali kuu ya Kenyatta.

Ukiwa na maswali kuhusu haki zako kama mshiriki wa utafiti unaweza kumpigia simu Professor M.L Chindia, katibu wa kamati ya maadili katika Chuo Kikuu cha Nairobi, simu: 020-2726300, extension 44102 or 44103.

Sahihi

Mtafiti…… Tarehe……

Kauli ya muhusika

Mimi……nimeelezwa na kuelewa madhumuni, utaratibu, hatari na manufaa ya utafiti huu na ninaushiriki kwa hiari yangu.

……

Sahihi ya muhusika/Alama ya kidole.

Appendix III: Laboratory Test Form

PREVALENCE, ANTIMICROBIAL SUSCEPTIBILITY AND SEROTYPES OF GROUP B STREPTOCOCCUS RECTO-VAGINAL ISOLATES FROM PREGNANT WOMEN AT KENYATTA NATIONAL HOSPITAL

Participant Serial Number _____ Collection Date _____

Culture results

Specimen type	*Streptococcus agalactiae* culture resu
Anorectal swab	
Vaginal Swab	

Antimicrobial susceptibility profile

Antimicrobial	Susceptibility results
Penicillin	
Ampicillin	
Clindamycin	
Vancomycin	

Serotypes Isolated

IA	Ib	II	III	IV	V	VI	VII	VIII	IX

Tests Done by _____ **Date** _____

Appendix VI: Questionnaire / Dodoso

PREVALENCE, ANTIMICROBIAL SUSCEPTIBILITY AND SEROTYPES OF GROUP B STREPTOCOCCUS RECTO-VAGINAL ISOLATES FROM PREGNANT WOMEN AT KENYATTA NATIONAL HOSPITAL /

KIWANGO CHA MAAMBUKIZI, USUGUDHIDI YA DAWA NA AINA YA VIJIDUDU VYA GROUP B STREPTOCOCCUS VINAVYOPATIKANA KATIKA SEHEME YA UKE - TUU WA WANAWAKE WAJAWAZITO KATIKA HOSPITALI KUU YA KENYATTA

Participant Study Number/ ***Namba ya Muhusika*** _____

Date of Interview / *Tarehe ya mahojiano* (Day/Month/Year) __ __/ __ __/ 2016

Obstetric questions / *Maswali ya uzazi*

1. Age/ *Umri wa mama* ____Years
2. Estimated gestational weeks / *Ujauzito huu una wiki ngapi?* _____
3. Number of times pregnant / *Umeshika mimba mara ngapi*? _____
4. Number of live birth / *Je,ni watoto wangapi umejifungua wakiwa hai?* _____
5. Number of still births / *Je, ni watoto wangapi umejifungua wakiwa wamefariki?* _____
6. Abortions or ectopic /*Je,umewahi kuavya mimba au kuwa na mimba ya mshipa na ni ngapi?*_____
7. Number of premature birth less than 37 weeks gestational / *Ni watoto wangapi umejifungua kabla ya kufikisha majuma 37 ya ujauzito*? _____

8. Have you ever had your water break more than 18 hours before the baby was actually born?/ *Je,maji yako ya chumba cha uzazi yamewahi kipasuka zaidi ya masaa 18 kabla ya mtoto kuzaliwa katika ujauzito uliopita?*

1. Yes 2. No 3. N/A

9. Has any of your babies died within the first 7 days of birth? / *Umewahi kumpoteza mwanao (kufa) ndani ya siku saba baada ya kuzaliwa* ?

1. Yes 2. No 3. N/A

10. Have you ever been told that your baby was born with infection after delivery? / *Je kwa mimba zahapo awali,uliwahi elezwa kuwa mwanao alizaliwa na maambukizwi?*

1. Yes 2. No 3. N/A

Present symptoms/ *Dalili za sasa*

11. Have your fore waters broken? / *Je, maji yako ya uzazi yamepasuka?*

1.Yes 2. No

12. Have you used antibiotics within the last two weeks? / *Je, umetumia dawa aina ya antibiotics kwa muda wa chini ya majuma mawili yaliyopita?*

1.Yes 2. No

13. Are you allergic to penicillin? / *Je, unamzio kwa dawa ya iaana ya penicillin?*

1.Yes 2. No 3. Don't know

Clinical examination/ *Ukaguzi wa kimatibabu*

14. Gestation by fundal height / *Umri wa mimba kwa kutumia urefu wa "fundal"*

_____

15. Uterine tenderness / *Utefu wa chumba cha uzazi*

1.Yes 2. No

16. Colour of discharge on swab / *Rangi ya majimaji ya uke*

1. Yellow/ *Manjano* 2. Brown/ *Kahawa* 3. White/ *Nyeupe* 4. Clear/*eupe* 5. Others/ *Nyingine* (specify/ *Taja*) _____

17. Antenatal profile as per ANC card / *Vipimo vya kliniki ya antenatal*

HIV/ *UKIMWI* 1. Positive 2. Negative

VDRL / *Kaswende* 1. Positive 2. Negative

Appendix V: Study Ethical Approval Letter

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
Tel:(254-020) 2726300 Ext 44355

KNH-UON ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://www.erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

KENYATTA NATIONAL HOSPITAL APPROVED 11 OCT 2016 KNH/UoN-ERC P.O. Box 20723-00202 NRB.

Ref: KNH-ERC/A/399

11th October 2016

Jisuvei Clayton Salano
W64/76432/2014
Institute of Tropical and Infectious Disease (UNITID)
College of Health Sciences
University of Nairobi

Dear Clayton

REVISED RESEARCH PROPOSAL: PREVALENCE, ANTIMICROBIAL SUSCEPTIBILITY AND SEROTYPES OF GROUP B STREPTOCOCCUS RECTO-VAGINAL ISOLATES FROM PREGNANT WOMEN AT KENYATTA NATIONAL HOSPITAL (P521/07/2016)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and **approved** your above revised proposal. The approval period is from 11th October 2016 – 10th October 2017.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH-UoN ERC before implementation.
c) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

For more details consult the KNH- UoN ERC website **http://www.erc.uonbi.ac.ke**

Protect to discover

Yours sincerely,

PROF M. L. CHINDIA
SECRETARY, KNH-UoN ERC

c.c. The Principal, College of Health Sciences, UoN
The Deputy Director, CS, KNH
The Assistant Director, Health Information, KNH
The Chairperson, KNH- UoN ERC
The Director, UNITID,UoN
Supervisors: Dr. Anne Maina, Dr.Alfred Osoti

Appendix VI: Approval letter from Reproductive health Head of Department

KENYATTA NATIONAL HOSPITAL,
P. O. BOX 20723-00202, NAIROBI
Tel: 2726300-9/2726450/2726550
Fax: 2725272
Email: knhadmin@knh.or.ke

KNH/RH/16/Vol.1

DATE: 16th November, 2016

To
Dr. Jisuvei Clayton Salano.
Principal Investigator
Department of OBS & GYN
U.o.N

RE: RESEARCH PROPOSAL: PREVALENCE, ANTIMICROBIAL SUSCEPTABILITY AND SEROTYPES OF GROUP B STREPTOCOAUS RECTO-VAGINAL ISOLATES FROM PREGNANT WOMEN AT KNH (ERC/A/399).

The Department of Reproductive Health has no objection for you to carry out the above study.

Please liaise with Senior Assistant Chief Nurse Reproductive Health Department and Incharge labour to facilitate your study.

Dr. I.S.O. Maranga
HEAD OF DEPARTMENT
REPRODUCTIVE HEALTH DEPARTMENT
KENYATTA NATIONAL HOSPITAL

KENYATTA NATIONAL HOSPITAL
16 NOV 2016
Box 20723 - 00202 NRB.
Assistant Director Reproductive Health

CC: SACN – Reproductive Health Department
Incharge Labour ward

Appendix VII: KNH Study Registration Certificate

KNH/R&P/FORM/01

KENYATTA NATIONAL HOSPITAL
P.O. Box 20723-00202 Nairobi

Tel.: 2726300/2726450/2726565
Research & Programs: Ext. 44705
Fax: 2725272
Email: knhresearch@gmail.com

Study Registration Certificate

1. Name of the Principal Investigator/Researcher
   JISUVEL CLAYTON SALAMO
2. Email address: salamo.clayton@gmail.com Tel No. 0720327025
3. Contact person (if different from PI).....
4. Email address: ..... Tel No. .....
5. Study Title
   PREVALENCE, ANTIMICROBIAL SUSCEPTIBILITY AND SEROTYPES OF GROUP B STREPTOCOCCUS RECTO-VAGINAL ISOLATES FROM PREGNANT WOMEN AT KENYATTA NATIONAL HOSPITAL
6. Department where the study will be conducted .....
   (Please attach copy of Abstract)
7. Endorsed by Research Coordinator of the Department where the study will be conducted.
   Name: DR. A. IKOL Signature ..... Date 17/11/16.
8. Endorsed by Head of Department where study will be conducted
   Name: Dr. Muranga F.O Signature ..... Date 17.11.16
9. KNH UoN Ethics Research Committee approved study number KNH-ERC/A/399
   (Please attach copy of ERC approval)
10. I JISUVEL CLAYTON SALAMO commit to submit a report of my study findings to the Department where the study will be conducted and to the Department of Research and Programs.
    Signature..... Date 15. NOV. 2016
11. Study Registration number (Dept/Number/Year) Reproductive Health/129/2016
    (To be completed by Research and Programs Department)
12. Research and Program Stamp

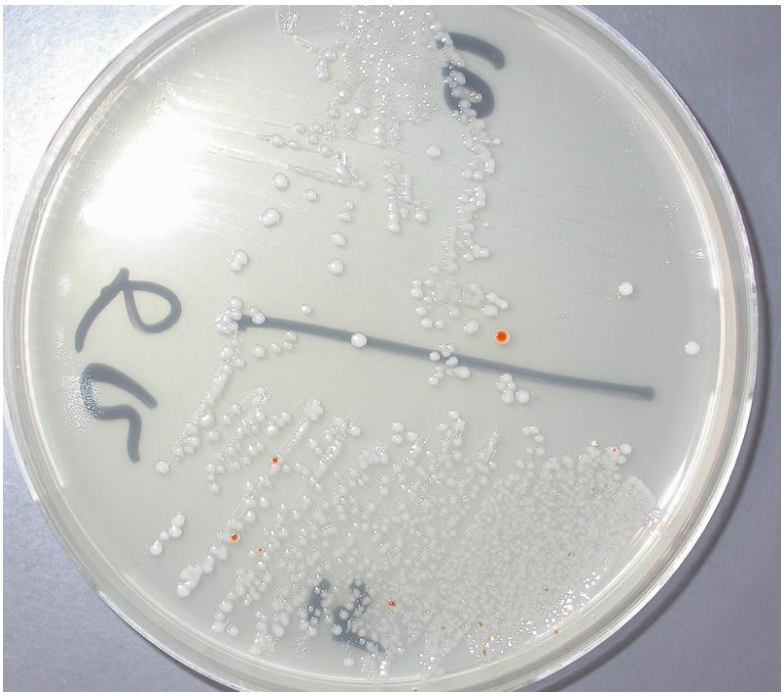
KENYATTA NATIONAL HOSPITAL H.O.D. RESEARCH & PROGRAMS 29 NOV 2016 P.O. BOX NAIROBI

All studies conducted at Kenyatta National Hospital **must** be registered with the Department of Research and Programs and investigators **must commit** to share results with the hospital.

Version 2: August, 2014

Appendix VIII: Illustrations of GBS growth on Granada Agar

Growth of *Streptococcus agalactiae* on Granada agar (Control culture)



Rectovaginal swab growth on Granada agar showing orange coloured colonies of *Streptococcus agalactiae* after 18 hours anaerobic incubation at 36^0C

*Photos borrowed from Wikipedia at https://en.wikipedia.org/wiki/Granada_medium