GROUP B *STREPTOCOCCUS* PREVALENCE, ANTIBIOTIC SUSCEPTIBILITY AND RISK FACTORS FOR COLONIZATION AMONG ANTENATAL WOMEN AT MBAGATHI HOSPITAL, NAIROBI CITY COUNTY, KENYA

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN INFECTIOUS DISEASES (BACTERIOLOGY) IN THE SCHOOL OF MEDICINE OF KENYATTA UNIVERSITY

OCTOBER, 2021

DECLARATION

This thesis is my original work and has not been presented for the award of a degree or any other award in any other University or institution

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DEDICATION

This work is dedicated to my loving wife Betty and our son Radley. For your patience and love during this worth course, God bless you!

ACKNOWLEDGEMENT

I thank the almighty God for enabling me to complete this academic journey faithfully. I also appreciate my supervisors, Dr. Nelson C. Menza and Dr. Scholastica G. Mathenge for their dedicated supported and guidance to ensure this thesis was completed. I am as well grateful to the entire fraternity of Mbagathi hospital for the conducive environment provided during the entire process of data collection. Special thanks to my parents Timothy and Lucy Mwambia, my sister Nelly, family and friends who supported, encouraged and constantly cheered me on during this journey. I also extend deep gratitude to the Kenyatta University ethics review committee for granting me the ethical clearance to conduct this research.

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LIST OF ACRONYMS AND ABBREVIATIONS

ANC	Antenatal Care
ACOG	American College of Obstetricians and Gynecologists
BA	Blood Agar
CAMP	Christie, Atkins and Munch- Petersen
CDC	Centers for Disease Control and Prevention
CFU/ mL	Colony Forming Units per Milliliter
CLSI	Clinical and Laboratory Standards Institute
EOD	Early Onset Disease
GBS	Group B *Streptococcus*
HIV	Human Immunodeficiency Virus
IAP	Intrapartum Antibiotic Prophylaxis
kDa	Kilodalton
LOD	Late Onset Disease
MIC	Minimum Inhibitory Concentration
NAAT	Nucleic Acid Amplification Testing
PCR	Polymerase Chain Reaction
PYR	Pyrrolidonyl Arylamidase
SSA	Sub-Saharan Africa
TAT	Turn-Around Time
U.S.A	United States of America
UTI	Urinary Tract Infection

OPERATIONAL TERMS

Bacteriuria: Presence of bacteria in urine.

Colonization: Presence of bacteria either in or on a host.

Intrapartum: Relating to delivery or childbirth.

Intrapartum antibiotic prophylaxis: The administration of antibiotics after labor has set in or rupturing of membranes has occurred, but just before delivery occurs.

Neonate: a newborn child who has not exceeded four weeks of age.

Neonatal sepsis: a bloodstream infection occurring in infants who are not more than 3 months old.

Puerperal sepsis: A genital tract bacterial infection that occurs following rupturing of membranes after miscarriage or childbirth.

ABSTRACT

Group B *Streptococcus* is recognized as the leading cause of neonatal sepsis as well as a significant cause of maternal post-partum sepsis globally. Maternal genitourinary colonization with the bacteria in pregnancy is the most significant risk factor for neonatal infection due to the risk of transmission to neonates during delivery. There is a gap in knowledge in the developing countries, Kenya included, regarding the prevalence of group B *Streptococcus* among the antenatal women. Therefore this study determined the prevalence of group B *Streptococcus* isolates and their antibiotic susceptibility patterns as well as identified the risk factors associated with group B *Streptococcus* colonization among antenatal women. The study was conducted at Mbagathi hospital, Nairobi City County, over a period of three months, adopting a cross- sectional study design. It included 323 systematically selected pregnant women. These participants provided 30-40 mls of mid-stream urine specimens which were then enriched in Todd-Hewitt broth and later cultured in strep B chromogenic agar and blood agar for bacteria isolation. The isolates were later identified using Gram staining and microscopy as well as a series of biochemical tests including the Christie Atkins and Munch Petersen test, catalase and pyrollidonyl arylamidase tests. The vitek 2 system was used to carry out antibiotic susceptibility testing and the resulting minimum inhibitory concentration values interpreted as either sensitive, intermediate or resistant with reference to Clinical and Laboratory Standards Institute 2020 guidelines. A questionnaire was used to collect data regarding the risk factors associated with maternal colonization with group B *Streptococcus*. The data was analyzed using the statistical packages for social sciences version 17. Percentages were used to show the prevalence of group B *Streptococcus* among participants and the differences in antibiotic susceptibility patterns of the isolates towards the various antibiotics that they were tested against. The Chi square test was used to test the association between the risk factors associated with maternal colonization with group B *Streptococcus* and the colonization status of the participants. The overall prevalence of group B *Streptococcus* established by this study was 15.2%, with 49 participants testing positive for group B *Streptococcus*. All isolates were 100% sensitive to Penicillin, Ampicillin, Ceftriaxone, Cefipime, Cefuroxime, Linezolid and Vancomycin. However, the isolates exhibited resistance towards Erythromycin 15 (30.6%), Azithromycin 9 (18.4%), Levofloxacin 7 (14.3%), Clindamycin 10 (20.4%) and Tetracycline 4 (8.2%). In addition, 10 (20.4%) of the isolates exhibited intermediate susceptibility towards Tetracycline. Age of a participant, place of residence and marital status (P= 0.000) were found to be significant. All obstetric related risk factors were also found to be significant; having a history of neonatal sepsis (P= 0.017), the parity status, history of: still birth, miscarriage, neonatal death and preterm delivery (P= 0.000). The human immunodeficiency virus status and dysuria (P= 0.000), lower abdominal pain (P= 0.001) and any form of vaginal infection (P=0.009) were also found to have significant associations with GBS colonization. No significant associations were found with the level of education of participants (P= 0.075) and the number of sexual partners (P= 0.083). Penicillin remains the antibiotic of choice for treatment of GBS infections and risk factors without individual routine screening are not sufficient for identifying candidates for intrapartum antibiotic prophylaxis. The study provided information regarding the most current effective antibiotics for group B streptococcal infections and advised on the criteria to identify candidates for intrapartum antibiotic prophylaxis. Further research is recommended to come up with a vaccine that would prevent women from being colonized with group B *Streptococcus* during pregnancy.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Initially, streptococci were classified as α-hemolytic, β-hemolytic and Ɣ-hemolytic, based on the ability of each group to lyse red blood cells on blood agar plates (Liu *et al.*, 2013). Based on carbohydrate antigens expressed on the bacteria's cell surface, Rebecca Lancefield in 1933 further classified the β-hemolytic streptococci into various groups. From this classification, *Streptococcus agalactiae,* is subsequently referred to as group B *Streptococcus* (GBS), since it is the sole species of streptococci that expresses the B antigen (Liu *et al.,* 2013). *Streptococcus agalactiae* is a non-motile, Gram- positive encapsulated coccus which grows as grayish mucoid colonies on blood agar plates. It differs from other streptococci by exhibiting much narrower zones of β- hemolysis on blood agar plates. It is also a facultative anaerobe (Melin and Efstratiou, 2013).

For a long time, GBS has been known to exist as a commensal of the genitourinary and lower gastrointestinal tract of about 10-30% of humans, particularly women. While vaginal colonization with GBS starts to become common in adolescence all the way to pregnancy, occurring either as persistent, transient or intermittent and accounting for 10-37% of recto-vaginal microbiota, it is unusual in childhood (Melin and Estraftiou, 2013). Variation in rates of GBS colonization among antenatal women occur majorly depending on the population studied and sample collection among performance of other microbiological procedures. Despite the huge colonization of the genitourinary tracts of

many women of child bearing age with GBS, this organism is only very significant in child birth, more so where there are signs of infection (Le Doare and Heath, 2013).

Presently, as recognized in the developed countries, GBS is attributed to many cases of neonatal sepsis, pre-term births, still births, very low weight deliveries and maternal puerperal sepsis which are subsequently responsible for substantial neonatal and maternal mortality rates. This thus shows that GBS infections are not restricted to neonates as it also affects pregnant and post-partum women (Melin and Efstratiou, 2013). Group B *Streptococcus* can cause either early or late onset disease, depending on the infant's age at the time which the disease manifests. Usually the early onset neonatal disease or sepsis (EOD), accounting for approximately 80% of the cases sets in within life's first week and is primarily associated with vertical transmission of bacteria from the maternal genitourinary tract to the neonate before or during delivery (Cools *et al.*, 2016).

On the other hand, late onset neonatal disease (LOD), sets in between life's first week and the 2^{nd} to 3^{rd} month of life, and is primarily associated with vertically or horizontally acquired bacteria (Cools *et al.*, 2016). This progressive unfolding over the recent decades of a clearer understanding of GBS with regard to its contribution to perinatal infections, overall disease burden, neonatal and maternal mortalities globally shows the need to conduct more research on how to identify, manage and prevent GBS related diseases more so in developing countries like Kenya.

1.2 Statement of the problem

Many neonates die globally each year due to neonatal sepsis associated with group B *Streptococcus*; *Streptococcus agalactiae*, which is now known to be the leading cause of neonatal sepsis. Screening and detection of colonization with GBS among antenatal women, which is the main predisposing factor of GBS associated neonatal and puerperal sepsis is not prioritized in the developing countries like Kenya, and thus no effective measures have been put in place to prevent GBS infections. On the other hand, developed countries like the United States of America have comprehensive epidemiological data regarding the disease burden associated with GBS prevalence and preventive measures put in place. However, this still remains to be an element of great uncertainty in the developing countries like Kenya.

Due to the scarcity of such information in our country, it is necessary to conduct more research targeting GBS right from maternal colonization to transmission to neonates. A study investigating the rates of maternal recto-vaginal colonization with GBS in late pregnancy was carried out in 2009 at the Kenyatta National Hospital. However, due to the increasing rates of GBS associated neonatal sepsis and mortalities, there is need to obtain the most current data on GBS prevalence among antenatal women. This study therefore identified the prevalence rate of GBS colonization among pregnant women as well as the antibiotic susceptibility profile of these GBS isolates. It also assessed the risk factors associated with maternal colonization with GBS focusing on urinary carriage of GBS at any point of the pregnancy which occurs concurrently with maternal genital tract colonization.

1.3 Justification

Group B *Streptococcus* associated neonatal and puerperal sepsis which greatly rely on maternal colonization with GBS contribute to a great extent to the overall disease burden and related mortalities in our country. Therefore, it is important to source comprehensive epidemiological data besides carrying out risk factor assessment encompassing the prevalence and distribution of GBS colonization among antenatal women, as there is limited data pertaining GBS prevalence in our country. This will be important in development of suitable, effective and practical intervention strategies that will go a long way in helping to reduce cases of GBS related neonatal and puerperal sepsis together with their associated mortalities.

1.4 Research questions

i. What is the prevalence of group B *Streptococcus* colonization among antenatal women at Mbagathi hospital?
ii. What is the antibiotic susceptibility profile of group B *Streptococcus* isolates obtained from antenatal women at Mbagathi hospital?
iii. What are the risk factors associated with group B *Streptococcus* colonization among antenatal women at Mbagathi hospital?

1.5 Hypotheses

i. Antenatal women at Mbagathi hospital are not colonized with group B *Streptococcus*.

ii. Isolates of group B *Streptococcus* obtained from antenatal women at Mbagathi hospital are not sensitive to the commonly prescribed antibiotics.
iii. There are no risk factors associated with group B *Streptococcus* colonization among antenatal women at Mbagathi hospital.

1.6 Objectives

1.6.1 General objective

To determine group B *Streptococcus* prevalence, antibiotic susceptibility profile and risk factors associated with colonization among antenatal women at Mbagathi hospital in Nairobi City County, Kenya.

1.6.2 Specific objectives

i. To determine the prevalence of group B *Streptococcus* colonization among antenatal women at Mbagathi hospital.
ii. To determine the antibiotic susceptibility profile of group B *Streptococcus* isolates obtained from antenatal women at Mbagathi hospital.
iii. To determine the risk factors associated with group B *Streptococcus* colonization among antenatal women at Mbagathi hospital.

1.7 Significance of the study

Data from the study will advise clinicians on the most current effective antibiotics for treating GBS infections and promote development of suitable intervention strategies focused on early detection and management of maternal colonization with GBS. Data

obtained from the study will also advise against the potential risk factors for GBS colonization among antenatal women.

CHAPTER TWO

LITERATURE REVIEW

2.1 Group B *Streptococcus* bacteriuria

Streptococcus agalactiae, commonly referred to as the group B *Streptococcus* (GBS), normally occurs as a commensal within the lower gastrointestinal tract as well as the epithelium of the vagina in healthy adults. The organism is only very significant in child birth, more so where there are signs of infection such as the infant breathing fast and with difficulty, mostly with grunting noises (Le Doare and Heath, 2013). These key signs could be indicative of fulminant pneumonia besides being suggestive of group B streptococcal disease (Le Doare and Heath, 2013). It is for this reason that routine screening for group B streptococci in urine is recommended during pregnancy. This is because as seen in most cases, GBS bacteriuria during pregnancy acts as a marker of heavy maternal genital tract colonization with GBS during the intrapartum period and consequently increases the risk of a newborn developing early onset disease (Cagno *et al.*, 2012).

Every woman found to have GBS bacteriuria during any trimester of the pregnancy should therefore receive intrapartum antibiotic prophylaxis (IAP) (Cagno *et al.*, 2012). Previously, the Centers for Disease Control and prevention (CDC) recommended that group B streptococci detected in urine at whatever amounts be considered a positive culture. However, the new 2010 guidelines recommend that a culture should be considered positive if the concentrations exceed at least 10^4 colony- forming units per milliliter (CFUs/ml) regardless of whether GBS has been isolated singly or together

with any other organism (Verani *et al.,* 2010). This is because, it is concentrations of above or equal to 10^4 CFUs/ml that have been found to have a direct correlation with causation of early onset neonatal disease (Cagno *et al.,* 2012).

Treating GBS bacteriuria in prenatal care should be guided by a combination of several factors. They include bacterial load, based on the isolated colony counts of the bacteria and either the absence or presence of urinary symptoms as expressed by the pregnant woman (Cagno *et al.,* 2012). Generally, treatment is recommended for only those who are symptomatic. However, treating asymptomatic bacteriuria is associated with reduction of pre- term births, low birth weight deliveries and risks of the patients progressing to develop pyelonephritis (Perez- Moreno *et al.,* 2017). Administration of antibiotics during pregnancy does not however completely guarantee clearance of the bacteria from the genitourinary tract. Thus, re-colonization is possible even after treatment with a course of antibiotics (Virraniemi *et al.,* 2019).

This suggests that colonization with GBS is discontinuous since a woman could have a positive GBS culture at the first trimester screening and a negative GBS culture at the third trimester screening or vice versa. Whatever the reason, colonization with GBS in one pregnancy increases the risk of the woman being colonized by the same bacteria in a subsequent pregnancy by 50% (Turrentine *et al.,* 2016). Generally, a GBS negative screen is usually considered to be viable over 5 weeks. In case a GBS culture is not performed at late pregnancy; 35-37 weeks, following a history of GBS bacteriuria at

any point of the pregnancy, empiric IAP should be administered based on the risk factor of antepartum GBS bacteriuria (Perez-Moreno *et al.,* 2017).

2.2 Group B *Streptococcus* epidemiology

Over time, rates of maternal colonization with GBS have shown to be almost quite similar across the world. Previous studies have come up with variations of prevalence ranging from 10%-30% in the U.S, 6%-7.1% in Asia, 6.5%-36% in Europe and roughly 11.9-31.6% in Africa (Verani *et al.,* 2010). Data from African countries is however poorly and inaccurately documented, due to either poor, inadequate or total lack of research done in majority of the African countries. Despite the almost similar prevalence in the rates of GBS colonization throughout different regions of the world, variation occurs among geographical distribution of different GBS serotypes (Shabayek *et al.,* 2018).

The documented serotypes whose geographical distribution and predominance varies over time are as many as ten serotypes namely; 1a, 1b and II- IX (Le Doare and Heath, 2013). The serotypes' predominance varies as follows; serotypes 1a, 1b, II, III, and V are more prevalent in Europe and the U.S, serotypes VI and VIII prevailing more in Japan, IV and V prevailing more in Egypt and United Arab Emirates respectively. Serotype IX has recently been reported in Denmark (Le Doare and Heath, 2013). In our country, there is little knowledge about the burden associated with perinatal colonization with GBS and infections that are associated with it at large.

During the ninety's, cases of early onset GBS disease were 1.7 per 1000 live births, while this has significantly recently dropped to 0.34 to 0.37 cases per 1000 live births, especially in the developed countries. This has majorly been enhanced by the developments in early detection through screening of maternal GBS colonization and administration of IAP, which are not yet well embraced in the developing countries (Verani *et al.,* 2010). It has been observed that up to 70% of cases of early onset GBS disease occur among term infants while 60% of these cases occur among infants whose mothers had a negative GBS culture along their course of pregnancy (Verani *et al.,* 2010). In some instances, it has also been noted that maternal colonization with GBS is discontinuous, since up to 33% of patients who have a positive GBS culture in the course of pregnancy have shown not to be colonized or rather have a negative GBS culture at delivery. On the other hand, approximately 10% of patients who turn out to be colonized with GBS at delivery may have exhibited negative GBS cultures throughout their course of pregnancy (Russel *et al.,* 2017). This thus shows that not all infants develop GBS disease despite being born of mothers who are colonized with GBS.

2.3 Colonization with group B *Streptococcus*

In systemic disease, GBS is capable of crossing the epithelium of the lungs and endothelial cell barriers, the blood- brain barrier as well as the epithelium of the intestines. This occurs through mechanisms such as paracellular translocation, transcytosis as well as damage of cells and tissues (Da Costa *et al.,* 2011). In combination, these mechanisms enable the bacteria to access the central nervous system, the amniotic compartment as well as the blood stream. Necrosis or apoptosis of

the epithelial cells that are infected occurs when these cells are invaded by the bacteria. On the other hand, the bacteria traverse the blood- brain barrier by induction of either transcytosis or injury of cells. Transcytosis is also the mechanism that the bacteria employs to traverse the monolayer cells of the chorion that is usually intact (Dos Santos *et al.,* 2013).

The bacteria is well adapted to survive in the host during the course of infection. Group B *Streptococcus* typically grows and multiplies to high concentrations causing infection in various body sites such as the amniotic fluid, bloodstream, lungs and vagina. Typically, these sites have varied conditions which range from the blood stream's aerobic conditions and a pH that is neutral to an environment that is micro- aerophilic accompanied by a low pH of the vagina (Rosisnski- Chupin *et al.,* 2013). Some of the adaptation features that the organism employs to survive in the host include having a fast rate of growth and a biomass that is also increased, which both promote the organism's ability to invade cells and resist clearance by the immune system. This is attributed to enhanced gene regulation of the growth phase (Rosisnski- Chupin *et al.,* 2013). The bacteria also has the capability to initiate death of the phagocytes as the loss of effector cells weakens the host's capability in clearing infection (Rosisnski- Chupin *et al.,* 2013). The bacteria utilizes several virulence factors to enable it achieve three important aspects which encompass its ability to colonize and penetrate the host's tissue barriers, evade the host's immune defenses and successfully exit from the host after a successful infection.

2.3.1 Surface proteins

The classification of serotypes is based on the capsular polysaccharide. The capsular polysaccharide is one of the most important surface antigens which contribute to the pathogenicity of the bacteria. The group B- specific antigen is common to all strains of GBS and it is a peptidoglycan anchored antigen composed of galactose, N-acetylglucosamine, rhamnose and glucocitol (Shabayek and Spellerberg, 2018). This capsular polysaccharide enables the bacteria to evade the host's immune defenses by mimicking the carbohydrate epitopes of the host. It also plays an antiphagocytic role and interferes with the functioning of complement dependent defense phagocytes. This helps the bacteria to easily survive inside phagocytic cells (Shabayek and Spellerberg, 2018). The capsular polysaccharide is also responsible for biofilm formation which is important in exhibiting the bacteria's resistance to antibiotics (Di Xia *et al.,* 2015). Despite the polysaccharide capsule exhibiting all the above important functions, not all isolates of GBS express it, as it is normally expressed by only 5%- 20% of the isolates (Ippolito *et al.,* 2010).

The α C- protein is also a surface associated antigen that exhibits repeated units of the α C- antigen. It provides sites that are natural and favorable for rearrangement of genes and this favors the generation of antigenic variants by the bacteria. Antigenic variation further helps the bacteria avoid antibody detection from the host (Ippolito *et al.,* 2010). Another surface associated antigen is the β C- protein which exhibits a unique ability to bind the F_c region of the human IgA. IgA, being the predominant protective immunoglobulin that counters infection by microbes at mucosal sites, is thus weakened

by this consequential binding of its F_c region by the bacteria's β C- protein. Hence the bacteria can easily invade the mucosal surface (Ippolito *et al.*, 2010).

Both the α and β C- proteins also help the bacteria to bind epithelial cells such as those lining the vaginal wall and further promote survival of the bacteria by preventing it from being killed intracellularly by neutrophils (Nuccitelli *et al.*, 2011). The Rib protein is also a surface protein which exhibits similar properties as the α C- protein and greatly plays a role in helping the bacteria to evade the host's immunity by conferring antigenic variation (Nuccitelli *et al.*, 2011). The Pep protein, a cell associated oligopeptidase hydrolyzes the host collagen-like substrate which is also synthetic, degrading bioactive peptidases and this makes it easy for the bacteria to invade host tissues (Nuccitelli *et al.*, 2011). Another protein, the surface immunogenic protein; SIP, induces the bacteria's immunogenicity. The Christie, Atkins and Munch- Petersen (CAMP) factor is also an extracellular protein which helps in the bacteria's penetration into the host by forming pores into the host cell membrane. It also binds to gylcosylphosphatidylinositol (GPI) anchored proteins which are a class of membrane proteins, further promoting the bacteria's entry into the host (Whidbey *et al.*, 2015).

2.3.2 Enzymes

There are various enzymes which play key roles in the bacteria's virulence. They include: the enzyme C5a peptidase. This enzyme has the ability to cleave the complement component C5a, and this consequently hinders the recruitment of neutrophils to the site of infection, making it possible for the bacteria to successfully

invade the host (Rosini and Margarit, 2015). This enzyme also facilitates the adherence of the bacteria to the host by binding to epithelial cells lining the mucosal surface lining the urogenital tract and extracellular matrix fibronectin (Nuccitelli *et al.,* 2011).

Another type of enzyme is the serine protease which has ability to hinder recruitment of neutrophils and subsequent phagocytosis of the bacteria. This enzyme also cleaves chemokines and fibrinogen, facilitating penetration of the bacteria into the host tissues (Rosini and Margarit, 2015). The enzyme hyaluronate lyase cleaves hyaluronate, a component of the host extracellular matrix, thus promoting further spread of the bacteria throughout the course of infection. β- hemolysin also known as cytolysin, which is also an enzyme secreted by the bacteria plays different roles such as triggering lysis of the host cells, which promotes invasion of the host cells by the bacteria. It also induces apoptosis and pro-inflammatory responses, besides impairing host's liver and cardiac functions (Rosini and Margarit, 2015). Induction of the pro-inflammatory responses by the β- hemolysin damages the host's tissues, thus promoting progression of disease. The hemolytic pigment of the enzyme also facilitates penetration of the amniotic barrier by the bacteria, which leads to injury of the fetus (Whidbey *et al.,* 2015).

2.3.3 Pili

The bacteria's pili help it to adhere to cells of the host as well as promote the bacteria's resistance to antibiotics (Whidbey *et al.,* 2015).

2.4 Group B *Streptococcus* diseases

2.4.1 Early onset disease

Neonatal early onset disease (EOD) which accounts for 80% of total neonatal infections occurs within the first week of a newborn's life (Nishiara *et al.,* 2017). Most infants presenting with EOD show initial symptoms of infection within 12 to 24 hours of age (Escobar *et al.,* 2014). Early onset disease is usually caused by vertical transmission of bacteria from the mother's genitourinary tract to the infant before or during delivery. This can occur due to the newborn inhaling amniotic fluid that is infected or aspiration of secretions from the genitalia into the lungs (Cools *et al.,* 2016). The bacteria majorly ascends the amniotic fluid from the vagina once labor has set in and membranes rupture. However, in other instances the bacteria traverses intact membranes (Cools *et al.,* 2016). Vertical transmission normally occurs in 30-70% of the cases at the time of delivery (Melin and Efstratiou, 2013).

Early onset disease usually manifests with fulminant pneumonia and respiratory failure which usually progresses into systemic bacteremia and septic shock syndrome, and ultimately death (Melin and Efstratiou, 2013). Bacteremia occurs due to the newborn inhaling amniotic fluid that is infected or aspiration of secretions from the genitalia into the fetal lungs during passage across the birth canal (Cools *et al.,* 2016). However, as seen in most cases, infants who get exposure to the bacteria in this manner only get colonized by the organism at mucosal sites in the respiratory and gastrointestinal tracts, but otherwise remain healthy (Cools *et al.,* 2016).

Early onset disease may at times lead to meningitis which accelerates the rate of neonatal death. However, the case fatality ratio associated with EOD has significantly declined from the overall high rates of up to 50% as seen in the 1970s to up to 4%-6% as seen in the recent years. This is majorly in the developed countries and is attributable to advanced neonatal care (Verani *et al.,* 2010). Majority of GBS associated EOD cases occur mostly among preterm infants, with a case fatality ratio of approximately 19.2% while that of term infants is approximately 2.1% (Nanduri *et al.,* 2016). In 2015, it is estimated that globally there were 200,000 cases of GBS associated EOD, exclusive of the U.S with the largest numbers of GBS associated perinatal deaths occurring in Africa (Puopolo *et al.,* 2019). A combination of GBS associated neonatal diseases and still births contribute to approximately 150,000 neonatal and fetal deaths globally (Seale *et al.,* 2017).

2.4.1.1 Risk factors for development of early onset disease

It is generally recognized that maternal genitourinary colonization with GBS during the intrapartum period is the key predisposing factor for neonatal early onset disease (Le Doare and Heath, 2013). If antibiotic prophylaxis is not provided during the intrapartum period, approximately 50% of newborns whose mothers are GBS positive become colonized with the bacteria. Out of these, 1%-2% progress to developing GBS associated EOD (Russell *et al.,* 2017). If the gestational age is lower than normal; < 37 weeks, the infant is more likely to develop early onset disease. This is because at this point, the protective maternal antibodies are in low concentrations and the neutrophil mediated defenses are much weaker (Collins *et al.,* 2018). Also, babies being born with

very low weight, as mostly seen with very young mothers are more predisposed to developing EOD (Nanduri *et al.,* 2019).

The prolonged rupture of membranes encourages the entire process of ascending colonization as well as the infection of the compartment of the uterus and fetus (Puopolo *et al.,* 2019). Intra-amniotic infections associated with group B streptococci serve as key predictors of early onset disease. These infections can be depicted by the mother having high fever during delivery as a result of inflammatory response to the bacterial infection. Maternal black race is associated with a lower socio-economic advantage as compared to other races like Caucasians and subsequent poor or lack of antenatal screening for GBS. This thus predisposes their infants more to developing EOD in the event that the mother is colonized with GBS (Mukhopadhyay, 2014).

If a mother has a history of delivering a child who developed invasive GBS disease through a previous pregnancy, then her infant during the current pregnancy is also at risk of developing EOD. This can be attributed to higher maternal antibodies responding poorly to the colonizing strains or other immune specific factors (Mukhopadhyay, 2014). Group B *Streptococcus* bacteriuria at any trimester of the pregnancy is also a risk factor associated with EOD. This is because it is attributed to high maternal intrapartum colonization with GBS as well as increased risk of the neonate being colonized and developing EOD (Kessous, 2012).

2.4.2 Late onset disease

Late onset disease (LOD), accounting for 20% of total neonatal infections occurs between the first week of life to the third month (Nishiara *et al.,* 2017). In very rare circumstances, LOD can occur even beyond 3 months of age. This mostly occurs among infants that are immunodeficient in nature or those that were born at a very early gestational age (Guilbert *et al.,* 2010). Generally, the prevalence rates of GBS associated LOD have not changed largely in the developed countries, which is associated especially with the use of IAP (Nanduri *et al.,* 2019). Preterm infants account for about 42% of GBS associated LOD cases. It has also been noted that the case fatality ratio is twice as much in preterm infants with GBS LOD as compared to term infants; 7.8% versus 3.4% respectively (Nanduri *et al.,* 2019).

Infants that develop GBS associated LOD meningitis have a higher fatality risk rate as compared to those with other syndromes (Puopolo *et al.,* 2019). Since maternal colonization is not a prerequisite for the infant developing GBS associated LOD, this suggests that the bacteria can be acquired horizontally from non-maternal caregivers. It is also suggested that an infant can develop GBS associated LOD out of consumption of breast milk (Nanduri *et al.,* 2019).

It is also however known that human breast milk associated GBS antibody is known to protect the infant against GBS LOD. Due to this contradiction, it is thus uncertain as to whether GBS presence in breast milk marks heavy infant and maternal colonization or it is simply a reservoir-like infection source (Le Doare and Kampmann, 2014).

2.4.3 Maternal GBS disease

The common maternal infections associated with GBS include urinary tract infections, endometritis, chorioamnionitis and post- partum bacteremia or septicemia (Edwards *et al.,* 2019).

2.4.3.1 Urinary tract infections

During pregnancy, GBS causes asymptomatic bacteriuria which occurs concomitantly with maternal genital tract colonization (Cagno *et al.,* 2012). This subsequently causes infection of the ascending urogenital tract; urosepsis and cystitis which may ultimately lead to pyelonephritis. In combination, these predispose a woman to maternal sepsis which can ultimately cause a miscarriage or pre- term delivery (Bako *et al.,* 2012).

2.4.3.2 Endometritis

This commonly occurs in cesarean more than vaginal deliveries. Women who are colonized with GBS during pregnancy are at a higher risk of developing post- partum endometritis (Bako *et al.,* 2012).

2.4.3.3 Chorioamnionitis

This clinical syndrome is usually associated with tenderness of the uterus, fetal and maternal tachycardia, leukocytosis and fever. It has over time been associated with heavy GBS colonization especially in the second trimester, premature deliveries and preterm labor (Tita and Andrews, 2010). It is also associated with amniocentesis, a

procedure that is invasive as well as one being co- infected with *Chlamydia*, *N. gonorrheae* and *E. coli.*

2.4.4.4 Puerperal sepsis and post-partum bacteremia

These usually manifest with increased body temperature of > 38°c on any successive two days post- delivery without any apparent cause, tenderness of the uterus. In addition, although uncommon, pelvic thrombophlebitis may set in and this increases the risk of pulmonary embolism occurring. Severe cases of puerperal sepsis or septic shock may involve endotoxic shock, ultimately being fatal (Bako *et al.*, 2012).

2.5 Host's immune response to GBS infection

The host is also well adapted to respond to GBS infection, as it similarly does with other bacterial infections. For example, the host has antibodies that are type- specific against the polysaccharide capsule of the bacteria. The capsule of the bacteria is known to be weakly immunogenic (Melin, 2011). These antibodies, in both adults and neonates are capable of opsonizing and phagocytosing the bacteria. However, only 10-20% of mothers express the capsule- specific antibodies in levels that can be protective, which leaves a majority of neonates at higher risks of infection (Melin, 2011).

The innate immune system encompassing soluble molecules in blood like antimicrobial enzymes and the complement as well as the myeloid lineage cells is the major line of defense against GBS infection. This is because it is triggered upon infection without necessarily requiring prior exposure to the antigen (Carey *et al.*, 2014). The innate

system's myeloid lineage cells such as the phagocytes, monocytes and macrophages, neutrophils and dendritic cells play a key role in ingesting and killing the organism as well as presenting the antigen to other cells of the immune system. When the bacteria get into the host, they encounter macrophages and are either phagocytosed immediately or the macrophages produce anti-inflammatory cytokines like interleukin-10; IL-10 (Carey *et al.,* 2014). The production of mediators of inflammation causes other cells like neutrophils which contain enzymes that have an antimicrobial and toxic effect to the organism to be recruited. The dendritic cells phagocytose the antigen and present it to other cells which can then kill it (Melin, 2011).

2.6 Management of GBS disease

The use of intrapartum antibiotic prophylaxis for all women whose GBS status is unknown is recommended in the case whereby a woman sets into labor and consequently gives birth before 37 weeks of gestation (Puopolo *et al.,* 2019). Generally, initiation of antibiotic prophylaxis 4 hours or more before delivery is known to be more effective in preventing the transmission of GBS disease to the fetus. This does not however rule out the effectiveness of initiating antibiotic prophylaxis at any other interval shorter than 4 hours before delivery. As a matter of fact, administration at any other shorter interval before delivery is also effective in prevention of transmission of GBS disease to the fetus (Morgan and Cooper, 2019).

Antibiotic prophylaxis is indicated for those women who have positive culture results or their GBS status is known as at the onset of labor, or they had a positive GBS culture in

their previous pregnancy. This thus explains the recommendation that chemoprophylaxis should be stopped in a situation where it had been initiated on a woman with preterm labor who later proceeds to true labor and their culture results turn out negative (Verani *et al.,* 2010).

2.6.1 Antibiotics used in management of GBS disease

Over time, group B streptococci have shown to be susceptible to β-lactam antibiotics. Penicilin G has remained the recommended antibiotic for intrapartum chemoprophylaxis of group B streptococcal disease (ACOG, 2019). Regardless of the fact that the group B streptococci are sensitive to a commonly prescribed antibiotic like Penicilin, their treatment in most cases is hampered by the high rate in which the disease development takes place (ACOG, 2019). Penicilin G readily crosses the placenta after intravenous administration and reaches peak cord blood concentrations within an hour but rapidly declines by 4 hours. This shows that the drug is easily eliminated by the kidney of the fetus into the amniotic fluid (Berardi *et al.,* 2018). In cases where Ampicilin has been used at least 24 hours before delivery, it has been shown to decrease the rates of maternal vaginal colonization with GBS during delivery as well as preventing neonatal surface colonization in 97% of cases (Kuzniewicz *et al.,* 2017).

Penicilin G is usually substituted with Ampicilin for patients who are allergic to Penicilin (Cagno *et al.,* 2012). Ampicilin reaches peak cord blood concentrations within half an hour and is detectable in amniotic fluid after 45 minutes from the time the drug

is administered to the mother (Berardi *et al.*, 2018). The Penicilin dose is administered intravenously as first a loading single dose of 5 million units, which is subsequently followed by a maintenance dose of 2.5 to 3 million units four- hourly until delivery. On the other hand, if Ampicilin is chosen, it should be administered intravenously as a single loading dose of 2g, followed 1g four- hourly until delivery (Cagno *et al.*, 2012). Either of the regimens are aimed at maintaining sufficient levels of drugs in circulation of the fetus and amniotic fluid, while at the same time cushioning against maternal toxicity (Puopolo *et al.*, 2019).

Cefazolin is the recommended alternative antibiotic for intrapartum chemoprophylaxis for women who are allergic to Penicilin who do not exhibit severe anaphylactic reactions following its administration, like development of urticarial symptoms, angioedema or even respiratory distress. It should be administered as a single intravenous loading dose of 2g, followed by 1g eight- hourly until delivery (Puopolo *et al.*, 2019). Cefazolin's mode of action and pharmacokinetics resemble those of Ampicilin and it is detectable in amniotic fluid and cord blood within 20 minutes of administration to the mother, at levels which are above the MIC for GBS (Groff *et al.*, 2017). Group B *Streptococcus* positive women who exhibit severe anaphylactic reactions to either a Penicilin or a cephalosporin such as Cefazolin should have an antibiotic susceptibility test conducted to evaluate if the GBS isolates exhibit resistance for Erythromycin and Clindamycin (Puopolo *et al.*, 2019).

In the case whereby the isolates exhibit susceptibility to Clindamycin and resistance to Erythromycin, they should be tested for inducible Clindamycin resistance. This is because resistance to Clindamycin can be induced by Erythromycin- resistant isolates (Cagno *et al.,* 2012). If the isolates are susceptible to both Clindamycin and Erythromycin, intravenous administration of 900mg Clindamycin 8-hourly until delivery is recommended for intrapartum chemophrophylaxis (Cagno *et al.,* 2012). If the drug susceptibility testing shows either resistance to both or inducible resistance to Clindamycin, 1g of Vancomycin should be administered 12- hourly until delivery.

Erythromycin is however not recommended for empirical prophylaxis, as it has been observed to increase resistance rates over time as well as poor placental kinetics (Morgan and Cooper, 2019). Clindamycin is also not largely preferred due to the fact that it has poor excretion in fetal urine after metabolism in the liver and also it has to be administered in multiple doses for adequate concentrations to be reached in amniotic fluid. Vancomycin should also be used if a woman is more likely to exhibit signs of anaphylaxis, if testing for both Clindamycin and Erythromycin resistance has not been done or the results are unavailable at the onset of labor (Cagno *et al.,* 2012).

2.6.1.1 Criteria for categorizing penicilin allergic patients

2.6.1.2 Low risk patients

These patients exhibit skin pruritis without necessarily developing rash, have maculopapular rash that is non- urticarial and is also not accompanied by systemic symptoms. The patients may also have a familial history of allergy to Penicilin but not

specifically having a personal history of allergy to Penicilin (Macy and Vyles, 2018). The patient may also report a personal history but have no recollection of either the treatment or symptoms. Finally, these patients may develop non- specific symptoms which are less likely to be allergic such as gastrointestinal distress and headaches (Macy and Vyles, 2018).

2.6.1.3 High risk patients

Patients who are at a high risk of allergy to Penicilin may have a history that is suggestive of an IgE mediated event, such as angioedema, pruritic rash accompanied with urticarial, respiratory distress and immediate flushing. These patients also exhibit reactions to various β-lactam antibiotics as well as turn out positive for Penicilin allergy testing (Macy and Vyles, 2018).

2.6.1.4 Special cases requiring IAP without GBS testing

There are special conditions in which IAP is indicated even without testing for GBS. They include a mother having either of the following; previously delivering a child who developed GBS disease, GBS bacteriuria at any trimester of the current pregnancy and unknown GBS status accompanied by fever without any other specified cause at the onset of labor (Virraniemi *et al.,* 2019). Other special conditions are; if a mother's GBS status in the current pregnancy is not known but she had a positive culture during the previous pregnancy, simply because treatment with a course of antibiotics does not guarantee 100% chance of elimination of the bacteria. In addition, chemoprophylaxis

should be initiated if preterm labor sets in and the GBS status is unknown as well as premature rupture of membranes (Virraniemi *et al.,* 2019).

For women who have planned cesarean birth, IAP is not recommended especially where labor and rupturing of membranes are not involved. This is because multistate surveillance has shown over time that GBS EOD is very unlikely to occur or rather at very low rates in this situation; approximately 3 per 1,000,000 live births (Nanduri *et al.,* 2019). However, such women are not excluded from GBS screening as membranes may rupture or even labor may set in before the date of the planned cesarean birth. In occurrence of such a case, a single dose of GBS prophylaxis and pre- surgical prohpylaxis should be administered. Cefazolin meets both of the criteria (Nanduri *et al.,* 2019).

2.6.2 Antibiotic resistance of GBS isolates

Concerns have been raised concerning development of resistance to antibiotics among isolates of GBS owing to the widespread usage of intrapartum chemoprophylaxis in prevention of early onset GBS disease, more so in developed countries (Bianco *et al.,* 2016). Generally, group B streptococci have over time shown susceptibility penicilins and cephalosporins of the first generation like Cefazolin. However, some GBS isolates have recently been reported from studies such as one conducted in Japan in 2012 to have reduced susceptibility to Penicilin and Cefazolin, besides having increased MICs to Ampicilin and Penicilin (Capanna *et al.,* 2013). Over the past 20 years, there have been increasing instances of reported resistance of GBS isolates to Clindamycin and

Erythromycin. Resistance to Erythromycin is frequently but not always associated with Clindamycin resistance (Verani *et al.,* 2010).

Resistance to macrolides like Erythromycin and Clarithromycin as well as to lincosamides like Clindamycin and Lincomycin majorly occurs by ribosomal methylation and antibiotic efflux (Bianco *et al.,* 2016). Resistance of GBS isolates to quinolones like Levofloxacin and Ciprofloxacin has also occasionally been reported. This majorly occurs through mechanisms like mutation of the target site, efflux, permeability and transmissible resistance mechanisms. Serotype 1b is the GBS serotype that has been greatly associated with quinolone resistance (Teatero *et al.,* 2017).

Group B *Streptococcus* isolates are also generally resistant to other drugs like Tetracycline and Chloramphenicol, in which the bacteria uses chloramphenicol acetyltransferases to inactivate the drug in the latter (Capanna *et al.,* 2013). Due to the rising concerns of increased cases of antibiotic resistance among GBS isolates, it is important to carry out antibiotic susceptibility testing for an individual patient during screening. This will go a long way in helping to evaluate the legitimacy and appropriateness of the new CDC intrapartum chemoprophylaxis recommendations in our local context.

2.6.3 Immunoprophylaxis for prevention of GBS neonatal disease

Initially, attempts to develop a vaccine that would be effective for GBS targeted the capsular polysaccharides. However, these attempts failed due to low and variable

response which was observed from ineffective increases in the titres of antibodies (Rodriguez-Granger *et al.,* 2012). Afterwards, other attempts followed, whereby there was conjugation of polysaccharides covalently with a protein, the tetanus toxoid. Trials for this vaccine were done across non-pregnant adults, pregnant women as well as their babies. The vaccine however proved to be ineffective, owing to the geographical variability of prevalent serotypes as well as the possibly but however rare events of capsular switching among different GBS strains (Rodriguez-Granger *et al.,* 2012).

Recent vaccine developments are targeting conserved surface antigens, like C5a peptidase and the surface immunogenic protein. This puts into consideration the fact that if antibodies are directed against the surface antigens, they can interfere with the virulence factors of the bacteria (Sakata, 2012). Reverse vaccinology which employs genome-based discovery of antigens by integration of genomes, molecular biology and bioinformatics is the most recent development in attempts to develop an effective vaccine for GBS (Sakata, 2012).

This has led to the discovery of possibility of developing a vaccine that is pilus- based which utilizes the pilus- like structures on the surface of the bacteria. Since these structures facilitate adhesion of the bacteria to host cells, an effective vaccine would work against bacterial adhesion to host cells and subsequent biofilm formation (Rinaudo *et al.,* 2010). Owing to all these recent trials, development of a universal vaccine for GBS would possibly be achieved by combining glycoconjugates representative of the most prevalent serotypes with a mix of the pilus proteins.

Unfortunately, despite vaccines for GBS showing a promise of effectively combating GBS disease in trials, there still isn't any approved vaccine in the market for GBS disease (Verani *et al.,* 2010).

2.7 Laboratory diagnosis of GBS

The clinical specimens that can be used to diagnose a GBS infection include urine, blood, cerebrospinal fluid and vaginal and rectal swabs. The gold standard testing for GBS relies on culture using recto-vaginal swabs or urine on chromogenic media like the strep B chromogenic agar, alongside blood agar; BA. When urine is cultured, a significant infection is defined as when organisms have a concentration of at least 10^4 CFUs/ ml (Cagno *et al.,* 2012).

2.7.1 Identification of GBS by phenotypic methods

Culture- based techniques have over time been the gold standard for GBS screening. In this, a critical step during culture is incubating the specimen in selective enrichment broth before proceeding to incubate the specimen onto agar plates. Selective broth like Todd- Hewitt broth supplemented with Gentamicin 8µg/ml and Nalidixic acid 15µg/ml has shown to be effective in maximizing identification of GBS in cultures (Couturier *et al.,* 2014).

Blood agar is used due to its ability to demonstrate traits of hemolysis as displayed by various bacteria, in this case the clear demonstration of the β- hemolytic trait of GBS. Strep B chromogenic agar is selective for GBS and enhances quicker and better growth

of the GBS isolates. It also allows easy identification of the GBS colonies which appear as uniquely colored mauve colonies (Rosa-Fraile and Spellerberg, 2017). Generally, as observed over time, most of the GBS isolates colonizing humans grow on blood agar after being incubated overnight. Typical GBS colonies appear as large grayish mucoid colonies on BA plates, which are normally 3 to 4 mm in diameter, exhibiting a zone of β- hemolysis that is narrow (Rosa- Fraile and Spellerberg, 2017).

However, since β- hemolysis of some strains of GBS may not be easy to detect on BA, it makes it necessary to identify the colonies by extra methods. In most cases, colonies that show morphology that is typical to that of GBS colonies are further subjected to tests that rely on latex agglutination (Stoner *et al.,* 2011). These tests rely on the specific detection of the Lancefield group B antigen, as *S. agalactiae* exists as the only species of the streptococci that expresses the B antigen. Therefore, if a test turns positive, the colonies are said to be those of GBS (Rosa- Fraile and Spellerberg, 2017). In very rare cases, *Streptococcus porcinus* which occasionally colonizes the genital tract as seen in pregnant women also exhibits β- hemolytic colonies on blood agar and may tend to cross- react with latex agglutination kits for GBS.

Thus, colonies presumptively identified as GBS by β- hemolysis on BA and positive latex agglutination testing still require testing that is more specific (Suwantarat *et al.,* 2015). Presumptive differentiation of *S. agalactiae* and *S. porcinus* can be done by comparing their zones of β- hemolysis, in which *S. porcinus* generally exhibits more pronounced and wider zones of β- hemolysis than *S. agalactiae* (Suwantarat *et al.,*

2015). GBS can specifically be distinguished from other streptococcal colonies exhibiting β- hemolysis by detection of granadene, a polyenic pigment that is only produced by *S. agalactiae* (Rosa- Fraile *et al.,* 2014).

For this reason, some people would prefer using Granada- type media; either Granada agar or broth. Incubation is done anaerobically and typical GBS colonies occur as pigmented colonies while all other organisms occur as white colonies (Rosa- Fraile *et al.,* 2014). Various chromogenic media such as CHROMagar strep B; Chrom ID- Strepto B, Strep B select and Brilliance GBS can also be used for culturing GBS. Chromogenic media rely on a combination of chromogens to facilitate differentiation of GBS isolates from other bacteria, by ensuring that the colonies of other bacteria are not similar to those of GBS (Morita *et al.,* 2014).

The chromogenic media have substrates of enzymes which are linked to chromogens of indoxyl. The microorganisms of target are defined by their ability to metabolize the indoxyl substrate hence releasing the chromogen. As a result, the indigoid dye that arises due to the oxidation and dimerization of indoxyl molecules in the presence of oxygen is able to precipitate within the colonies. The colonies therefore acquire different bright colors that aid in their differentiation (Orenga *et al.,* 2009). Chromogenic media should not be incubated anaerobically as this would prevent the compounds of indoxyl from undergoing oxidation. The result is that the development of differently colored colonies would occur poorly (Morita *et al.,* 2014). Storage and incubation of chromogenic media should preferably be done in dark conditions to avoid

degradation of their substrates of chromogens as this would also alter proper development of colors on the isolates (Orenga *et al.,* 2009).

Despite these media enabling quick and easy identification of GBS, their specificity and sensitivity is not 100%, as they do not consist of any chromogen that is exclusively specific for GBS. As a result of this, colonies that are presumptively identified on chromogenic media as GBS require further confirmatory tests majority of which are biochemical in nature. These tests include the pyrrolidonyl arylamidase; PYR test, and the CAMP factor test among others (Morita *et al.,* 2014).

2.7.2 Biochemical identification of GBS

2.7.2.1 Pyrrolidonyl arylamidase test

This test relies on detection of the enzyme pyrrolidonyl arylamidase. It yields invariable results that are negative for GBS, which makes it possible to differentiate GBS from enterococci that exhibit β- hemolysis, group A Streptococci that exhibit morphology that is atypical and *S. porcinus* which are all positive for PYR (Buchan and Ledeboer, 2014).

2.7.2.2 Christie Atkins and Munch Petersen test

The CAMP factor, a cytolytic toxin, is produced by nearly all isolates of GBS with clinical significance. The CAMP test entails streaking the suspect colonies perpendicular to a *S. aureus* streak on a blood agar plate plate followed by overnight incubation at 35-37ºc (Rosa- Fraile and Spellerberg, 2017). A positive test is indicated

by the formation of an arrow-head hemolysis zone which occurs adjacent to the meeting point of the two streak lines as shown in figure 2.1.

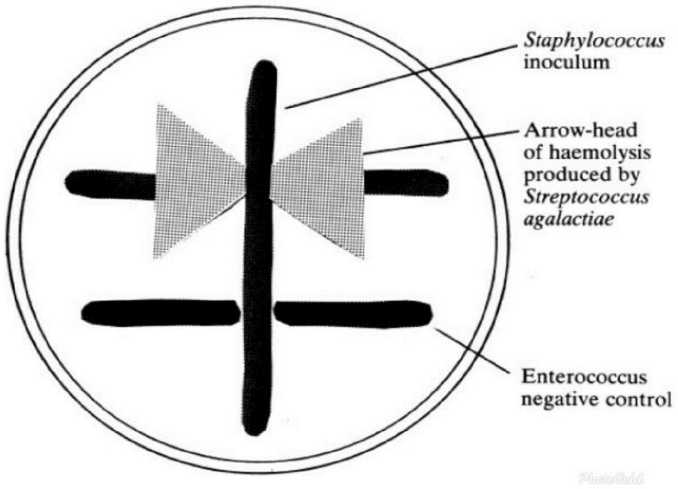

Figure 2.1: Formation of an arrow-head hemolysis zone

Source: (Cheesbrough, 2006)

2.7.2.3 Hippurate hydrolysis test

Hippurate is hydrolyzed by most of the strains of GBS to produce glycine. This test has poor specificity however, as other streptococci such as enterococci react positively for hippurate hydrolysis (Buchan and Ledeboer, 2014).

2.7.3 Maldi-Tof mass spectrophotometry

This is a quick tool for identifying microbes that has emerged over the recent past. It relies on analyzing protein profiles arising from non- fragmenting or 'soft ionization' techniques which allow macromolecules that are specific to each microorganism to be analyzed (Suwantarat *et al.,* 2015). The protein fingerprints; 2-20kDa which act as the attached spectra are compared with a database of spectra that are known. This technique

however requires the use of pure colonies as generating a mass of spectrum that is satisfactory requires deposition of sufficient sample onto a target for analysis. The software programs that are currently available are incapable of analyzing spectra arising from cultures that are mixed (Buchan and Ledeboer, 2014). This expensive but quick technique has an accuracy of 100% in detection of GBS (Clark *et al.*, 2013).

2.7.4 Immunological methods

These methods are quick, simple and specific for detection of GBS. They are serology based methods that rely on determining carbohydrate cell wall antigens that are group-specific. The commercially available kits for GBS identification are largely dependent on coagglutination and latex agglutination (Suwantarat *et al.,* 2015). Usually, streptococcal cells are put together with either latex particles or non-viable staphylococci layered with a coat of hyperimmune antiserum that is also group-specific. If either the staphylococcal cells or latex particles get to clump together, the reaction is interpreted as positive and vice versa. Some test kits are also specifically made to use CSF and they are majorly used when diagnosing neonatal meningitis that is suspected to be GBS- associated (Rosa- Fraile and Spellerberg, 2017).

2.7.5 Molecular methods

These are slowly becoming the most widely acceptable methods for diagnosis in clinical microbiology and they are based on polymerase chain reaction (PCR). Besides using the conventional cultures for antepartum screening of GBS, nucleic acid amplification testing (NAAT) has been recently on increased use and shown to be either almost

equivalent or better (Curry *et al.,* 2018). This is especially the case if before carrying out the NAAT analysis, the sample was incubated in enrichment broth for about 18-24 hours which is also the routine practice in the conventional methods of performing cultures. This is as opposed to just conducting amplification directly from clinical swabs or other specimens (Curry *et al.,* 2018). This makes NAAT based analysis an alternative that is a more potentially sensitive technique as compared to the conventional culture- based screening.

However, the limitation with this technique is that if NAAT which is molecular based is used, the organism is not isolated as with conventional culture, hence hampering the process of carrying out antibiotic susceptibility testing especially for women who have Penicillin allergy (Curry *et al.,* 2018). This thus necessitates the performance of an extra culture whereby the organism will be isolated and AST conducted, if a patient turns GBS positive by NAAT, especially if the patient has a history of allergy to Penicillin. For women whose GBS status is not known at the onset of labor, NAAT is the rapid test of choice as it has a short term turn- around time (TAT) of 1- 2 hours as compared to the conventional cultures that would require 24- 72 hours (Curry *et al.,* 2018).

2.8 Risk factors associated with maternal colonization with GBS

In many circumstances, vaginal carriage of GBS is more associated with younger mothers as compared to more elderly mothers. Some previous studies have revealed that females of maternal age 18-39 years are at a greater risk of being more colonized with GBS as compared to those having an age of > 40 (Kunze *et al.,* 2011). Contrary to this

observation, other studies have reported that higher rates were among much older mothers as compared to the younger mothers. Over time, it has also been observed that the level of education and place of residence have some significant contribution to the likelihood of the mother being colonized with GBS. This can be attributed to a mother's exposure to awareness of antenatal care and better hygiene practices associated with one's education level as well as the locality they live in as these go hand in hand in most cases (Rick *et al.,* 2017).

In most instances, as observed with varied populations, those who have a higher education level of at least above 13 years of school are less likely to be colonized with GBS as opposed to those who have spent a lesser number of years or none at all in school (Rick *et al.,* 2017). While practically controversial, some studies have reported that prevalence of maternal colonization with GBS is low in women of low socio-economic status who subsequently may have limited access to advanced antenatal care. Infection with HIV as a risk factor is also conflicting among different populations; in South Africa for example, lower rates of colonization with GBS were found among HIV infected mothers, in Malawi it was only among mothers who were infected with HIV while no association at all was observed between GBS colonization and HIV infection in both Zimbabwe and the U. S (Seale *et al.,* 2017).

Practices like usage of chlorhexidine based vaginal washing agents which are broad spectrum microbicides, especially in late pregnancy have also been largely associated with maternal colonization with GBS (Cools *et al.,* 2016). Other significant risk factors

include ectopy of the cervix, having multiple sexual partners or participating in commercial sex, which increases the risk of one's colonization with GBS due to easier transfer of the bacteria from one partner to another. This is despite the fact that GBS does not rely on sexual transmission. Also, a previous delivery of a sibling who had EOD or a still birth heightens a mother's chances of continually being colonized with GBS (Nishiara *et al.,* 2017).

This is also observed in mothers who have previously had bacterial vaginosis associated with GBS alongside other bacteria like *Gardnerella vaginalis*, as well as having candidiasis (Nishiara *et al.,* 2017). Colonization with GBS is also a common observation in cases where the mother has a history of previous recurrent UTI in previous pregnancies. A positive association between carriage of GBS and *E. coli* has also been shown in several instances (Cools *et al.,* 2016). Although not yet clearly understood, it has also been noted that mothers who have ever had a previous miscarriage are at a lower risk of being colonized with GBS as opposed to those who have never had a previous or more recent miscarriage (Chen *et al.,* 2018).

In as much as multiple gestation is thought to be associated with higher likelihood of a mother being colonized with GBS, no statistical difference has been found in prior studies between association of parity status with GBS colonization (Khan *et al.,* 2015). Previous studies in Tanzania and Netherlands established that women whose parity status was zero had GBS colonization rates of 23.9% and 21% respectively, without any statistical significance among mothers with different parity statuses. Another study in

Turkey revealed more colonization rates in women who had only delivered once as compared to women who had delivered thrice (Khan *et al.*, 2015). Since no substantial reasons are available to explain the varying colonization rates, it shows that there is need to do further studies that can provide clearer answers pertaining the risk factors associated with maternal colonization with GBS.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The study was conducted in Mbagathi hospital, Nairobi City County. Being the county's referral hospital, the hospital serves an average of 30-35 clients at the antenatal clinic per day according to hospital records. It serves a population of both city residents and informal settlement residents as well as an overflow population of the country's main referral hospital; Kenyatta National Hospital.

3.2 Study design

The study adopted a cross- sectional study design among antenatal women at Mbagathi hospital as it sought to establish the relationship between GBS status among pregnant women and the risk factors associated with GBS colonization over a period of three months.

3.3 Study population

The study involved pregnant women attending ANC clinic at Mbagathi hospital who consented to participate in the study.

3.3.1 Inclusion criteria

For a pregnant woman attending the ANC clinic to be considered eligible for the study, she had to pass the following criteria: she had to be willing and provide consent of participating in the study by filling in and signing the study participants consent form;

appendix v and not have a history of having been on antibiotic therapy for two weeks before the commencement of the study. A participant was also required to not have had a recent history of vaginal bleeding or have genital warts of any form.

3.3.2 Exclusion criteria

The study excluded antenatal women who did not consent to participate in the study as well as those that had a history of having been on antibiotic therapy within the last two weeks before commencement of the study. Also, those that had a recent history of vaginal bleeding or had genital warts of any form were excluded from the study.

3.4 Sample size determination

The sample size was determined by the Fisher's formula (Fisher, 1998). The study assumed a prevalence of 30%, which is the estimated prevalence of maternal colonization with GBS in pregnancy as provided by the revised CDC 2010 guidelines (Verani *et al.*, 2010).

$$n = \frac{Z^2 P (1-P)}{d^2}$$

Where: n= the required size of the sample

Z= the level of confidence, at 95% (standard value of 1.96).

P= the estimated prevalence of maternal colonization with GBS; 30% (0.3).

d= the error margin at 5% (standard value of 0.05)

Substituting; $n = \frac{1.96^2 \times 0.3 (1-0.3)}{0.05^2}$

n= 323 participants.

Therefore 323 samples were collected and analyzed in this study.

3.5 Sampling technique

Systematic sampling was done until the desired sample size was obtained. From previous records, the ANC clinic served an average of 600 clients per month. In projection, an average of 1800 clients was expected to be served during the 3-month study period. Dividing 1800 by 323 which was the target population, 6 samples were collected each day, at intervals of every 5^{th} respondent, considering an average daily clientele of 30.

3.6 Participant recruitment

With the assistance of a female nurse attending at the ANC clinic, the principal investigator informed the women visiting the clinic about the study. A pre-eligibility questionnaire (appendix ii) was filled by those who were interested in participating in the study. Those who willingly consented to participate in the study after having met the requirements of the inclusion criteria were considered eligible.

The recruited members were individually called in a private consultation room whereby all the information on the consent form (appendix iv) was reviewed to enlighten the participant on the study's relevance and the study benefits further explained to her. The specimen collection process was also explained to the participant. It is at this point that a participant was allowed to sign her consent form, followed by filling in of the

questionnaire that was designed to assess the risk factors that are associated with maternal colonization with GBS.

3.7 Data collection tool

Data regarding risk factors associated with maternal colonization with GBS was collected using a coded questionnaire. The appendix iii captured relevant details ranging from socio-demographic factors to obstetric related factors. They included factors like age, place of residence, level of education, history about previous pregnancies such as birth of GBS infected babies, any cases of miscarriages, still births, pre-term births and recurrent UTIs or candidiasis in either the previous or both previous and current pregnancy. The questionnaire also assessed other important risk factors like the number of sexual partners, the HIV status, the parity status as well as the marital status of a participant (Mohamed, 2009).

3.8 Sample collection

The participants were each required to collect a sample of mid-stream urine in the uniquely coded sterile urine containers provided and hand them in just after collection to the laboratory processing area. To ensure properly collected mid–stream urine samples were provided by the participants, they were to follow the collection procedure explained to them during the recruitment.

The proper sample collection was achieved by the participant first voiding a small amount of urine in the toilet bowl, then while holding the sterile container a few

centimeters from the urethra, void urine into the container until it was half way or almost full; 30-40 mls. This was then followed by tight screwing of the container cap back to its position (Hooton *et al.,* 2013).

3.9 Laboratory analysis

3.9.1 Culture

All culture media that were used were prepared under sterile conditions to ensure that there was no or minimal contamination of the media prior to inoculation. Before inoculation on any batch of plates or broth tubes prepared, a pair of each type of media; two of blood agar and two of *Streptococcus* B chromogenic agar as well as two tubes of Todd- Hewitt broth were incubated overnight at 35°C-37°C and examined for any growth the following morning. This was to serve as a control for sterility, since if no growth was observed on the plates or broth tubes after overnight incubation, it confirmed that the media was indeed sterile and only colonies growing from the samples to be inoculated would be observed and rather not as a result of contamination. A sterile wire loop was used at each point of inoculation to ensure sterility was maintained.

Upon receipt in the laboratory, the urine samples were separately inoculated using a sterile wire loop onto correspondingly labeled Todd-Hewitt broth tubes having a supplementation of Gentamycin 8mg/ml, Nalidixic acid 15mg/ml and 5% sheep blood. This was then followed by aerobic incubation at 35°C-37°C for 18-24 hours in 5% CO_2. Sub-culturing was then done on correspondingly labeled blood agar and strep B

chromogenic agar plates, followed by aerobic incubation at 35°C-37°C for 18-24 hours in 5% CO_2 (Rosa-Fraile and Spellerberg, 2017).

All mauve colonies on chromogenic agar plates as well as β-hemolytic colonies on blood agar obtained after enrichment in the Todd-Hewitt broth underwent further sub - culturing on blood agar followed by aerobic incubation at 35°C-37°C for 18-24 hours to obtain pure colonies (Rosa-Fraile and Spellerberg, 2017).

3.9.2 Gram staining and microscopy

Colonies were Gram stained to aid their presumptive identification by observing their Gram reaction. Smears of the colonies were made on clean grease-free slides, air-dried and flooded with crystal violet, Gram's iodine, acetone and safranin reagents, followed by washing after 1 minute of each reagent application. The slides were then observed using x100 objective lens under oil immersion (Strasbourg, 2019).

3.9.3 Biochemical tests

Several biochemical tests were performed for identity confirmation of the presumptively identified colonies through evaluating their known enzymatic reactions. The various tests that were carried out included catalase test, Christie Atkins and Munch-Petersen (CAMP) test, and the pyrrolidonyl arylamidase (PYR) test (Rosa-Fraile and Spellerberg, 2017).

The catalase test was done by suspending a portion of the suspected colonies using a sterile swab from blood agar or strep B chromogenic media in a tube with hydrogen peroxide (Rahbar *et al.,* 2012). These isolates were then subjected to the CAMP test which entailed streaking of the suspect colonies perpendicular to a *Staphylococcus aureus* streak on a blood agar plate followed by overnight incubation at 35 ºC -37ºC (Rosa-Fraile and Spellerberg, 2017). The CAMP test was performed using the recommended protocol as illustrated in figure 3.1.

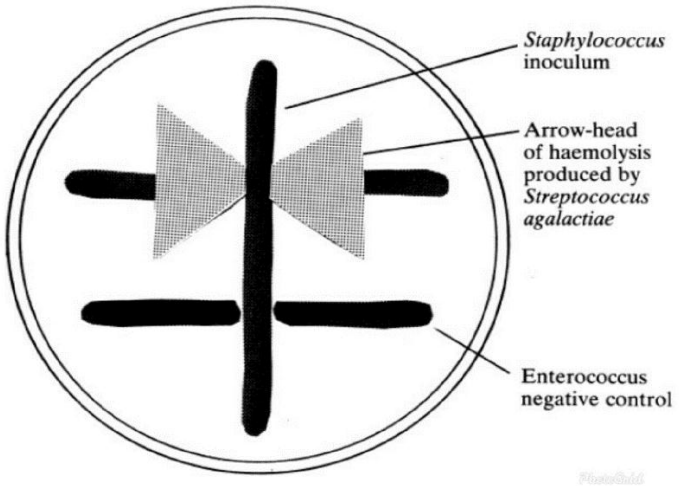

Figure 3.1: Formation of an arrow-head hemolysis zone

Source: (Cheesbrough, 2006)

Typical GBS colonies were positive for the CAMP test. This was indicated by the formation of an arrow-head hemolysis zone which occurred adjacent to the meeting point of the two streak lines as shown above. The CAMP test was controlled by running a parallel test with commercially acquired colonies of *Enterococcus faecalis;* ATCC 29212, whose intersection with the *Staphylococcus aureus* streak failed to produce a zone of arrow- head hemolysis as expected.

The final confirmatory test of the GBS isolates was the PYR test as this test always gives a negative result for GBS. This was performed by picking up the suspect colonies using a sterile wire loop and emulsifying them in a small volume of PYR broth. This was then followed by incubation of the tube at 35°C for 4 hours. After 4 hours of incubation, a drop of the PYR reagent was then added to the tube and the tube observed for color change within one minute. A yellow-orange color appeared in the tube, indicating a negative result, as it is with typical GBS colonies. This made it suitable for differentiation of GBS from all other β-hemolytic streptococci including *Streptococcus pyogenes* (Rosa-Fraile and Spellerberg, 2017). The PYR test was controlled by running a parallel test with commercially acquired colonies of *Enterococcus faecalis;* ATCC 29212, in whose tube a cherry red color occurred after addition of the PYR reagent as expected.

3.9.4 Antibiotic susceptibility testing

This was done using the bioMérieux VITEK® 2 system (Pincus, 2010). Quality control for AST was performed using *Streptococcus pneumoniae* ATCC 49619 as per the CLSI recommendations (CLSI, 2020). Morphologically similar colonies were picked from a labeled plate using a sterile swab and transferred into a tube with 3mls of saline. These were then mixed to form a homogenous suspension. The turbidity of the suspension was checked using a turbidimeter and the density adjusted to 0.5 McFarland units. Using a 280ml pipette, an aliquot of the organism suspension was then transferred to another tube containing saline (Gherardi *et al.,* 2012).

The Vitek 2 *Streptococcus* AST card transfer tube was then placed into the AST suspension. The cassette was now placed into the system for carrying out of AST and MIC determination. The remainder of the organism suspension was then discarded. The system then automatically carried out the antibiotic susceptibility testing and the resulting minimum inhibitory concentration (MIC) values were translated into the clinical breakpoints as susceptible, intermediate or resistant, with reference to the Clinical and Laboratory Standards Institute; CLSI 2020 guidelines (CLSI, 2020).

The interpretive categories and MIC breakpoints in µg/mL as per CLSI 2020 guidelines for the drugs used were as follows: Penicilin; ≤ 0.12 - sensitive, Ampicilin; ≤ 0.25 – sensitive, Cefepime, Cefotaxime and Ceftriaxone; ≤ 0.5 – sensitive, Vancomycin; ≤ 1 – sensitive. Linezolid; ≤ 2 –sensitive. Intermediate and resistant breakpoints were not provided for the above drugs, as it has been shown over time that all strains of GBS are 100% sensitive to all the above drugs, with deviations from this occurring very rarely (CLSI, 2020). The breakpoints for the other drugs used were: Erythromycin and Clindamycin; ≤ 0.25 – sensitive, 0.5 – intermediate, ≥ 1 - resistant. Azithromycin; ≤ 0.5 – sensitive, 1 – intermediate, ≥ 2 - resistant. Tetracycline and Levofloxacin; ≤ 2 – sensitive, 4 – intermediate, ≥ 8 – resistant. It was noted that susceptibility and resistance to Azithromycin could be predicted by testing Erythromycin. This has been summarized in table 3.1.

Table 3.1: Interpretive categories and MIC breakpoints; µg/mL

Interpretive Categories and MIC Breakpoints; µg/mL			
Antimicrobial Agent	**Sensitive (S)**	**Intermediate (I)**	**Resistant (R)**
Penicilin	≤ 0.12	-	-
Ampicilin	≤ 0.25	-	-
Cefipime, Cefotaxime, Ceftriaxone	≤ 0.5 ≤ 0.5 ≤ 0.5	- - -	- - -
Vancomycin	≤ 1	-	-
Erythromycin	≤ 0.25	0.5	≥ 1
Azithromycin	≤ 0.5	1	≥ 2
Tetracycline	≤ 2	4	≥ 8
Levofloxaxin	≤ 2	4	≥ 8
Clindamycin	≤ 0.25	0.5	≥ 1
Linezolid	≤ 2	-	-

Source: (CLSI 2020)

3.10 Data presentation, analysis and dissemination of research findings

The data was analyzed using the statistical packages for social sciences version 17. Data was presented using figures, graphs and tables to show the overall prevalence of GBS, antibiotic susceptibility patterns as well as prevalence of GBS alongside each risk factor assessed. Percentages were used to compare the differences in prevalence of group B *Streptococcus* among participants across various categories as well as the differences in antibiotic susceptibility patterns of the bacteria against the various antibiotics that they were tested against. The Chi square test was used to test the association between the risk factors associated with maternal colonization with group B *Streptococcus* and the group

B *Streptococcus* status of the participants. The research findings after completion of the study were disseminated by publication.

3.11 Ethical considerations and confidentiality

Ethical approval was obtained from Kenyatta University ethics review committee and a research permit was obtained from the National Commission for Science, Technology & Innovation (NACOSTI). Permission was also obtained from Mbagathi hospital management before commencement of the study. All participants that had not attained legal age but were willing to participate in the study had their parents or guardians give consent on their behalf. All participants were provided with their culture results during subsequent ANC visits. In addition, all study materials were coded to ensure confidentiality.

CHAPTER FOUR

RESULTS

4.1 Demographic characteristics of study population

A total of 323 pregnant women at Mbagathi hospital ranging from age 16 years-41 years were enrolled in the study. These women were from across all trimesters of their pregnancies. One hundred and seventy nine; 179 (55.4%) of the participants had ≤25 years of age while one hundred and forty four; 144 (44.6%) of the participants had ≥26 years of age. Two hundred and four; 204 (63.2%) of the participants resided within Kibra constituency while one hundred and nineteen; 119 (36.8%) of them resided outside Kibra constituency. Majority of the participants; 132 (40.9%) were in their first trimester of pregnancy, while the second trimester of pregnancy had 106 (32.8%) participants. The least number of the participants; 85 (26.3%) were in their last trimester of pregnancy. The distribution of study participants based on their individual trimesters is shown in figure 4.1.

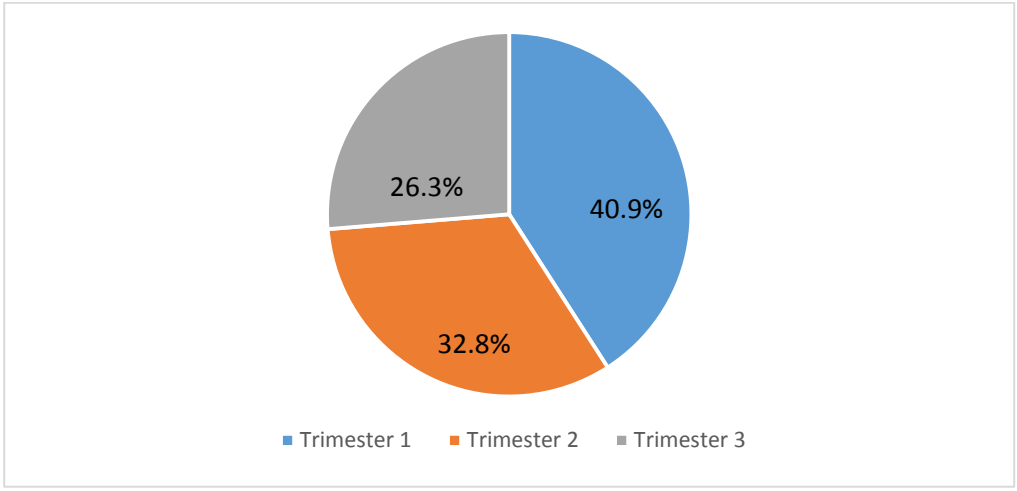

Figure 4.1: The distribution of study participants in percentage according to their trimester of the pregnancy

4.1.1 Socio-demographic characteristics of the study population

These characteristics included age, place of residence, the level of education, marital status and the number of sexual partners.

4.1.1.1 Age of the study population

The age of the study population ranged from 16yrs - 41yrs. The participants were categorized into two groups according to age; those who were aged ≤ 25yrs and those who were aged ≥ 26yrs. Those aged ≤ 25yrs were 179 (55.4%) participants, while those aged ≥ 26yrs were 144 (44.6%) participants.

4.1.1.2 Place of residence of the study population

The study population was grouped into two groups according to their place of residence; those who resided within Kibra constituency and those who were not residents of Kibra constituency. A majority of the participants; 204 (63.2%) resided within Kibra constituency while the minority group; 119 (36.8%) resided outside Kibra constituency.

4.1.1.3 Education level of study the study population

According to the level of education, the study population was grouped into 3 categories. These categories were; those who had attained university/ college education, those who had attained secondary school education and those who had attained primary school education. Only 42 (13%) of the participants had attained university/ college education,

while 193 (59.8%) had attained secondary school education and 88 (27.2%) of them had attained primary school education.

4.1.1.4 Marital status of the study population

The study population was again categorized into either those that were single or those that were married. Only 83 (25.7%) of the participants were single, while a majority of them; 240 (74.3%) were married.

4.1.1.5 Number of sexual partners the study population

With regard to this factor, the study population was categorized into either those having one sexual partner or those having multiple sexual partners. A majority of them; 271 (83.9%) had one sexual partner while only 52 (16.1%) had multiple sexual partners.

4.2 Prevalence of group B *Streptococcus*

A total of 49 participants out of the 323 participants were found to have been colonized with GBS. Therefore the prevalence of GBS from the study was 15.2 %. Of the 49 participants that were found to be colonized with GBS, 20 (40.8%) were those from the third trimester, 18 (36.7%) were from the second trimester and 11 (22.4%) were from the first trimester. This clearly showed that there was a significant association between a participant's gestational age and their GBS status ($p= 0.024$, $\chi^2= 7.446$, $df= 2$).

4.2.1 Prevalence of GBS according to age of the participants

The prevalence of GBS among participants who had ≤ 25yrs was (15.1%) with 27 out of the 179 participants who had ≤ 25yrs of age having been colonized with GBS. On the other hand, out of the 144 participants who had ≥ 26yrs of age, 22 (15.3%) were found to be colonized with GBS.

4.2.2 Prevalence of GBS according to place of residence of the participants

Maternal colonization with GBS among participants that were residents of Kibra constituency was (14.2%) with 29 out of the 204 participants residing within Kibra constituency having been colonized. The rate of maternal colonization with GBS among non- Kibra constituency residents was (16.8%) with 20 participants out of the 119 who were residing outside Kibra constituency having been colonized with GBS.

4.2.3 Prevalence of GBS according to level of education of the participants

Only 5 (11.9%) of the 42 participants who had attained university/ college education were found to be colonized with GBS while 29 (15%) of the 193 participants who had attained secondary school education were found to be colonized with GBS. On the other hand, only 15 (17%) of the 88 participants who had attained secondary school education were found to be colonized with GBS.

4.2.4 Prevalence of GBS according to number of sexual partners of the participants

The prevalence of GBS among participants who had one sexual partner was 14.8%, with 40 out of the 271 participants who had one sexual partner being found to be GBS positive. Their counterparts; those who had multiple sexual partners had a GBS colonization rate of 17.3%, with 9 out of the 52 participants in this category having been colonized with GBS.

4.2.5 Prevalence of GBS according to marital status of the participants

Participants who were married had a colonization rate of 15.4%, with 37 out of the 240 who were married being found to be colonized with GBS. On the other hand, those that were single had a colonization rate of 14.5%, with 12 out of the 83 who were single being found to be colonized with GBS.

4.2.6 Prevalence of GBS according to parity status of the participants

Multiparous participants; those that had given birth more than once were found to be more colonized with GBS than their nulliparous counterparts; those that had not given birth previously. Of the 183 multiparous participants, 30 (16.4%) were found to be GBS positive, while only 19 (13.6%) of the 140 nulliparous participants were found to be GBS positive.

4.2.7 Prevalence of GBS among participants with history of still birth

Of the 3 participants who had a history of still birth, 2 (66.7%) were found to be GBS positive while only 1 (33.3%) of these participants was not colonized with GBS.

4.2.8 Prevalence of GBS among participants with history of neonatal sepsis

Of the 34 participants who had a history of neonatal sepsis, 10 (29.4%) were found to be GBS positive while 24 (70.6%) of these participants were not colonized with GBS.

4.2.9 Prevalence of GBS among participants with history of miscarriage

7 (35%) of the 20 participants who had a history of miscarriage were colonized with GBS while 13 (65%) were not colonized with GBS.

4.2.10 Prevalence of GBS among participants with history of neonatal death

Only 2 (33.3%) of the 6 participants who had a history of neonatal death were found to be GBS positive, while the rest 4 (66.7%) were found to be negative for GBS.

4.2.11 Prevalence of GBS among participants with history of preterm delivery

Of the 12 participants who had a history of preterm delivery, 8 (66.7%) were found to be GBS positive while 4 (33.3%) of them were found to be negative for GBS.

4.2.12 Prevalence of GBS among participants with dysuria

A high percentage of those who had dysuria were found to be GBS positive, with 11 (78.6%) of the 14 participants who had dysuria were found to be colonized with GBS while only 3 (21.4%) of these were not colonized with GBS.

4.2.13 Prevalence of GBS among participants with any form of vaginal infection

A high percentage of those who had any form of vaginal infection were colonized with GBS. 24 (68.6%) of the 35 who fell in this category were colonized with GBS while only 11 (31.4%) among them were not colonized with GBS.

4.2.14 Prevalence of GBS among participants with lower abdominal pain

A high percentage of the 39 participants who had lower abdominal pain were found to be colonized with GBS, with 18 (62.1%) being found to be GBS positive while only 11 (37.9%) of those who were experiencing lower abdominal pain were found to be GBS negative.

4.2.15 Prevalence of GBS among HIV positive participants

Two (66.7%) of the 3 participants who were HIV positive were colonized with GBS while 1 (33.3%) were found not to be colonized with GBS.

4.3 Antibiotic susceptibility profile of GBS isolates

Antibiotic susceptibility testing for the GBS colonies was also performed and results translated into clinical breakpoints as either sensitive, intermediate or resistant

according to the CLSI 2020 guidelines. All isolates; 100% were sensitive to Penicillin, Ampicillin, Ceftriaxone, Cefuroxime, Cefotaxime, Cefepime, Vancomycin and Linezolid. The sensitivity rates for Erythromycin, Azithromycin, Levofloxacin, Clindamycin and Tetracycline were 69.4%, 81.6%, 85.7%, 79.6% and 71.4% respectively. It was also noted that Erythromycin had the highest resistance among the isolates; 30.6%. The isolates also exhibited resistance towards Azithromycin; 18.4%, Levofloxacin; 14.3%, Clindamycin 20.4% and Tetracycline; 8.2%. Tetracycline had the most unique susceptibility pattern amongst all antibiotics. This is because 20.4% of the isolates exhibited intermediate susceptibility towards the antibiotic, while no other antibiotic used for AST had such a pattern. The antibiotic susceptibility patterns of the GBS isolates are shown in table 4.1.

Table 4.1: Antibiotic susceptibility patterns of GBS isolates (%)

Antibiotic	Sensitive (S)	Intermediate (I)	Resistant (R)
*Penicillin	100	-	-
*Ampicillin	100	-	-
Ceftriaxone	100	-	-
Cefuroxime	100	-	-
Cefepime	100	-	-
*Azithromycin	81.6	-	18.4
Levofloxacin	85.7	-	14.3
Erythromycin	69.4	-	30.6
Clindamycin	79.6	-	20.4
Tetracycline	71.4	20.4	8.2
Vancomycin	100	-	-
Linezolid	100	-	-

* Penicillin, Ampicillin and Azithromycin are the commonly used antibiotics for treatment of GBS associated infections in pregnant women.

4.3.1 Actual counts of the GBS isolates subjected to AST

In terms of actual counts of the bacterial isolates when subjected to AST, all isolates that were subjected to AST; 49 exhibited sensitivity towards Penicillin, Ampicillin, Ceftriaxone, Cefuroxime, Cefotaxime, Cefepime, Vancomycin and Linezolid. However, a number of the isolates exhibited resistance towards the antibiotics used as follows; Erythromycin; 15, Azithromycin; 9, Levofloxacin; 7, Clindamycin; 10 and

Tetracycline; 4. Tetracycline also had 10 of the isolates exhibiting intermediate sensitivity towards it. This is demonstrated in figure 4.2.

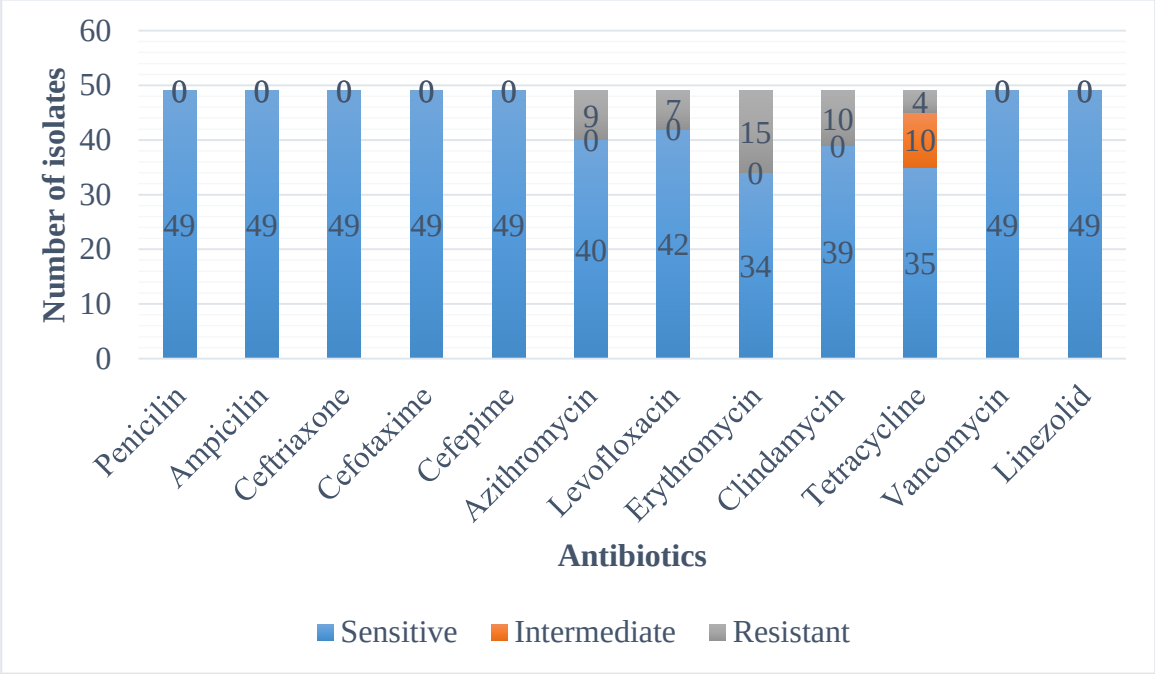

Figure 4.2: Actual counts of the GBS isolates subjected to AST

4.4 Risk factors associated with maternal colonization with GBS

The Chi square test was used to test the association between a participant's GBS status and the evaluated risk factors. P≤ 0.05 was considered statistically significant. McNemar's values were considered. The risk factors evaluated included those that were socio-demographic related, those that were obstetric related such as parity status of the participants, as well as bad outcomes associated with previous pregnancies. The bad outcomes associated with previous pregnancies encompass events such as having a history of still birth, having a history of a baby with neonatal sepsis, having a history of neonatal death or even miscarriage and also having a history of preterm delivery. Only 75 (23.2%) of the total participants had bad outcomes in their previous pregnancies.

Other risk factors that were evaluated were those that were related to clinical symptoms of a urogenital tract infection in the current pregnancy. Only 78 (24.1%) of the total participants had the clinical symptoms that were under evaluation during the period of the study. The HIV status of participants was also evaluated independently as a risk factor for maternal colonization with GBS. Each of the risk factor's association with maternal colonization with GBS has been discussed below.

4.4.1 Socio-demographic factors associated with GBS colonization

4.4.1.1 Age of the study participants

A slight difference of 0.2% in prevalence rate of GBS between the two age categories that participants were placed into; 15.3% being found among those who were ≥ 26yrs of age, and a 15.1% prevalence of GBS being found among participants who were ≤ 25yrs of age was noted. However, age was considered a significant risk factor for maternal colonization with GBS. This is because a p-value of 0.000 was obtained for this risk factor, despite the slight difference with regard to prevalence rate per each category of participants.

4.4.1.2 Place of residence of the study participants

With the participants who resided outside Kibra constituency having a higher prevalence rate of GBS colonization of 16.8% as compared to their counterparts who resided within Kibra constituency who had a prevalence rate of 14.2%, a difference of 2.6% with regard to prevalence rate was noted. However, a statistically significant

association between maternal colonization with GBS and the participants' place of residence was found, with a p-value of 0.000 being obtained for this risk factor.

4.4.1.3 Education level of the study participants

Despite the varying prevalence rates of 11.9%, 15% and 17% of maternal colonization with GBS among participants who had attained university/ college education, secondary school education and primary school education respectively, the differences were found to have no statistical significant association with maternal colonization with GBS. This is because a p-value of 0.075 was obtained for this risk factor.

4.4.1.4 Number of sexual partners of the participants

A difference of 2.5% in prevalence rate of GBS was obtained between participants who had multiple sexual partners; 17.3% and those who had one sexual partner; 14.8%. However, this difference was not found to have a statistically significant association with GBS, with a p-value of 0.083 being reported.

4.4.1.5 Marital status of the study participants

The slight difference of 0.9% in prevalence rate of GBS between participants who were married; 15.4% and participants who were single; 14.5% was found to have a significant association with maternal colonization with GBS, as a p-value of 0.000 was reported for the marital status as a risk factor. Associations between socio-demographic related risk factors associated with maternal colonization with GBS have been summarized in table 4.2.

Table 4.2: Associations between socio-demographic related risk factors with GBS status

Risk factor	GBS (+) N= 49	GBS (–) N= 274	P-value
Age (yrs)			
≤ 25	27 (15.1%)	152 (84.9%)	0.000
≥ 26	22 (15.3%)	122 (84.7)	
Place of residence			
Within Kibra constituency	29 (14.2%)	175 (85.8%)	
Outside Kibra constituency	20 (16.8%)	99 (83.2%)	0.000
Education level			
University/ college	5 (11.9%)	37 (88.1%)	
Secondary school	29 (15%)	164 (85%)	0.075
Primary school	15 (17%)	73 (83%)	
No. of sexual partners			
One	40 (14.8%)	231 (85.2%)	0.083
Multiple	9 (17.3%)	43 (82.7%)	
Marital status			
Single	12 (14.5%)	71 (85.5%)	0.000
Married	37 (15.4%)	203 (84.6%)	

The number of sexual partners and the level of education were the only factors among the risk factors that were socio-demographic related that showed no significant association with maternal colonization with GBS.

4.4.2 Obstetric related factors

4.4.2.1 Parity status of the study participants

The participants were grouped as either being nulliparous; those that had not given birth previously and multiparous; those that had given birth more than once. The difference of 2.8% in GBS prevalence rates between multiparous participants; 16.4% and nulliparous participants; 13.6% was found to have a significant association with GBS, as a p-value of 0.000 was obtained.

4.4.2.2 History of still birth

This risk factor assessed how losing a baby before or during delivery in a previous pregnancy was associated with a participant being colonized with GBS. With a big difference in the high GBS prevalence rate of 66.7% among those who had a history of still birth as compared to the 14.7% prevalence rate of those that never had such a history, a significant association between maternal colonization with GBS and this risk factor was established. This association could be termed as highly statistically significant, with a p-value of 0.000.

4.4.2.3 History of neonatal sepsis

This risk factor assessed how having a neonate who developed a blood infection within either the first week of their life or within the first three months of life from a previous pregnancy was associated with the mother being currently being colonized with GBS. A statistically significant association with a p-value of 0.017 was found between the history of neonatal sepsis and the GBS status of the mother. A huge difference of 15.9%

between the GBS prevalence rates of 29.4% among those that had a history of neonatal sepsis and 13.5% among those that did not have a history of neonatal sepsis was found among the study population.

4.4.2.4 History of neonatal death

Having delivered a child out of a previous pregnancy who later died was found to be significantly associated with maternal colonization with GBS, with a p-value of 0.000 being obtained for this risk factor. Notably, a huge difference of 18.5% between the GBS prevalence rates of 33.3% among those that had a history of neonatal death and 14.8% among those that did not have a history of neonatal death was found among the study population.

4.4.2.5 History of miscarriage

Having lost a baby who was less than 20 weeks in a previous pregnancy was found to have a highly statistically significant association between a mother's GBS status and this history (p=0.000). Notably, a huge difference of 21.1% between the GBS prevalence rates of 35% among those that had a history of neonatal miscarriage and 13.9% among those that did not have a history of miscarriage was found among the study population.

4.4.2.6 History of preterm delivery

The association between having delivered a child before 37 weeks of pregnancy in a previous pregnancy and a mother's GBS status also showed to be of high statistical

significance (p=0.000). Remarkably, a difference of 53.5% between the GBS prevalence rates of 66.7% among those that had a history of preterm delivery and 13.2% among those that did not have a history of preterm delivery was found among the study population. The associations between the obstetric related risk factors and a mother's GBS status have been summarized in table 4.3.

Table 4.3: Associations between obstetric related risk factors and GBS status

Risk factor	**GBS (+) N= 49**	**GBS (–) N= 274**	**P-value**
Parity status			
Nulliparous	19 (13.6%)	121 (86.4%)	0.000
Multiparous	30 (16.4%)	153 (83.6%)	
History of still birth			
Yes	2 (66.7%)	1 (33.3%)	0.000
No	47 (14.7%)	273 (85.3%)	
History of neonatal sepsis			
Yes	10 (29.4%)	24 (70.6%)	0.017
No	39 (13.5%)	250 (86.5%)	
History of neonatal death			
Yes	2 (33.3%)	4 (66.7%)	0.000
No	47 (14.8%)	270 (85.29%)	
History of miscarriage			
Yes	7 (35%)	13 (65%)	0.000
No	42 (13.9%)	261 (86.1%)	
History of preterm deliveries			
Yes	8 (66.7%)	4 (33.3%)	0.000
No	41 (13.2%)	270 (86.8%)	

4.4.3 Factors related to symptoms of urogenital tract infections in current pregnancy

These were evaluated as factors that would signify presence of a urogenital tract infection in the current pregnancy, that are also associated with GBS colonization. They included dysuria; difficulty in passing urine, lower abdominal pain and any form of vaginal infection such as candidiasis or vaginitis. Only 78 (24.1%) of the total participants were experiencing any of the above mentioned clinical symptoms in their current pregnancy during the period of the study.

4.4.3.1 Dysuria

Having difficulty in passing urine was found to have a significant association (p=0.00) with GBS colonization. A notable huge difference of 66.3% in GBS prevalence rate was observed between those that had dysuria; 78.6% and 12.3% among those that were not experiencing dysuria.

4.4.3.2 Lower abdominal pain

Having lower abdominal pain as a factor associated with colonization with GBS was found to be statistically significant, as a p- value of 0.001 was obtained for this factor. Again, a notable huge difference of 51.6% in GBS prevalence rate was observed between those that were experiencing lower abdominal pain; 62.1% and 10.5% among those that were not.

4.4.3.3 Any form of vaginal infection

This risk factor being the last among those that were related to clinical symptoms of a urogenital tract infection in the current pregnancy was also found to be significantly associated with maternal colonization with GBS. A p-value of 0.009 was obtained for this risk factor.

4.4.3.4 Human immunodeficiency virus status of the participants

This risk factor was assessed as an independent risk factor associated with maternal colonization with GBS. Once more, a notable huge difference of 52% in GBS prevalence rate was observed between those that were HIV positive; 66.7% and 14.7% among those that were negative. Therefore, the association between being HIV positive and being colonized with GBS was found to have an association of high statistical significance, with a p-value of 0.000 being obtained for this risk factor. The associations between clinical symptoms of a urogenital tract infection as well as the HIV status of participants and the GBS status have been summarized in table 4.4.

Table 4.4: Associations between clinical symptoms of a urogenital tract infection and HIV with GBS status

Risk factor	GBS (+) N= 49	GBS (–) N= 274	P-value
Dysuria			
Yes	11 (78.6%)	3 (21.4%)	0.000
No	38 (12.3%)	271 (87.7%)	
Lower abdominal pain			
Yes	18 (62.1%)	11 (37.9%)	0.001
No	31 (10.5%)	263 (89.5%)	
Any form of vaginal infection			
Yes	24 (68.6%)	11 (31.4%)	0.009
No	25 (8.7%)	263 (89.5%)	
HIV status			
Positive	2 (66.7%)	1 (33.3%)	0.000
Negative	47 (14.7%)	273 (85.3%)	

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

This study determined the prevalence of group B *Streptococcus* among the study population. It further determined the most recent antibiotic susceptibility patterns of the GBS isolates as well as established the factors that are significantly associated with GBS colonization among antenatal women.

5.1.1 Prevalence of group B *Streptococcus*

This study found the prevalence of colonization with GBS among the study population to be 15.2%. These findings are higher in comparison to those of a similar study conducted in Tortosa, Spain, where a prevalence of 10.8% was reported (Pérez-Moreno *et al.,* 2017). This study's findings are however comparable to those of a study conducted in Makkah, Saudi Arabia where a prevalence of 13.4% was reported (Khan *et al.,* 2015). Another study, conducted in Iran, reported a much lower prevalence of 8.92% (Shayanfar *et al.,* 2012). However, the findings of this study were lower as compared to those reported from a similar study carried out at Kenyatta National Hospital, where a prevalence of 25.2% was reported (Mohammed, 2009).

The differences in the rates of colonization reported from the various studies could have been as a result of several factors. These factors include using study populations from different parts of the world, using different techniques for culture as well as using study populations whose gestational ages vary. The study conducted at Kenyatta National

Hospital screened pregnant women for GBS during late pregnancy; 35-37 weeks of gestation while using recto-vaginal samples as stipulated by the CDC guidelines when screening is done during late pregnancy. On the other hand, this study as well as the studies done in Spain and Iran screened pregnant women for colonization with GBS from across all trimesters of their pregnancy using urine as the sample of choice, as provided for in the updated CDC guidelines. The current guidelines provide for GBS bacteriuria screening at any point of pregnancy, as this is considered a surrogate marker for maternal intrapartum colonization with GBS (Verani *et al.,* 2010). Therefore, this study prospectively predicted the possibility of maternal colonization with GBS during the intrapartum period.

The study conducted in Tortosa, Spain showed that GBS bacteriuria at any point of pregnancy was a significant predictor of maternal intrapartum colonization with GBS. This is because women who had GBS bacteriuria were found to have an increased likelihood of 5-6 times more of being colonized with GBS during late pregnancy and concomitant intrapartum colonization than those who did not have GBS bacteriuria (Pérez-Moreno *et al.,* 2017). This current study observed that majority of participants being colonized with GBS were in their last trimester of pregnancy, a finding that is concurrent with findings from a study conducted in Egypt (Sadaka *et al.,* 2018). This finding further qualifies the CDC's recommendation that screening for GBS should be done during late pregnancy, as cultures performed during this time of gestation have the highest negative predictive value; 95%-99%. This is in comparison to cultures

performed during any other point of gestation, which may have lower negative predictive values (Verani *et al.,* 2010).

5.1.1.1 Prevalence of GBS as per the age of the participants

This study established prevalence rates for GBS of 15.1% among participants who were ≤ 25yrs and 15.3% for participants who were ≥ 26yrs of age. A similar study conducted in Saudi Arabia found the prevalence of GBS for participants aged 20-24yrs to be 15.2%, a finding that was comparable with that established by our study for participants who fell in the same age bracket. However, the study in Saudi Arabia established prevalence rates of 14.3%, 9.7% and 9.5% for participants aged 25-29yrs, 30-34yrs and 35-40yrs respectively (Khan *et al.,* 2015). This was an average prevalence of 11.2% for participants above 26yrs of age, which was lower than our study's finding for participants above ≥ 26yrs of age. Other studies have reported that a young maternal age increases the likelihood of an infant getting early onset disease (Kim *et al.,* 2011). This is attributable to the fact that generally, high vaginal carriage of GBS is more associated with younger mothers as compared to much elderly mothers (Kunze *et al.,* 2011). Other studies have reported that increasing age is associated with increased rates of GBS colonization (Joachim *et al.,* 2009).

5.1.1.2 Prevalence as per the place of residence of the study participants

Similar to findings of other studies, this study found that higher colonization rates with GBS were among residents of formal settlement areas that are generally known to be occupied by people who are of either medium or high socio-economic status who can

afford better antenatal care. This is as compared to the informal settlement areas which are largely occupied by people of the low socio-economic status (Kim *et al.,* 2011). In our study, those that resided outside Kibra constituency were considered to be residing in better formal settlements as opposed to their counterparts who were residing within Kibra constituency, where majority of occupants have informal settlements.

5.1.1.3 Prevalence as per the education level of the participants

This study established the lowest GBS prevalence rate among those who had attained university/ college education, with increasing rates of colonization being found among those who had attained secondary school and primary school education respectively. This can be associated with a mother's exposure to awareness of better antenatal care and better hygienic practices like vaginal washing practices, which are directly attributable to one's level of education. Contrary to findings of our study, higher colonization rates were found among those with higher levels of education as compared to those who had lower levels of education in a study conducted in Iran (Dashitizade *et al.,* 2020).

5.1.1.4 Prevalence as per the number of sexual partners of the study participants

This study established that higher rates of colonization were among participants who had multiple sexual partners as opposed to those who had one sexual partner. This can be explained by the fact that by being found on the urogenital tract, sexual contact favors the transmission of the bacteria from one individual to the other, and hence those having multiple sexual partners having a higher probability of acquiring the bacteria

from their partners. This is despite the fact that GBS is not sexually transmitted (Seo *et al.,* 2010). This finding was consistent with that of a study conducted in a South Africa that established higher colonization rates of GBS among those with multiple sexual partners (Cools *et al.,* 2016).

5.1.1.5 Prevalence as per the marital status of the study participants

This study established that the prevalence of GBS was higher among participants that were married as compared to those that were single. As explained above, sexual contact promotes the transmission of GBS. Hence, as it is more likely for married participants to be more exposed to sexual contact as compared to their single counterparts, they end up having higher extents of colonization than those who are single (Seo *et al.,* 2010). This finding was consistent with those of a similar study in South Africa which found that a higher colonization rate was among the married as well (Cools *et al.,* 2016).

5.1.1.6 Prevalence as per the parity status of the study participants

This study established that multiparous women had a higher colonization rate than nulliparous women, a finding that was consistent with findings of a study conducted in India which had a similar observation (Sharmilla *et al.,* 2011). Another study in Tanzania established that a higher rate of GBS colonization; 50% was among women who had delivered more than 5 times when compared to those who had delivered fewer times and substantial colonization rates among nulliparous women; 23.9% (Khan *et al.,* 2015). In as much as multiple gestation is thought to be associated with higher

likelihood of a mother being colonized with GBS, no clear reasons for this have yet been established, which calls for further research to get a scientific reason for the same.

5.1.1.7 Prevalence as per the bad outcomes of previous pregnancies

This study found higher rates of GBS colonization among participants that had histories of bad outcomes of previous pregnancies; histories of still birth, neonatal sepsis, neonatal death, miscarriage and preterm delivery. This can be explained by the fact that GBS is attributed to many cases of neonatal sepsis, pre-term births, still births, very low weight deliveries and maternal puerperal sepsis which are subsequently responsible for substantial neonatal mortality rates (Melin and Efstratiou, 2013). This finding was contrary to findings of a similar study conducted in KNH which found higher rates of GBS colonization among participants who did not have histories of bad outcomes of previous pregnancies as opposed to those that did not (Mohammed, 2009).

5.1.1.8 Prevalence as per the symptoms of urogenital tract infections

This study established low rates of GBS colonization among participants who did not have any form of vaginal infection, such as vaginitis or candidiasis as opposed to those who had vaginal infections. Existing infections are associated with compromised immune defenses. Conversely, the non-existence of other forms of vaginal infections like vaginitis and hence non-compromised immune defenses among some participants explains why lower rates of colonization with the bacteria existed among participants who did not have other urogenital tract infections. This finding was consistent with

findings of a similar study in Korea that reported similar observations (Kim *et al.*, 2011).

The prevalence of GBS was found to be higher among those that had dysuria and lower abdominal pain. Common bacteria like *Escherichia coli* have been previously frequently isolated from most of people with urogenital tract infections. With the knowledge that a positive association between carriage of GBS and *E. coli* has been shown in several instances, then this could be the probable reason why higher prevalence rates were among those expressing symptoms of urogenital tract infections, where bacteria like *E.coli* are usually implicated (Cools *et al.,* 2016). On the other hand, other studies in Iran reported higher rates of GBS colonization among those that did not have these symptoms (Dashitizade *et al.,* 2020).

5.1.1.9 Prevalence as per the HIV status of the study participants

In this study, a high GBS prevalence rate was among participants who were HIV positive as opposed to the other studies where higher rates of colonization with GBS were reported among those that were HIV negative (Cools *et al.,* 2016). The high prevalence rates among HIV positive participants are attributable to a weakened immune system associated with HIV, which favors infection even by commensals which turn to be pathogenic (Gray *et al.,* 2011).

5.1.2 Antibiotic susceptibility profile of GBS isolates

As for antibiotic susceptibility testing, all isolates that were tested demonstrated 100% susceptibility to Penicillin, Ampicillin, Ceftriaxone, Cefepime, Vancomycin and Linezolid, findings that are similar to those reported by a study in Egypt (Sadaka *et al.*, 2018). This clearly shows that these antibiotics, preferably Penicillin due to its ease of accessibility even in resource poor settings can be effectively used for intrapartum management of GBS as recommended by the CDC in our local settings as it is in other parts of the world.

The GBS isolates also showed resistance towards Azithromycin (18.4%), Levofloxacin (14.2%), Tetracycline (8.2%), Erythromycin (30.6%) and Clindamycin (22.4%). These findings are comparable to those reported from the study conducted in Egypt, as the isolates had demonstrated resistance to the same antibiotics. However, the resistance rates for the same antibiotics reported from Egypt were much higher than those reported from this study, with resistance rates of 28.3% and 43.4%, being reported for Azithromycin and Levofloxacin respectively. The resistance rates reported for Erythromycin and Clindamycin were 22.6% and 15% respectively (Sadaka *et al.*, 2018).

Both studies also reported intermediate sensitivity for the antibiotic Tetracycline, with this study reporting an intermediate sensitivity rate of 20.4% while the study from Egypt reported an intermediate sensitivity rate of 20.8%. The resistance rates towards Erythromycin and Clindamycin for both studies were comparable and of keen interest to note, as either of the two antibiotics or Vancomycin are recommended by the CDC for

intrapartum antibiotic prophylaxis in women who exhibit allergy towards Penicillin which is the first line drug of choice in those who are not allergic to it (Verani *et al.*, 2010). This emphasizes the need for susceptibility testing before administering either Erythromycin or Clindamycin so as to ensure the activity of the drug on the isolate, which should advisably be accompanied with Penicillin-allergy testing as recommended by the American College of Obstetricians and Gynecologists (ACOG, 2019).

In this study, the resistance rates for the second line drugs of choice for IAP; Clindamycin and Erythromycin which were 22.4% and 30.6% respectively are comparable to rates reported across other parts of the world. This is because the average rates reported for resistance towards Clindamycin are 2.3% - 57.9% and those for Erythromycin are 4% - 58.3% (Shabayek and Spellerberg 2018). Interestingly, 10 of the isolates that were resistant to Erythromycin were also resistant to Clindamycin. Resistance to Erythromycin being higher than Clindamycin, makes the drug Erythromycin to be no longer recommended for use as an alternative for Penicillin-allergic women. Besides, the drug has been observed to induce resistance to Clindamycin as well as being noted to have a poor ability to cross the placenta. Its inability to cross the placenta effectively causes it to fail to produce levels of drugs that are therapeutic in either the blood of the fetus or amniotic fluid (ACOG, 2019).

The fact that 100% of the isolates were susceptible to Vancomycin justifies the CDC's recommendations on usage of the drug for IAP for Penicillin-allergic patients whose GBS isolates are resistant to Clindamycin (Onwuchuruba *et al.*, 2014). However,

Vancomycin should be used as an option of last resort since its general use has been associated with other adverse effects such as emergence of organisms that are resistant, for example Vancomycin resistant enterococci (VRE). This goes a long way in having significant negative implications in public health (Phillips *et al.*, 2018).

5.1.3 Risk factors associated with maternal colonization with GBS

Contrary to the finding of our study where age was found to have a significant association with maternal colonization with GBS, a similar study conducted in Saudi Arabia found no statistically significant association between maternal age and GBS colonization (Khan *et al.,* 2015). Some studies have reported that increasing age is associated with increased rates of GBS colonization (Matee *et al.,* 2009). Other studies have reported that a young maternal age increases the likelihood of an infant getting early onset disease (Kim *et al.,* 2011).The differences in colonization rates can be explained by the fact that different studies draw their subjects from populations of varying geographical location.

While our study found the association between GBS colonization and a participant's place of residence to be significant, other studies found no statistical association between the two (Cools *et al.,* 2016). However, Similar to findings of other studies, this study found that higher colonization rates with GBS were among residents of formal settlement areas that are generally known to be occupied by people who are of either medium or high socio-economic status. This is as compared to the informal settlement areas which are largely occupied by people of the low socio-economic status (Kim *et*

al., 2011). Residents of formal settlements who are generally people of the medium or high socio-economic status are believed to be more likely exposed to practices like usage of chlorhexidine based vaginal washing agents. These agents are broad spectrum microbicides and are largely associated with maternal colonization with GBS, hence their users have higher colonization rates than those who do not use them (Cools *et al.*, 2016).

This study found no significant association between the education level of a participant and their GBS status; a finding that was consistent with findings of other studies (Dashtizade *et al.,* 2020). This study established that higher rates of colonization were among participants who had multiple sexual partners as opposed to those who had one sexual partner. This finding was consistent with that of a study conducted in South Africa that established higher colonization rates of GBS among those with multiple sexual partners (Cools *et al.,* 2016). However, in both studies, no statistical association was found between a participant's number of sexual partners and their GBS status. This can be explained by the fact that GBS is not exclusively sexually transmitted, and hence the impact of sexual behavior on transmission and consequently colonization by GBS is debatable (Seo *et al.,* 2010).

On the other hand, this study established that the relationship between a participant's marital status and their GBS status was statistically significant. This could be explained by the fact that since married participants are more likely to have sexual contact as opposed to their single counterparts, then sexual contact may promote the transmission

of the bacteria, as it is generally found on the genital tract. This is regardless of the fact that the bacteria is not sexually transmitted, but whose transmission still relates to that of other microorganisms like *Candida* spp whose transmission is promoted by sexual contact. The contribution of sexual activity to colonization with GBS can be hypothesized to be one that leads to only a temporal vaginal colonization by GBS (Cools *et al.,* 2016).

This study established that the relationship between a participant's parity status and their GBS status was statistically significant, while other studies have found no significant association between the two (Khan *et al.,* 2015). The effect of parity status on colonization with GBS is not yet clear, since some studies report significant associations with increasing parity while others report the vice versa. These differences would be due to ethnic, geographical or socio-economic variations and show the need for further studies to confirm how the parity status of women from different ethnic, geographical and socio-economic background is correlated with GBS colonization (Dashtizade *et al.,* 2020).

This study found all bad outcomes of previous pregnancies like history of still birth, history of miscarriage and history of neonatal sepsis to be significantly associated with GBS colonization. These findings were contrary to findings of a similar study conducted in similar settings which established only still birth among all the outcomes of previous pregnancies to have a significant association with GBS colonization (Mohammed, 2009). The findings of this study are however justifiable, as they are in

line with the knowledge that GBS is associated with causation of miscarriages, preterm deliveries, still births, neonatal sepsis and neonatal death (Nan *et al.,* 2015).

Notably, in this study all clinical symptoms of urogenital infections; dysuria, lower abdominal pain and having any form of vaginal infection such as candidiasis were found to have significant associations with GBS colonization. This finding has also been reported in mothers who have previously had bacterial vaginosis associated with GBS alongside other bacteria like *Gardnerella vaginalis*, as well as having candidiasis (Nishiara *et al.,* 2017). The assessed clinical symptoms were meant to evaluate the impact of how having a urogenital tract infection during pregnancy can serve as a predictor for maternal colonization with GBS (Nan *et al.,* 2015). The finding of this study is however contrary to the findings of a study conducted in a geographical setting similar to that of this study that found indicators of a urogenital tract infection like abnormal vaginal discharge, cervical mucus and pyuria not to have a significant association with GBS colonization (Cools *et al.,* 2016).

This study also found the association between a participant's HIV status and their GBS status significant, which others did not. This is attributable to a weakened immune system, hence allowing easy colonization with GBS of the pregnant mothers. This is simply because the immune system during pregnancy is generally weaker than when in a non- pregnant state, hence further weakening by being HIV positive sets in favorable conditions even for commensals to become pathogenic (Gray *et al.,* 2011).

Infection with HIV as a risk factor for colonization with GBS is however conflicting as reported from different studies, with some reporting higher colonization rates among those who are HIV positive and others reporting lower colonization rates or none at all among those who are HIV positive. Having a low colonization rate or none at all among those who are infected with HIV can be justified on grounds that these people are usually on Co-trimoxazole prophylaxis which is largely active against GBS (Matee *et al.,* 2009). The study limitations included a small number of the cases, underreporting of some factors such as the number of sexual partners as well as the lack of pre-gestational examination record in the cases included in the study. More so, our study did not investigate the effect of anal sexual manipulations. This is regardless of the knowledge that genitourinary infections are caused by anal sexual intercourse and manipulations which promote transfer of the infections towards the vulva (Hegazy, 2015).

5.2 Conclusions

i. Out of all the women who attend antenatal clinic at Mbagathi hospital, 15.2% among them are colonized with group B *Streptococcus*.
ii. Penicillin is still the antibiotic of choice for treatment of GBS infections due to 100% sensitivity of all GBS isolates towards it.
iii. Colonization with group B *Streptococcus* is significantly associated with an individual's age, place of residence, marital status, bad outcomes of pregnancy such as still birth, neonatal sepsis, neonatal death, miscarriage and preterm delivery. Being HIV positive and having clinical symptoms suggestive of a

urogenital infection such as dysuria, lower abdominal pain and vaginal infections are also significantly associated with GBS colonization. An individual's level of education and the number of sexual partners do not have any significant association with GBS colonization.

5.3 Recommendations

i. There is need to conduct screening for GBS on all women attending antenatal clinic at any point in the course of pregnancy without necessarily having to wait for screening at late pregnancy.

ii. In absence of Penicillin, Ampicillin or Azithromycin should be used as surrogates for treatment of GBS infections in pregnancy.

iii. Since a risk-based strategy for identifying candidates who are eligible for intrapartum antibiotic prophylaxis is not sufficient, due to lack of specificity, individual screening for colonization with GBS should be done. In the absence of individual screening for GBS, any pregnant woman found to have a risk factor that is considered to be significantly associated with GBS colonization should receive intrapartum antibiotic prophylaxis.

5.3.1 Further research

There is need to conduct further research that would lead to development of an affordable and safe vaccine that covers all strains of GBS.

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APPENDICES

APPENDIX I: GROUP B *STREPTOCOCCUS* PLATES

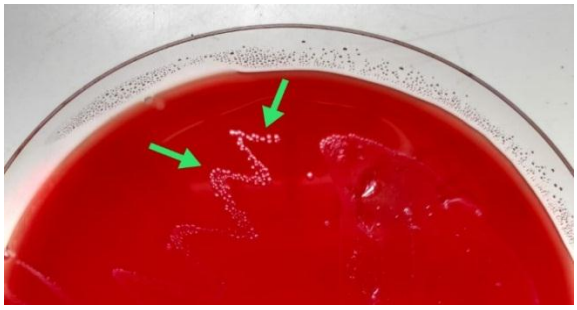


Plate a: GBS colonies on Blood Agar

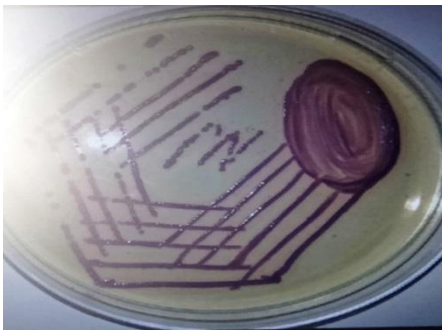


Plate b: GBS colonies on strep B CHROMAgar

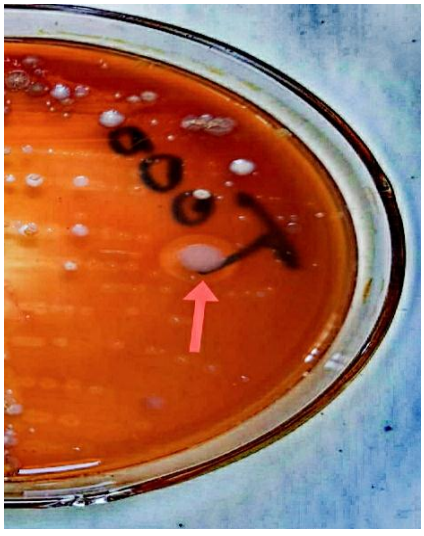


Plate c

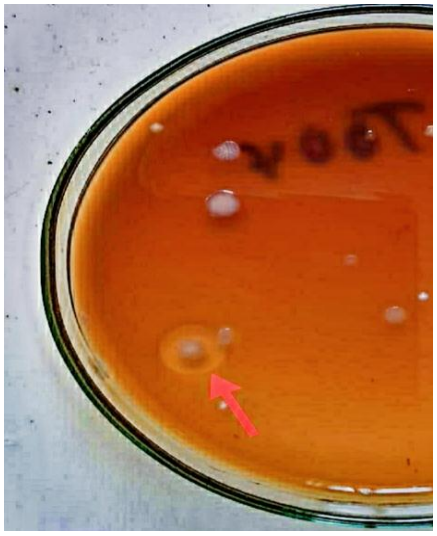


Plate d

Plates c and d: Clear zones of beta-hemolysis demonstrated by GBS colonies on blood agar.

APPENDIX II: PRE-ELIGIBILITY QUESTIONNNAIRE

Please indicate your choice by ticking (√) the most appropriate.

i. Have you been on antibiotic medication within the past two weeks? **(A)** YES **(B)** NO

ii. Have you recently experienced vaginal bleeding? **(A)** YES **(B)** NO

iii. Have you recently had or currently have any form of genital wart? **(A)** YES **(B)** NO

APPENDIX III: QUESTIONNAIRE FOR GBS COLONIZATION RISK FACTOR ASSESSMENT

Questionnaire For Assessment Of Risk Factors Associated With Group B *Streptococcus* Colonization Among Antenatal Women At Mbagathi Hospital.

Please indicate your choice by ticking (√) the most appropriate.

1. How old are you? **(A)**15-20yrs **(B)** 21-25yrs **(C)** 26-30yrs **(D)** 31-35yrs **(E)** Above 35yrs.
2. What is your place of residence? .....
3. What is your highest level of education? **(A)** University **(B)** College/ Technical institute **(C)** High school **(D)** Primary school.
4. How many children do you have? **(A)** 1 **(B)** 2 **(C)** 3 **(D)** 4 **(E)** 5 or more.
5. What is your marital status? **(A)** Married **(B)** Single **(C)** Divorced.
6. How many sexual partners do you have? **(A)** 1 **(B)** 2 or more.
7. Have you contracted a urinary tract infection (UTI) within the course of your pregnancy? **(A)** YES **(B)** NO.
8. Apart from a urinary tract infection (UTI), have you ever experienced any form of vaginal discharge that lead to treatment within the course of your pregnancy? **(A)** YES **(B)** NO.
9. Have you ever given birth to a child who developed pneumonia or meningitis within a week up to three (3) months after birth? **(A)** YES **(B)** NO.
10. Have you ever delivered a pre-term baby? **(A)** YES **(B)** NO.
11. Have you ever had a miscarriage or a stillbirth? **(A)** YES **(B)** NO.

12. Are you aware of your HIV/AIDS status? If **YES**, please provide details……

……..

APPENDIX IV: STUDY PARTICIPANTS CONSENT FORM

My name is Tony Munene, a masters student from Kenyatta University. I am conducting a study on "Group B *Streptococcus* prevalence, antibiotic susceptibility and risk factors for colonization among antenatal women at Mbagathi hospital, Nairobi City County, Kenya". This will contribute to a great extent in the development of strategies to help solve the problem of the ever rising cases of neonatal and maternal post-partum sepsis and mortalities associated with group B *Streptococcus*.

Procedures to be followed during the Study

Participating in this study entails answering a simple questionnaire regarding risk factors associated with group B *Streptococcus* colonization among antenatal women as well as giving a urine sample of 30-40 mls for culture and sensitivity testing.

Voluntarism

Your participation in this study is voluntary, and failure to participate will not affect the services offered to you at the clinic or any other facility either during the study or thereafter. You are at liberty to ask questions pertaining the study or even withdraw from the study regardless of having given your consent to participate.

Discomforts and Risks

Some questions in the questionnaire may target responses that may tend to be a little bit personal or embarrassing. Should you feel that some of the questions in the questionnaire are of this nature, you are free to leave them unanswered. Also, participating in the study will delay your visit to the clinic by about 15 extra minutes to allow for sample collection and recording purposes.

Benefits of participating in the Study

By participating in the study you will help us to develop appropriate interventions on screening and management of group B streptococcal disease. In case you are found positive on screening, advice on the most effective treatment will be given.

Rewards

Participation in the study will not attract any form of reward or payment.

Confidentiality

You will be allocated a unique code that shall identify you rather than your name. Also, all materials throughout the study will be coded and kept in a room under lock and key to ensure confidentiality and safety. Any information regarding you as a participant will only be shared with the supervisors.

Contact Information

Should you have questions pertaining to the study call me on 0727-219626 or the co-investigators; Dr. Menza on 0725-011570 or Dr Mathenge on 0722-936884 or enquire about your rights as a study participant on Kenyatta University Ethical Review Committee Secretariat on chairman.kuerc@ku.ac.ke.

Participant's Statement

All the information pertaining participation in the study is clear to me and I have voluntarily agreed to participate in the study. All queries have been clarified to me satisfactorily. It is also in my knowledge that all my records are confidential and safe,

and that I can choose to quit from participating in the study at any point. By so doing, I am also aware that not even this action will affect the services I receive at this facility either during the study or thereafter.

Name……

Signature or Thumbprint…… Date…….

Investigator's Statement

I, Tony Munene, have disclosed all the necessary information to the volunteer using the easiest comprehensible terms regarding the procedures, benefits and risks involved in the study.

Signature……..… Date……

APPENDIX V: PROTECTION AND CONFIDENTIALITY OF RESEARCH PARTICIPANTS

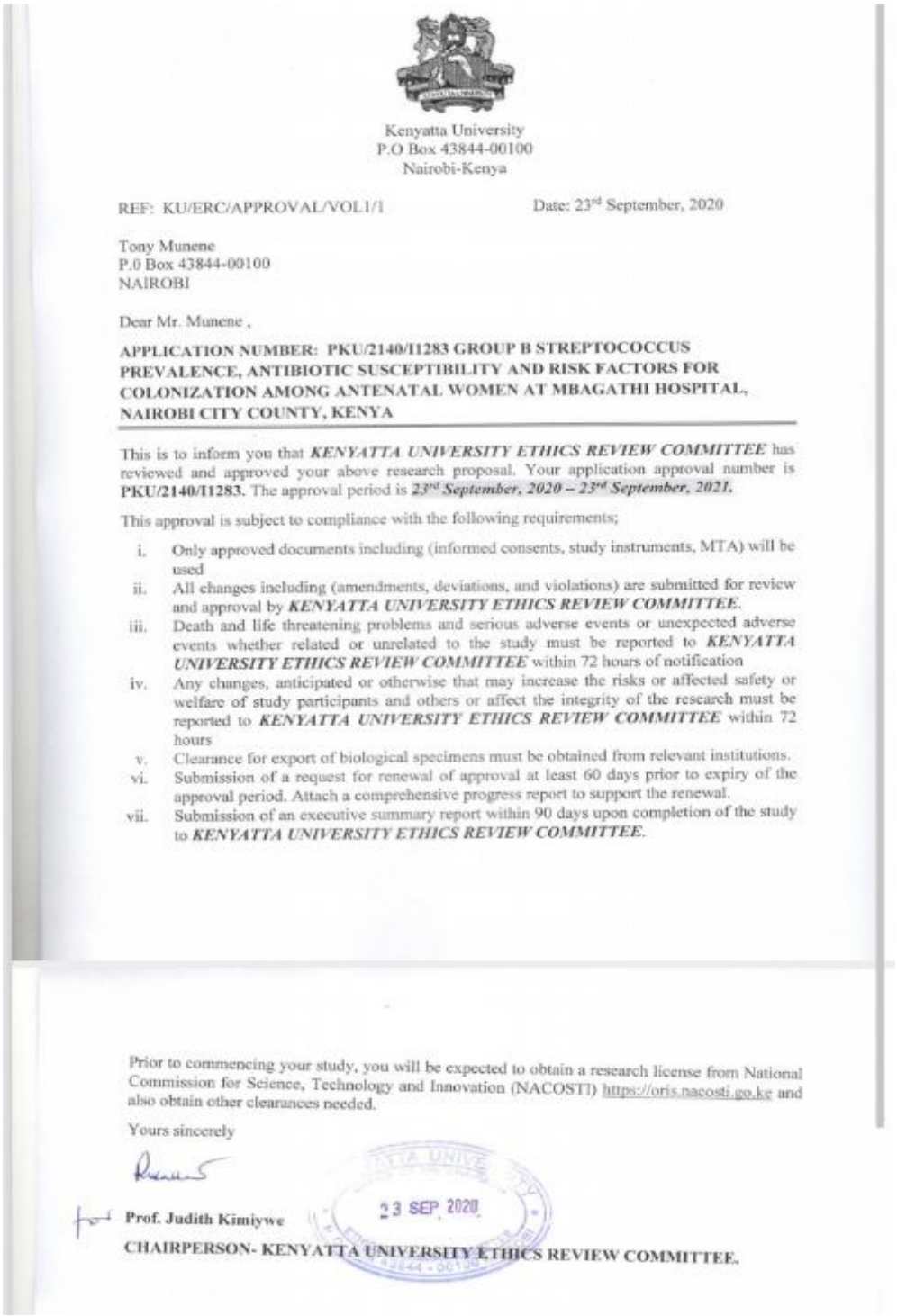
Protection of research Participants

All participants were provided with an informed consent before commencement of the study and their rights as study participants explained to them. The participant (s) who had not attained 18 years of age and were willing to participate in the study had their parents or guardians give consent on their behalf. Participants who chose to withdraw from the study even after giving consent had their decisions respected and this did not in any way affect the services that they received at the clinic during the study or thereafter. All queries raised by the participants pertaining the study were addressed to satisfactory levels. The participant recruitment process was conducted within the minimum time possible to ensure that the participants did not take too long during their visit to the clinic. Further, information provided by the participants was only used to facilitate the study and not for any negative or malicious purposes. A female nurse attending at the clinic was available during the participant recruitment exercise to ensure the women felt comfortable.

Confidentiality of research participants

Recruitment of participants was done privately. All participants were identified using unique codes rather than their actual names during the study. Also, all research materials including the questionnaires were correspondingly labeled with the unique identification codes. These materials were then kept in a cabinet under lock and key to ensure limited access to only the principal investigator and the supervisors.

APPENDIX VI: KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE APPROVAL



Kenyatta University
P.O Box 43844-00100
Nairobi-Kenya

REF: KU/ERC/APPROVAL/VOL1/1 Date: 23rd September, 2020

Tony Munene
P.0 Box 43844-00100
NAIROBI

Dear Mr. Munene ,

APPLICATION NUMBER: PKU/2140/I1283 GROUP B STREPTOCOCCUS PREVALENCE, ANTIBIOTIC SUSCEPTIBILITY AND RISK FACTORS FOR COLONIZATION AMONG ANTENATAL WOMEN AT MBAGATHI HOSPITAL, NAIROBI CITY COUNTY, KENYA

This is to inform you that ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE*** has reviewed and approved your above research proposal. Your application approval number is **PKU/2140/I1283.** The approval period is ***23rd September, 2020 – 23rd September, 2021.***

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE***.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE*** within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE*** within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. 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.
vii. Submission of an executive summary report within 90 days upon completion of the study to ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE***.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely

23 SEP 2020

Prof. Judith Kimiywe

CHAIRPERSON- KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE.

APPENDIX VII: NATIONAL COMMISION FOR SCIENCE, TECHNOLOGY AND INNOVATION (NACOSTI) PERMIT

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **845233**

Date of Issue: **05/October/2020**

RESEARCH LICENSE

This is to Certify that Mr.. tony mwambia munene of Kenyatta University, has been licensed to conduct research in Nairobi on the topic: GROUP B STREPTOCOCCUS PREVALENCE, ANTIBIOTIC SUSCEPTIBILITY AND RISK FACTORS FOR COLONIZATION AMONG ANTENATAL WOMEN AT MBAGATHI HOSPITAL, NAIROBI CITY COUNTY, KENYA. for the period ending : 05/October/2021.

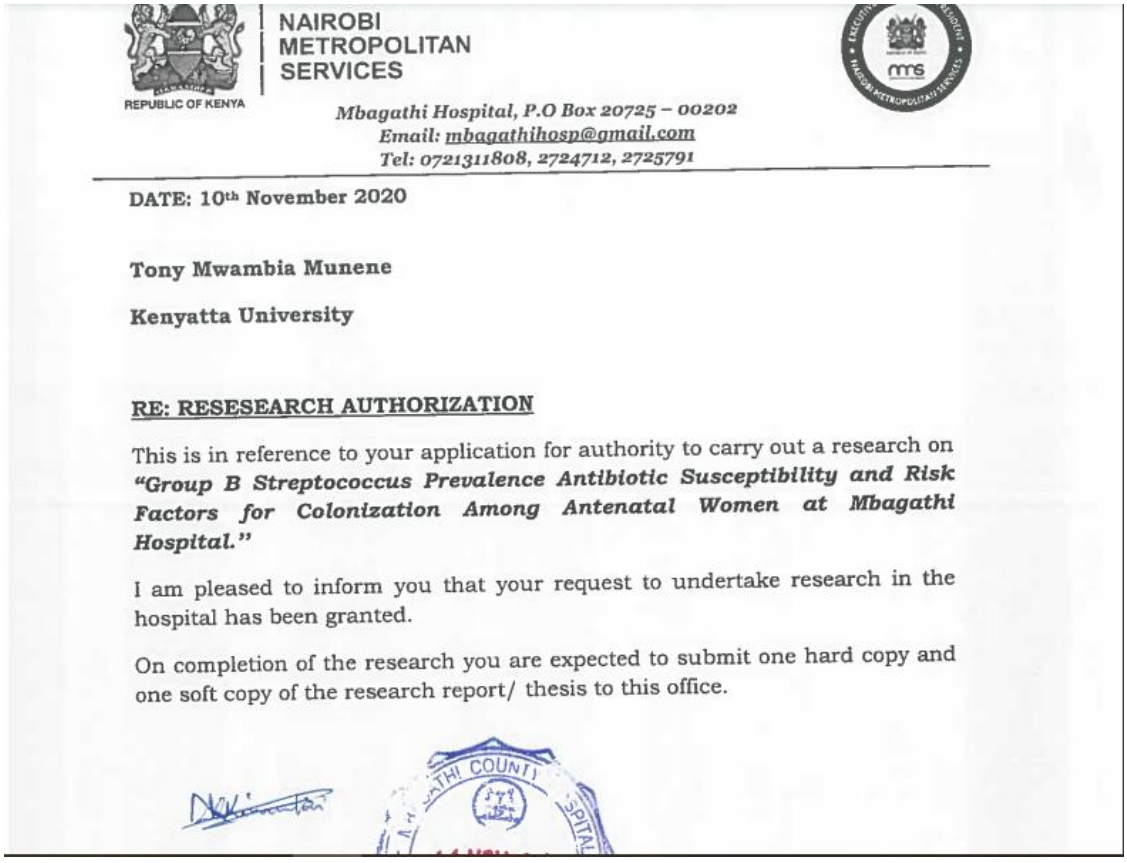
License No: **NACOSTI/P/20/6939**

845233

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

APPENDIX VIII: MBAGATHI HOSPITAL PERMIT



NAIROBI METROPOLITAN SERVICES

REPUBLIC OF KENYA

Mbagathi Hospital, P.O Box 20725 – 00202
Email: mbagathihosp@gmail.com
Tel: 0721311808, 2724712, 2725791

DATE: 10th November 2020

Tony Mwambia Munene

Kenyatta University

RE: RESESEARCH AUTHORIZATION

This is in reference to your application for authority to carry out a research on ***"Group B Streptococcus Prevalence Antibiotic Susceptibility and Risk Factors for Colonization Among Antenatal Women at Mbagathi Hospital."***

I am pleased to inform you that your request to undertake research in the hospital has been granted.

On completion of the research you are expected to submit one hard copy and one soft copy of the research report/ thesis to this office.