PREVALENCE OF BACTERIA IN INTRAAMNIOTIC INFECTIONS AMONG WOMEN IN SPONTANEOUS PRETERM LABOUR WITH INTACT MEMBRANES AT KENYATTA NATIONAL HOSPITAL: AN EXPLORATORY CROSS-SECTIONAL STUDY.

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DECLARATION

This dissertation is my original work done with guidance of my supervisors and has not been presented for the award of any degree in any other university.

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DEDICATION

This work is dedicated to my mothers (Rosemary Auma Amenge and Wylfrida Apiyo Murunga) and every woman who has ever experienced the burden of preterm birth.

LIST OF ABREVIATIONS

DEFINITION OF TERMS

Preterm labour - Labour that occurs before 37 weeks gestation.

Term labour - Labour that occurs at 37 weeks and beyond.

Intact membranes- Prior to rupture of fetal membranes (amnion, chorion)

Subclinical Intraamniotic infections- Presence of micro-organisms in the amniotic cavity with no clinical signs and symptoms.

Intraamniotic infection (chorioamnionitis)- infection that result in the inflammation of any combination of amniotic fluid, placenta, fetus, fetal membranes or decidua.

List of figures

List of tables

Table of Contents

ABSTRACT

Background and Objectives: Preterm births are a major public health concern with global estimates at 10.6% of all births. It is estimated at 13.5% at the Kenyatta National Hospital, Nairobi Kenya. Asia and Sub-Saharan Africa accounts for about 80% of all preterm births. It remains an obstetric dilemma. Intraamniotic bacterial invasion causes spontaneous preterm labour, most commonly from ascending infections. It's associated with increased maternal and neonatal morbidities, decreased preterm-labour onset to delivery time and poor response to tocolysis. There is paucity of information on identification and antimicrobial susceptibility patterns of the involved bacteria. The aim of this study was to determine the prevalence of subclinical intraamniotic bacterial infection in women in spontaneous preterm labour with intact membranes presenting at a tertiary teaching hospital located in SubSahara Africa, the identity of the bacterial isolates and their antimicrobial sensitivity.

Design: this was an exploratory cross-sectional study involving 22 gravid women consecutively recruited from the antenatal and labour wards of Kenyatta National Hospital, Nairobi, Kenya.

Methodology: 22 women with singleton pregnancies in spontaneous preterm labour with intact membranes were consecutively sampled and recruited in the study. Informed consent was obtained. Pretested questionnaires used to collect demographic and obstetric data. Continuous ultrasound guided amniocentesis was performed aseptically and samples cultured forr presence of bacteria and their antibacterial sensitivity. Descriptive analysis was applied and prevalence of subclinical intra-amniotic bacterial infection calculated. Bacterial isolates and their antimicrobial susceptibility were described in proportions.

Results: 22 gravid mothers in spontaneous preterm labour with intact membranes were recruited with a mean age of 26.7 years (SD 6.3). 36% and 41% had primary and secondary level of education respectively. 36% were primi-gravida. 68% presented at gestational ages

of 32-36weeks. There was bacterial growth in amniotic fluid samples from 2 of the 22 participants. Enterococci feacalis and Staphylococcus epidermidis were the identified bacteria with varied anti-microbial susceptibility.

Conclusion: The prevalence of subclinical bacterial intra-amniotic infection amongst women in spontaneous preterm labour with intact membranes was 9%. Spontaneous preterm labour with intact membranes is potentially associated with subclinical intraamniotic bacterial invasion and needs to be further evaluated to ascertain causative association and to guide its management.

Key words: 'Preterm-labour', preterm –births, 'intra-amniotic invasion', 'intact membranes', bacteria.

CHAPTER 1

INTRODUCTION

Background and Justification

Preterm labour remains a challenge in obstetrics care with majority being inexplicable (1). An increasing body of evidence suggest intra-amniotic infections being very prevalent amongst women with spontaneous preterm labour. This infection is often by the ascending microbes from the vaginal and gut flora and not uncommonly by sexually transmitted infections such as chlamydia trachomatis (2). In a systemic review on bacterial aetiological agents of intraamniotic infection and preterm birth in pregnant women, Mendz L. et al (2013) found that the most common bacterial phylla were Firmicutes, and Fusobacteria. Other phyla identified included proteobacteria, actinobacteria and bacteriodes. Mycoplasmatales (*mycoplasma hominis, ureaplasma parvum, ureaplasma urealyticum* among other taxa) and lactobacillates (*streptococcus agalactiae, streptococcus aeroginosus,* and *Streptococcus mitis* among other taxa in this order) were found in 58.9% and 25% of women respectively (2). The rate of preterm deliveries, peri-partum sepsis, neonatal sepsis and other neonatal morbidities amongst premature babies maybe reduced significantly with the right care. This right care requires adequate knowledge of intraamniotic space bacterial pattern in the setting of preterm labour in terms of prevalence of and identification of bacterial isolates and the antibacterial sensitivity of the isolates

There was paucity of information on this subject in Kenya and Kenyatta National Hospital. This study tried to unravel the prevalence of bacterial isolates and their antimicrobial sensitivity patterns in subclinical intra-amniotic infections in singleton women with spontaneous preterm labour.

Studies on use of antibiotics in spontaneous preterm labour have largely been inconclusive. However common factor to the choice of the antibiotic in these was that they were not informed by any antibacterial sensitivity studies, a key component of antibacterial stewardship.

This study was intended to provide insight into possible cause of spontaneous labour and form basis for consideration in the development of protocols on management of preterm labour in KNH. The study involved identifying women with singleton pregnancy in spontaneous preterm labour with intact membranes in KNH labour ward and antenatal wards. Consenting participants underwent ultrasound guided amniocentesis. The collected sample were submitted for microbial culture and sensitivity.

CHAPTER 2:

LITERATURE REVIEW

Labour before 37 completed weeks (259days) of gestation is preterm labour as defined by the World Health Organisation (1970). This is calculated from the first day of last menstrual period (4). Over 80% of preterm births occur in Africa and Middle East with worse survival chances in low and middle income countries (5, 6). Preterm births contribute significantly to infant mortalities globally. Preterm birth survivors are prone to numerous complications such as intra-ventricular haemorrhage, neonatal sepsis and jaundice and kernicterus, feeding difficulty, failure to thrive and delayed milestones. They also face a lifetime disability including learning disability, visual and hearing problems (5, 6). Infection contributes largely to this process. Amniotic fluid compartment is considered a sterile environment. Studies from cultivation and bio-molecular studies shows that amniotic cavities are devoid of bacteria (8-9). In preterm labour, the prevalence of intra-amniotic bacteria invasion and infections is reportedly high. Various ranges are mentioned from 2% to 50%, most of which are subclinical (11-13).

According to World Health Organisation (WHO) about 15 million babies are born too soon. Furthermore, the poorer families have higher rates of preterm births than their richer counterparts (5, 6). Developed countries seem to have increasing prevalence of preterm births due to increasing use of assisted reproductive technology. Preterm birth is a public health issue as it contributes significantly to neonatal and under five mortality but also creates a huge healthcare burden with a potential worse economic implication as the surviving preterm with various complication grow old (15,16).

In Africa, studies done in various institutions provide varied prevalence of preterm births. The constant factor is preterm births are associated with high perinatal morbidities and mortalities. In a study on characteristics and risk factors of preterm births in a tertiary centre in Lagos,

Nigeria, Azeez Butali et al (2016) found that the prevalence of preterm birth was 16.8% amongst singleton pregnancies in the Lagos University Teaching Hospital in the period 2011 to 2013. Older maternal age, hypertension, and premature rupture of membrane being significant risk factors. In the study however it was not clear the contribution of infection to the spontaneous preterm labour (17).

At the university teaching hospital, Enugu in Naigeria, Iyoke C et al (2013) found a similar trend of preterm birth to the Lagos group with a prevalence of 16.9% in the period 1st January 2009 and 31st December 2013 with 57% reported as spontaneous. In the study, the adjusted perinatal mortality rate for preterm babies was 46.1%. Adjusted early neonatal death rate were 24.0%. This figures confirm the high contribution preterm births make towards perinatal mortality.

Two studies that are unpublished done at Kenyatta National hospital exist in the university of Nairobi repository. Ondari et al in 2010 did a study on factors associated with preterm birth, a hospital based comparative cross sectional non interventional study. This study compared characteristics of women who had preterm delivery to those who delivered at term in the period 15th June to 29th August 2000. He found a prevalence of spontaneous preterm births of 8.7% with decreasing perinatal mortality from 79.3% (28-30 weeks gestation) to 4.5% (38 weeks gestation). This study observed no difference in socio-demographic characteristics of the two groups but had antenatal clinic attendance significantly different between the groups with 100% attendance for those women who delivered at term compared to 87% attendance among the preterm births. There was no statistically significant differences between the two groups in parity, previous preterm delivery nor inter-pregnancy interval. Similarly, Wagura et al (2011) found a preterm birth prevalence of 18.3%. It included medically induced preterm births thus the high prevalence of preterm births. In this study by Wagura, he additionally attempted to analyse factors that were strongly associated with early compared to late preterm birth using

expanded univariate analysis and found antepartum haemorrhage (three fold risk amongst early preterm births with OR=4.7 versus 1.7), multiple gestation (seven fold increase in risk for early preterm with OR=6.7), urinary tract infection in pregnancy (OR=2.5 for late preterm versus 1.3 for early preterm), and high parity of 4 or more had increased the early preterm birth by nearly two folds (OR=6.2 versus 3.9). Wagura concluded based on his study findings that biological factors such as high parity, previous preterm delivery, pregnancy associated hypertension, urinary tract infection in pregnancy, antepartum haemorrhage and preterm premature rupture of membranes were significantly associated with preterm births. Most of this factors can be assessed early and prompt treatment provided in addition to early pregnancy screening and treatment for urinary tract infection and hypertensive diseases in pregnancy (10).

Labour is a process that normally occurs at term, which is from 37 completed weeks. There is increasing evidence of decreased maternal morbidity and neonatal morbidity and mortality when mothers deliver between 39 weeks and 0 day to 40 weeks and 6 days. This has been called full term According to the American College Of Obstetrics and Gynecologist, adopted from 2012 work group, the arbitrary 'term pregnancy' has been replaced with early term (between 37 weeks Zero day to 38weeks 6 days), full term (39 weeks 0 days to 40 weeks 6 days), late term (41 weeks 0 day to 41 weeks 6 days) and post term (42 weeks and beyond) (18, 19). The result of this natural process is the expected delivery of a live baby and a stable mother who is able to enjoy the fruit of her pregnancy and motherhood as desired.

The initiation process of labour is yet to be clear. It is however postulated that both maternal and foetal contributions are key. The hormonal changes that occur include reduction of progesterone receptors, increase in estrogen concentration relative to the progesterone levels and increase in production of prostaglandins through the action of phospholipase A2 especially

the secretory phospholipase A2 (sPLA2) (20). However some studies still dispute this role (21). The oxytocin receptors increase in number and concentration. Oxytocin levels increase towards term. It's been shown that the activity of oxytocinase get depressed due to increasing amniotic fluid acidosis towards term that is thought to reduce the removal of oxytocin in the amniotic space and thus allow diffusion of this important chemical through the decidua and chorion into the myometrium to cause uterine contractions. The foetal pituitary releases oxytocin and the adrenal glands release cortisol, factors that play a role in initiation of labour at term (22-25). New information potent that the aging process of foetal tissues could also be the basis of labour initiation. This line of thought harbours that senescence initiates inflammatory process that triggers release of immunological factors such as cytokines and chemokine that are proinflammatory resulting in labour initiation (26, 27). With increasing evidence supporting this theory, other factors such as intra-amniotic bacterial invasion that may initiate intra-amniotic inflammation may therefore result in labour. Infection elicits inflammation which is associated with increasing production of inflammatory cytokines and proteins. To this, high concentration of inflammatory proteins and cytokines such as interluikin (IL) 6, IL 1a, microphage inflammatory proteins 1a, IL 1b and interleukin 4, are present in the intraamniotic fluid of women with intramnioitc inflammation in preterm labour. These are even much higher in amniotic fluid from women with spontaneous labour with intact membranes with evidence of intraamniotic microbial associated inflammation (28).

Labour both at term and before term is an inflammatory process. It is associated with increasing release of cytokines, chemokines, prostaglandins and matrix degrading enzymes. The inflammatory process triggers uterine contraction, membrane rupture and cervical changes that results in ripening of the cervix and labour. Both innate and specific immune systems play a role. The dendritic cells that bridge the two immune systems also play a role in releasing inflammatory molecules (29. 30). The trigger to inflammatory process before term pregnancy

remains a difficult dilemma to unravel. It's however been shown that intraamniotic infection is a trigger before term.

Amniotic fluid and the membranes are largely sterile in uncomplicated pregnancy with intact membranes before labour sets in. There are however instances when the intra-amniotic content and membranes are invaded by microbes. A number of routes for pathogenic entry of microbes into intra-amniotic space exist. These are the vaginal, haematogenous spread from other body sites and iatrogenic. The intra-amniotic infection has been found to majorly arise from ascending infection from the genitourinary tract. In a systemic review on bacterial aetiological agents of intraamniotic infection and preterm birth in pregnant women, Mendz L. et al (2013) found that the most common bacterial phylla were Firmicutes, and Fusobacteria while proteobacteria, actinobacteria and bacteriodes were also implicated. Mycoplasmatales (*Mycoplasma hominis, Ureaplasma parvum, Ureaplasma urealyticum* among other taxa) and lactobacillates (*Streptococcus agalactiae, Streptococcus aeroginosus, Streptococcus mitis* among other taxa in this order) were found in 58.9% and 25% of women respectively (4).

Neonatal sepsis have been proven to be majorly from mixed genital flora. These are majorly genitourinary bacterial flora, an evidence of ascending infection as a mode of intraamniotic infections (IAM). Loss of cervical protection such as loss of the cervical plug in labour with cervical dilatation increases the risk of intra-amniotic infection. This result in infected amniotic fluid culture often yielding microbial pattern similar to the vaginal flora. Abnormal flora from genitourinary infections and repeated vaginal examinations increase the risk. Equally the virulence of the microorganism indigenous in the genital tract or causing genital tract infection determines the rate, presentation and severity of infection (31).

Trans-placental passage of microbes into the intra-amniotic compartment may occur. This follows systemic bacteraemia following maternal infections such as pneumonia, urinary tract

infections, pyelonephritis and meningitis amongst other bacterial infections. Numerous micro-organisms are involved. However, trans-placental passage of *Listeria monocytogens* tend to be more common. This may occur as epidemics or as isolated cases following ingestion of contaminated dairy products. Reduced body immunity as seen in advanced human immunodefiency viral infection, metastatic malignancies, anaemia and malnutrition increase the risk of systemic infection with higher rates of transplacental transfer of microcrobes.

Invasive medical procedures such as amniocentesis, cordocentesis, intra-amniotic transfusion and cerclage may result in direct inoculation of bacterial pathogens into the intra-amniotic space causing infection. These are rare in low socio-economic countries as these procedure are rarely done due to limited resources and skills required.

Intraamniotic infections increase maternal and neonatal morbidity and mortality. Clinical chorioamnionitis is associated with poor neonatal outcomes. According to the American College Of Obstetrics and Gynecology (RCOG), intraamniotic infection also called chorioamnionitis is that infection that result in the inflammation of any combination of amniotic fluid, placenta, fetus, fetal membranes or decidua. Common neonatal complications associated with IAI are neonatal meningitis, sepsis, pneumonia, and death. For the survivor neonates, bronchopulmonary dysplasia and cerebral palsy may occur. The American College of Obstetrician and Gynecologists (ACOG) provides the diagnostic criteria chorioamnionitis to be maternal fever of ≥39°C or maternal fever of between 38°C to 38.9°C with at least one or more risk factors. The factors are foul smelling vaginal discharge, uterine tenderness, fetal tachycardia >160 beats per minute, maternal tachycardia >120 beats per minute (32-34).

Subclinical intra-amniotic infection equally increase risks for maternal morbidities. Mothers with subclinical IAI have been shown to have a higher risk of developing clinical chorioamnionitis with its associated complications including poor neonatal outcomes. Women with intra amniotic infection tend to develop preterm labour that is refractory to tocolytics, higher risk of spontaneous preterm premature rupture of membranes and a short time interval between presentation and delivery thus theoretically reducing the time for lung maturity effect of corticosteroids if used and increasing prevalence of preterm births. The preterm labours with IAI that result in preterm delivery are associated with increased postpartum endometritis and early neonatal sepsis. Pathologic examination of placenta and membranes of women with preterm delivery show as high as over 60% histologic chorioamnionitis. Comparatively, only about 20% of placenta and membranes pathologic examination following term delivery have histologic chorioamnionitis. Acute histologic chorioamnionitis is associated with preterm labour with evidence of inflammation as underlying pathophysiology resulting in preterm labour.

Ken Miyazaki, et al (2007) did a study involving data review for the period July 2001 to March 2006 of women with singleton pregnancy between 22 to 28 weeks gestation diagnosed with subclinical chorioamnionitis. The placentae of these patients who delivered preterm were examined histologically. Subclinical chorioamnionitis was diagnosed by amniotic fluid neutrophil elastase levels and grouped based on severity as group A (0.15-1microg/ml), group B (1-10 microg/ml) and group C (≥10 microg/ml). The study found that the latency period was longer with less severe forms of subclinical chorioamnionitis. Histologic chorioamnionitis and funisitis were 90.4% and 65.5 % respectively. Intrauterine deliveries were four and postnatal neonatal deaths were ten. Bronchopulmonary dysplasia was found to be the most common

major neonatal morbidity in groups B and C. This study intended to show the presence of intraamniotic infection in patients with PPROM (34).

There are no reliable methods or factors that can predict preterm labour and birth with certainty. However various factors have been studied with statistical significance. These include ferritin levels, Fibronectin levels above 0.05ug/ml, interleukin 6 levels in cervical secretion and transvaginal cervical length of less than 1.5 centimetre. Malaak T et al (1996) showed fibronectin levels of 0.05 microgram per millilitre predicted preterm birth with sensitivity of 63%, specificity of 95.6%, positive predictive value of 77.3%, and negative predictive value of 91.6% (36-40, 46-51).

Diagnosis of preterm labour is challenge as the symptoms of preterm labour are nonspecific. According to the American Family physician the symptoms and signs that may suggest preterm labour are frequent uterine contraction (four and above contractions per hour), cramping pain, pelvic pressure, backache and low back pain in a patient whose gestational age is less than 37 completed weeks. These clinical features are nonspecific. Creasy and Herron criteria of preterm labour where uterine contractions of four per 20 minutes or eight per 60 minutes that are accompanied by one of: preterm premature rupture of membranes (PROM),cervical dilatation greater than two centimetres, more than 50 percent effacement, or change in cervical dilatation or effacement detected by serial vaginal examination may be used for diagnosis of preterm labour as done by the University of Alabama at Birmingham. This criteria is however not widespread in use. The difficulty in diagnosis of preterm labour is also reflected in the National Institute of Health and Care Excellence (NICE) guidelines of preterm labour and birth (41, 42). Preterm labour is diagnosed through series of clinical findings. Patient presents with intermittent lower abdominal pains radiating to the back with increasing frequency and intensity or palpable uterine contractions. Examination finding may reveal palpable uterine

contractions with or without cervical changes on vaginal exam such as effacement and dilatation of the cervix.

Amniocentesis is an invasive procedure that involves drawing of amniotic fluid from the intrauterine cavity. It's a common procedure used for diagnostic purposes such as prenatal genetic diagnosis. Other indications includes cases of evaluating preterm premature rupture of membranes and preterm labour with intact membranes especially where infection is suspected. Various routes have been employed such as trans-abdominal and trans-vaginal ideally under ultrasound guidance. This has significantly reduced the risks associated with amniocentesis such as needle trauma to the foetus and foetal loss, risk for vertical transmission of HIV in HIV infected women (43-45). In a systemic review and meta-analysis about procedure related risk of miscarriage following amniocentesis, Akolekar R et al (2015) revealed that risk of pregnancy loss below 24 weeks gestation was 0.81% among women who underwent amniocentesis. This was comparable to the background rate of miscarriage in women from control group who did not undergo the procedure (44).

The post procedure precautions are key. Pregnant women with Rhesus D negative blood group should have anti-D immunoglobulin administered after amniocentesis. Follow up for foetal viability using either cardiotocography or follow up ultrasound are recommended. The study will however exclude all HIV positive women.

The procedure of amniocentesis is also faced with risks of foetal loss, with figures of 1-2% reported (45). Though minimal and comparable to general pregnancy losses without the procedure, it is of concern. Amniocentesis though invasive still remains the only reliable means of studying intra-amniotic cavity.

The use of prophylactic antibiotics in spontaneous preterm labour with intact membranes remains a controversy. The general consensus is that without evidence of infection no antibiotics should be given. The most land mark study, broad spectrum antibiotic for spontaneous preterm labour, the ORACLE 2 collaborative group, in a randomised multicentre study randomised 6295 women with preterm labour with intact membranes with no evidence of infection and assigned them to three treatment arms of 250mg erythromycin, 325 mg Co-amoxyclav (250mg amoxicillin plus 125mg clavulinic acid), both antibiotics and placebo. The primary outcome of focus were composite of neonatal death, chronic lung disease, or major cerebral abnormality on ultrasound before discharge from the hospital with intention to treat analysis. The results did not show any lower rates of the composite outcomes with any of the trial antibiotics (57, 58). This study has formed a major backbone in formulation of recommendations and advice by professional bodies. The RCOG does not recommend routine use of antibiotics in spontaneous preterm labour without evidence of infection. A systemic review by Flenady V et al (2013) involving 14 trials with 7837 randomised women failed to show any significant differences in perinatal or infant mortality between infants of women who were given prophylactic antibiotic compared to those who received none. There was also no significant reduction in preterm births. In their conclusion and recommendations, the antibacterial spectrum of the antibiotics used was questioned as no prior studies had been done to inform the antibiotic choices. The study recommended similar study in local set ups to further unravel the uncertainty surrounding the use of antibiotic amongst women with preterm labour with intact membranes. Significant questions however arise, what informed the choice of erythromycin and co-amoxiclav? Were the doses used optimum? Can this findings mostly carried out in different countries apply in KNH set up where infectious diseases are still among the leading cause of deaths with puerperal sepsis to which endometritis is a common contributor remains the third leading cause of maternal mortality? The local practice is different

as use of antibiotics in spontaneous preterm labour with intact membranes occur from anecdotal evidence. This begged the need for more information as to the prevalence of bacterial subclinical IAI, the bacterial spectrum involved and the antibiotic sensitivity pattern. If IAI is proven to be common, the study will help determine the antibiotic choice to be used on selected patients.

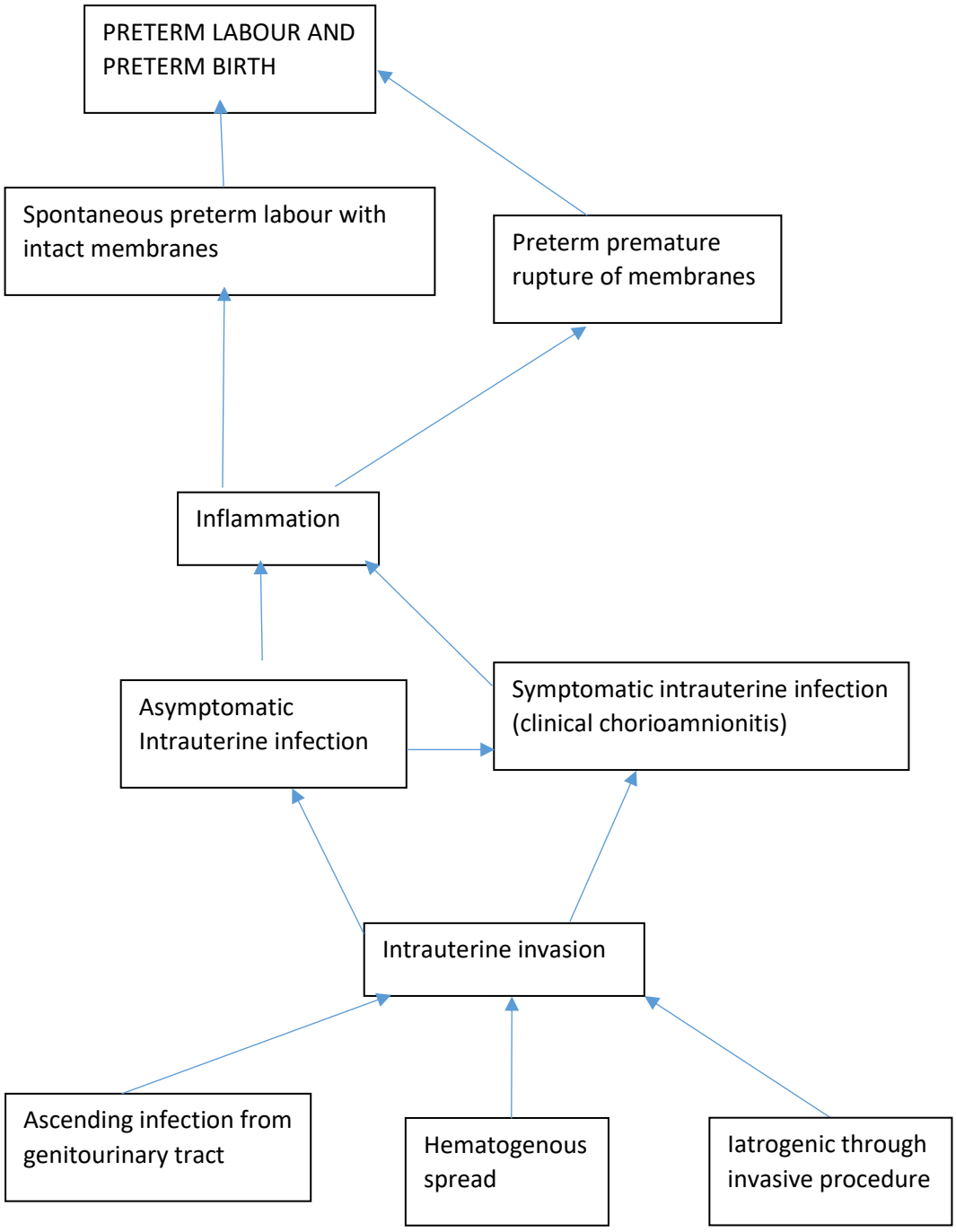


Figure1: Pathophysiologic conceptual framework of intraamniotic bacterial infection in preterm labour

Conceptual framework narrative

Intraamniotic infections (IAI) arise from ascending infections from genitourinary and gut, haematogenous spread from body areas remote from the genital organs including pneumonia and dental carriers and from iatrogenic inoculation of microbes during invasive procedures

such as amniocentesis, fetal cord blood sampling, and chorionic villi sampling. The amniotic space is sterile. Presence of bacteria is considered infective, intraamniotic infection. These can present with overt symptoms such as fever (≥38.1°C), foul smelling vaginal discharge, leucocytosis, fetal (≥160b/min) and maternal (>120b/min) tachycardia, maternal tachypnoea (≥20) and uterine tenderness on palpation. This is symptomatic IAI. More often the IAI are without symptoms forming asymptomatic IAI. Intraamnionic bacteria are recognised by Toll Like Receptors (TLR) and other pattern recognition receptors and activate innate immune reaction. This results in production of cytokines (interleukin, IL, 1 and 6 and Tumor necrotic factor-α) and chemokines (IL-8), prostaglandins, proteases and other enzymes that induce uterine contractions. Other effects may include placental detachment, ripening and weakening of the fetal membranes with rupture of membranes. Preterm labour results either with intact fetal membranes or with preterm premature rupture of membranes which may result in preterm birth unless labour progress is successfully terminated by tocolysis.

In this study of prevalence of intraamniotic bacterial infection amongst women in spontaneous preterm labour with intact membranes presenting in KNH, culture methods were used to identify presence of bacteria in amniotic fluid from consenting women who met the inclusion criteria of the study.

Study justification

Preterm labour remains a challenge in obstetrics. It is a public health dilemma. Intra-amniotic infections has been shown to be prevalent amongst women with preterm labour often from the ascending microbes. Also reported is haematogenous spread from other focus of infection and rarely iatrogenic inoculation of pathologic organisms during invasive procedures. Most of these intra-amniotic bacterial invasion are asymptomatic. The IAI has been associated with refractory uterine contractions despite use of tocolytics, results in short labour onset to delivery

time and associated with endometritis with resultant puerperal sepsis. Babies born preterm are at risk of severe neonatal complications and mortality. The survivors suffers short and long term complications. With improved preterm care more of the preterm babies survive but not without complications. The cost burden to family and the country in taking care of preterm babies is enormous. Equal to improving preterm care, there is need to control and manage the preterm labour. Adequate knowledge of the causes would be key. With over 50% of preterm labour considered idiopathic, unravelling this will form a key step in understanding preterm labour. Intrauterine infections have been postulated and proven to play major role in the idiopathic labour. The prevalence and the identification of the causative organism will be very informative. In the tropics where infections are significant cause of morbidity knowledge on IAI cannot be emphasised. The prevalence of subclinical intra-amniotic infection and pattern of micro-bacterial involvement in preterm labour is not known in the KNH set up.

This study will help provide information that will help inform the obstetric care provided to women preterm labour with intact membranes especially in KNH. It will also play a role in antibiotic stewardship.

Research Question

What is the prevalence of bacteria in subclinical intra-amniotic infections amongst women in spontaneous preterm labour with intact membranes in Kenyatta National Hospital during the period of April 2019 to June 2019?

Objectives

Broad objective;

To determine the prevalence of bacteria in amniotic fluid amongst women in spontaneous preterm labour with intact membranes who presented at KNH during the period of April 2019 to June 2019.

Specific objectives;

Amongst women with singleton pregnancy in spontaneous preterm labour with intact membranes presenting at KNH during the period of April 2019 to June 2019,

To determine the proportion of patients whose amniotic fluid grew bacteria on culture,

To identify the bacterial isolates cultured from amniotic fluid,

To determine antibiotic susceptibility of bacteria cultured from the amniotic fluid.

CHAPTER 3:

METHODOLOGY

Study design

This was an exploratory cross sectional study in which consenting women with singleton pregnancy in spontaneous preterm labour with intact membranes who met the inclusion criteria underwent continuous ultrasound guided amniocentesis and their amniotic fluid cultured for bacteria. This was a first study of its kind. The number of patient who present with spontaneous preterm labour with intact membranes are often few. This was confirmed by secondary data review during a similar period in 2017 during which it was noted that 2 to 3 patients in spontaneous labour with intact membranes presented per week. The study participation was once ending with the amniocentesis. Only the participants whose amniotic fluid yielded positive cultures were counselled and started on relevant treatments. This was however not part of the objectives.

Study site and setting

The study was conducted in labour ward and antenatal wards of Kenyatta National Hospital, KNH. KNH is a public, tertiary and referral hospital located in the immediate west of Upper Hill in Nairobi. It is the largest national referral and teaching hospital in Kenya. It's a teaching hospital to College of Health Sciences of the University of Nairobi and Kenya Medical Training College, Nairobi campus. It has bed capacity of 1800. It offers specialised and subspecialised care making it a first choice hospital for many patients both self and institutional referrals. KNH labour ward handles over 10000 deliveries annually. It cares for patients from the low and middle socioeconomic class. It serves the Nairobi and neighbouring counties of Machakos, Kiambu, and Kajiado but receives referrals from nearly every county within the republic of Kenya. The labour ward has a point of care ultrasound that was used in the study.

The hospital's radiology department had ultrasound machines that were also availed for this study. KNH medical laboratory department runs a separate ISO-certified microbiology laboratory with internal and external quality control systems assurance. The laboratory has Vitek 2, a high technological machine with ability to accurately and rapidly identify microbial susceptibility. The technology allows for rapid susceptibility testing of bacteria grown from culture media.

Study population

The study participants were consenting women with singleton pregnancies in spontaneous preterm labour presenting with intact membranes at gestational ages between 24 weeks and <37 weeks in KNH labour ward and antenatal wards.

Eligibility criteria

Inclusion criteria were:

1. Pregnant women with singleton pregnancy at gestational ages between 24 weeks to 36weeks + 6days confirmed by ultrasound or calculated using last menstrual period;
2. Provided informed consent and could afford the cost of ultrasound.
3. Regular coordinated uterine contractions of at least 2 in 10 minutes and or labour pains associated with any cervical changes such as cervical dilatation, effacement, shortening or softening with intact membranes
4. Negative serology for Human Immunodeficiency Virus

Exclusion criteria

1. overt maternal sepsis or chorioamnionitis (fetal tachycardia >160beats per minute, maternal tachycardia above 120 beats/minute, foul smelling vaginal discharge, uterine tenderness, maternal fever >39.0 degrees Celsius)
2. fetal malformations confirmed on obstetric ultrasound
3. iatrogenic preterm labor
4. Medical complications in pregnancy: poorly controlled diabetes mellitus, Gestational diabetes mellitus.

Sample size calculation

Sample size (N) was calculated using Fischer's formula as shown below

$N=Z\alpha^2\ p\ (1 - p) \div d^2$

Where,

Zα is standard normal variate (at 5% type 1 error ($p<0.05$) it is 1.96, and at 1% type 1 error ($p=<0.01$) it is 2.58). In majority of studies, the p value is considered significant if <0.05. This will be considered thus Zα will be 1.96 in the formula.

P is the expected proportion in the population based on previous studies.

d =absolute error or precision, will be chosen as 0.05

Sample size=$1.96^2\times0.10(0.90)/0.05^2\div$

=138.2976, which translate to 139 study participants

Using p=11% as the expected prevalence of asymptomatic intraamniotic infection amongst women with preterm labour as was the finding by Michael G Gravet et al (1986) in his study 'preterm labour associated with subclinical amniotic fluid infection and bacterial vaginosis'. In his study, Gravet studied 54 consecutive afebrile women in preterm labour with singleton gestations and intact fetal membranes. And found bacteria or *Candida albicans* in about 11% of the patients.

I settled for the lower proportion due to the following reasons:

1. Preterm labor with intact membranes that meets the set inclusion and exclusion criteria were not common in KNH. This was because preterm labor get complicated with premature rupture of membranes and clinical symptomatic infections.
2. Amniocentesis though ultrasound guided to improve on fetal safety was an invasive procedure with risks of fetal loss and iatrogenic introduction of infection into intrauterine cavity.

Based on the above reasons, the sample size was down sized further using the formula for finite population: $n = no \div n/ (1 + (no-1)/N)$

Where n was the sample size, no, the sample size calculated from Fischer's formula above i.e. 139 and N the finite population i.e. the target population of mothers who met the inclusion criteria.

From the secondary data sources, the labour ward discharge register, there were about 2 to 3 patients per week in the period between April to June 2016, who presented with preterm labour having excluded those who had induction of labour due to pre-eclampsia, preterm premature rupture of membranes, intrauterine foetal demise, and other comorbidities such as overt sepsis. The months used corresponded to the period I collected data in 2019. The year 2016 had more semblance to normalcy in terms of healthcare provision without major interferences with

national labour strikes such as that seen in 2017 where doctors, clinical officers, and nurses agitated for better terms of employment causing significant service disruption nationally. This resulted in Kenyatta National Hospital experiencing marked upsurge in number of patients seeking care.

Based on the above, the new sample size after the correction:

No = 139 ÷ (1+138⁄32)

= 27 patients

Considering attrition or accidental spillage, a 10% extra numbers will be added resulting in a new sample size of 30.

Sampling procedure

This was a consecutive sampling owing to the limited number of participants who met the inclusion criteria.

Every pregnant women who presented to KNH labour ward or antenatal ward in spontaneous preterm labour with no reported preterm rupture of membranes were approached, informed of the study and asked to voluntarily participate. Those who consented were recruited into the study. The recruited patients were assessed to rule out any exclusion criteria and those who didn't meet the criteria were excluded. The patients recruited into the study and who met the inclusion criteria with no exclusion factor were sequentially enrolled into the study through consecutive sampling. Every participant who met the inclusion criteria were informed further of the study detail, its objectives and significance, the procedures involved and the associated risks and asked to voluntarily provide an informed written consent. They were made to understand that they could withdraw from the study at any point without suffering loss of

quality care they deserved as patients of KNH. Those who declined to provide further consent were excluded from the study.

Recruitment and Consenting

The recruitment was done from the triage area where the first contact with the patients happened. Those diagnosed with preterm labour with intact foetal membranes were linked up the principal investigator and or research assistant who then explained to the patient about the study and clarified any questions or concerns of the patient factually and truthfully. Those who were willing to participate were assessed for the criteria and allowed to provide written consent. The discussions were done bedside with privacy and confidentiality ensured. The patients continued with the standard care of preterm labour offered in KNH. The study participants were taken to the ultrasound room within KNH labour ward or radiology departments where a trained radiology resident with the assistance of the investigator performed obstetric ultrasound to confirm foetal wellbeing and ruled out any foetal anomaly then through continuous ultrasound guidance amniocentesis was done using aseptic technique. Any patient who chose to withdraw from the study at any point was allowed, thanked for her decision and allowed to proceed with her care as per KNH protocols.

Data collection and management

Women who presented with preterm labour with intact membranes were recruited and after obtaining informed consent were interviewed using pretested questionnaire to extract bio-data, clinical and reproductive health information. This was done by the principal investigator and 2 trained research assistants who were medical officer and a medical officer intern working in Kenyatta National Hospital. Each participant then underwent amniocentesis where 3-5ml amniotic fluid were collected. The samples were labelled using unique codes similar to that

written on the questionnaire and submitted to the microbiology laboratory at KNH for culture and sensitivity. The samples once analysed through microbiologic processes described, the results were entered into the questionnaire.

The data from the questionnaires were entered into Statistical Package for Social Sciences (SPSS) version 23, cleaned and analysed. Applicable statistical methods as discussed in data analysis were applied.

Amniocentesis procedure

Clear information regarding the amniocentesis procedure; the indication being to determine presence of and identity of bacteria in intraamniotic infections among women in spontaneous preterm labour with intact membranes; the procedure itself; possible complications; and follow up ultrasound to confirm foetal wellbeing before and after the procedure were provided to the patient and informed consent obtained.

The patient was asked to lie in dorsal lithotomy position with a 15 degree tilt to the left lateral position.

The abdomen was exposed up to the waistline.

An obstetric ultrasound was performed to check the foetal wellbeing, placental position, foetal and cord position, and to locate amniotic fluid pocket devoid of fetal parts and cord.

The ultrasound transducer was removed and covered with a sterile glove and the patient's skin above the amniotic fluid pocket prepared with povidone iodine six times and draped with sterile O-towel. The sterilised area at the centre of the drape i.e. O centre was cleaned with sterile normal saline to remove possible bactericidal effect of the antiseptic on the intraamniotic bacteria as the needle is introduced into the intraamniotic space. The operator and principal investigator used sterile gloves and maintained sterile technique. The patient was informed not

to touch the sterile area during the procedure. The sterile gel was applied onto the sterile glove covered transducer and used to guide the spinal needle.

A spinal needle sizes 25G was inserted through the skin, abdominal wall, uterine wall and into the amniotic sac under continuous ultrasound guidance. Patient was reassured of the cramping feeling that occur during penetration through the uterine wall.

Five millilitres of amniotic fluid was aspirated into a sterile 10 ml syringe and the spinal needle rapidly removed under direct ultrasound view. The entry site was dressed with an adhesive bandage. The samples were put into well labelled sterile sample containers with a tight lid and submitted for culture and sensitivity within 30 minutes. The samples were transported in a cooler box.

A maximum two attempts for successful amniocentesis were allowed with patient promptly informed of any failure to get amniotic fluid sample. Any complication such as blood stained fluid, dry drainage were made known to the patient. However none of these untoward outcomes occurred.

After the amniocentesis, an immediate fetal reassessment for foetal wellbeing was done. The patient care continued as per hospital protocol of preterm labour.

Patients who were Rhesus negative were given antiRhesus D immunoglobulin 300 micrograms to prevent isoimmunisation.

The amniotic fluid samples were subjected to bacterial culture using various media for both gram positive and gram negative bacteria and the identified growths were analysed for antibiotic sensitivity using a Vitek 2 machine.

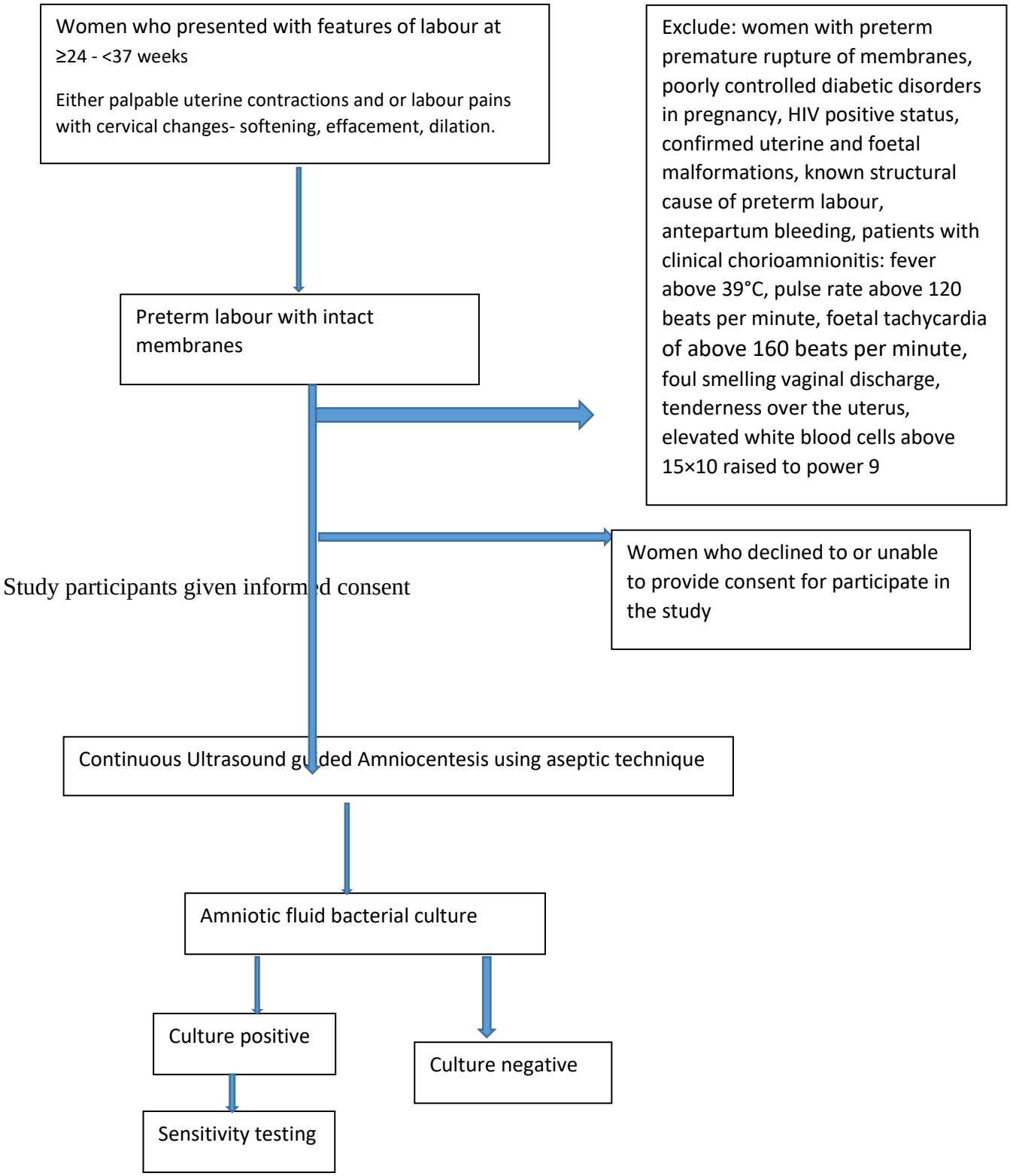


Figure 1 Methodological flow chart

Table 1: Data variables

SPECIFIC OBJECTIVES	EXPOSURE VARIABLE	OUTCOME VARIABLE	SOURCES OF DATA
determine the proportion of patients whose amniotic fluid grow bacteria on culture	Number of women presented with preterm labour with intact membranes recruited into the study.	Number of women who underwent amniocentesis. Proportion of patients whose amniotic fluid culture grew colonies of bacteria	Labour ward patient data entry book, patient files laboratory report
To identify the bacterial isolates in subclinical IAI by culture method	Number amniotic fluid specimen whose culture grew bacterial colonies	Identity of the bacterial isolates that grew from amniotic fluid specimen	Patient's files Microbiology report
to determine antimicrobial sensitivity of bacterial isolates in subclinical IAI	Amniotic fluid whose culture grew bacterial colonies. Specific bacterial isolates that grew on culture.	Antimicrobes to which each bacterial isolates were sensitive to using vitek machine. Bacterial isolates with resistance to antibacterial molecules of common antibiotics	Patient files Filled questionnaires Microbiology report

Data analysis methods

The data from questionnaires were entered into Statistical Package for Social Sciences, SPSS version 21. Data cleaning was done and data analysis done.

The mean, median and mode were used in describing numerical data that is age, parity, gestational age and presented in tables and pie charts.

In calculating prevalence, the number of patients whose amniotic fluid grew or showed bacterial colonies on culture were divided by the total number of patients with spontaneous preterm labour with intact membranes who underwent amniocentesis and their amniotic fluid sample analysed in the laboratory.

Subgroup analysis of the patients whose amniotic fluid grew bacteria on culture were analysed and specific bacterial colonies identified, expressed in proportions and presented in form of tables and bar charts.

Ethical Considerations

Approval was sought from the Department of Obstetrics and Gynaecology of The University of Nairobi and the Kenyatta National Hospital - University of Nairobi Research and Ethics committee.

Authorisation from Kenyatta National Hospital administration and departments of Obstetrics and Gynecology, Laboratory medicine and radiology departments were obtained.

An informed consent was obtained from study participants. An elaborate consent form with brief description of the study and its objectives including the benefits and risks that may result from the study procedures was provided to the participants. The study research assistants, who were midwives, and Medical Officer were recruited from KNH and trained. The research assistants provided precise explanation and answered every participant's questions before taking consent.

Ultrasound guided ultrasound was done by postgraduate masters of medicine in Radiology students in the UoN and selected sonographers from KNH who were recruited into the study to do ultrasounds and ultrasound guided amniocentesis to minimise the risk of injury to the foetus. As a common practice in management of preterm labour, obstetrics ultrasound is usually done to assess the foetal wellbeing as well as confirm gestation age. During the study, the participants who met the inclusion criteria and provided informed consent voluntarily underwent amniocentesis under continuous ultrasound guidance. This was to allow direct visualisation of the needle as it accessed the identified amniotic fluid pocket with no foetal parts and ensured that no unnecessary depth into the pocket was traversed. This eliminated the risk of injury to the foetus.

Spontaneous preterm labour was an inclusion criteria. The small risk of the uterine muscle excitation during amniocentesis resulting in preterm labour did not apply as spontaneous preterm labour had already set in in the recruited patients.

Confidentiality was maintained in handling study participants and their study records. The records were assigned unique serial numbers and all identifiers to the patient omitted in the study records. These records were only be used for research purposes and accessed by the principal investigator and the supervisors only.

The cost of ultrasound and anti-D for rhesus negative mothers who are not isoimmunised were met by the patients as these were part of their routine care for preterm labour.

Participants whose amniotic fluid grew bacteria were informed of the results and appropriate treatment instituted.

Important information gained from this study will be disseminated to the participants, policy makers at the ministry of Health, KNH and the department of Obstetrics and Gynaecology-University of Nairobi.

Study Limitations

The study involved amniocentesis to collect amniotic fluid samples for analysis. The procedure though a routine obstetric procedure was invasive and associated with some risks. These included pregnancy losses, and iatrogenic introduction of infection into the intra-amniotic space. These factors were made clear to the study participants during consent taking resulting in reduction of number of participants giving consent or being anxious during the procedure. This limited the sample size target. This risks were mitigated by ensuring that well trained personnel carried out the amniocentesis. Strict sterility during the procedure were adhered to following the protocol. Amniocentesis was ultrasound guided to reduce risk of injury to the fetus. The principal investigator with the help of radiologist ensured that safety and adherence

to protocol was followed. The amniocentesis was done by radiology masters of medicine students and selected sonographers who were recruited into the study and trained on the procedure.

There was risk of blood stained amniotic fluid being collected. This was minimized by ultrasound guidance and application of correct anatomy by avoiding major abdominal wall vessels.

The samples collected were transported to the KNH microbiology laboratory. This did portend a risk of delaying analysis as well as prone to accidents of spillage. This was minimized by ensuring that the sample collected was submitted to the laboratory within 30 minutes to ensure quality yield. The samples in a well labeled sample container with tight lid were transported in a cooler box.

The presence of bacterial isolates confirmed presence of bacterial IAI.

Small risk of skin contaminants yielding normal flora during the microbial studies existed. This was mitigated by strict adherence to aseptic technique. Skin preparation with povidone iodine was done.

CHAPTER 4:

RESULTS

Study Flow

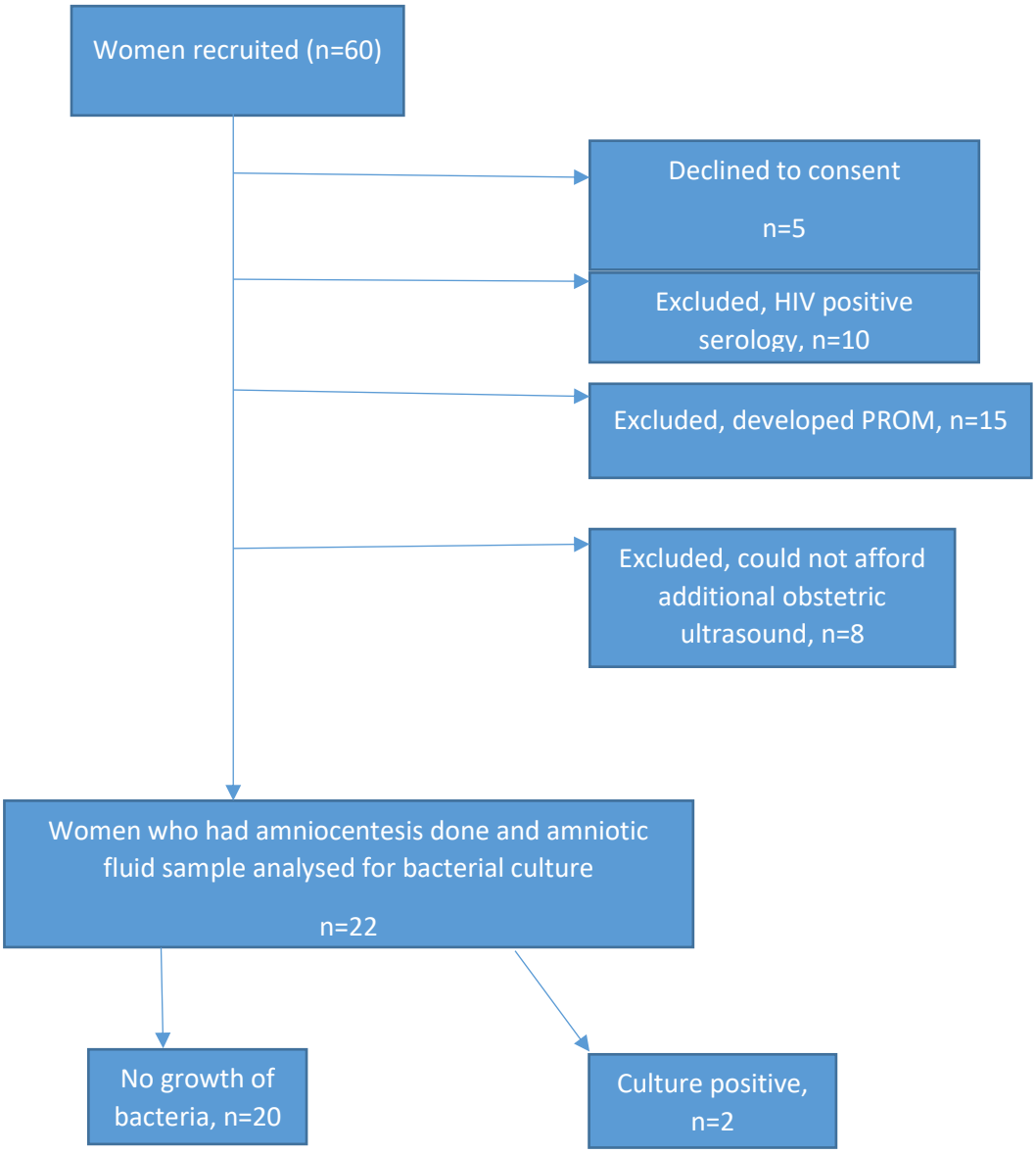


Figure 3: Study flow **of women with singleton pregnancy with spontaneous preterm labour with intact membranes presenting Kenyatta National Hospital, April – June 2019. PROM – preterm rupture of membranes, HIV- Human Immunodeficiency Virus.**

Study Participants Characteristics

Out of 60 patients who were approached having presented with spontaneous preterm labour with intact membranes during the study period of April to June 2019, only 22 had amniocentesis done and their amniotic fluid samples submitted for bacterial culture and sensitivity.

The mean age of study participants was 26.7 (SD 6.3) years. 3 participants were adolescents (19 years and below) and 3 were aged 35 years and above. 77% were married (table 2). 10 of the study participants had income of their own with 8 being housewives with no economic activity of their own. All the participants presented with intermittent lower abdominal pains radiating to the back with increasing frequency and intensity with 13 out of the 22 (58%) presenting within 10 hours from symptom onset. Of the 14 who had previous viable pregnancies, 3 (21.4%) had history of preterm labour in past pregnancy. One study participant had a history of preterm labour in current pregnancy.

Table 2: Socio-demographic characteristics of women with singleton pregnancy in spontaneous preterm labour who presented with intact membranes in Kenyatta National Hospital, April-June 2019

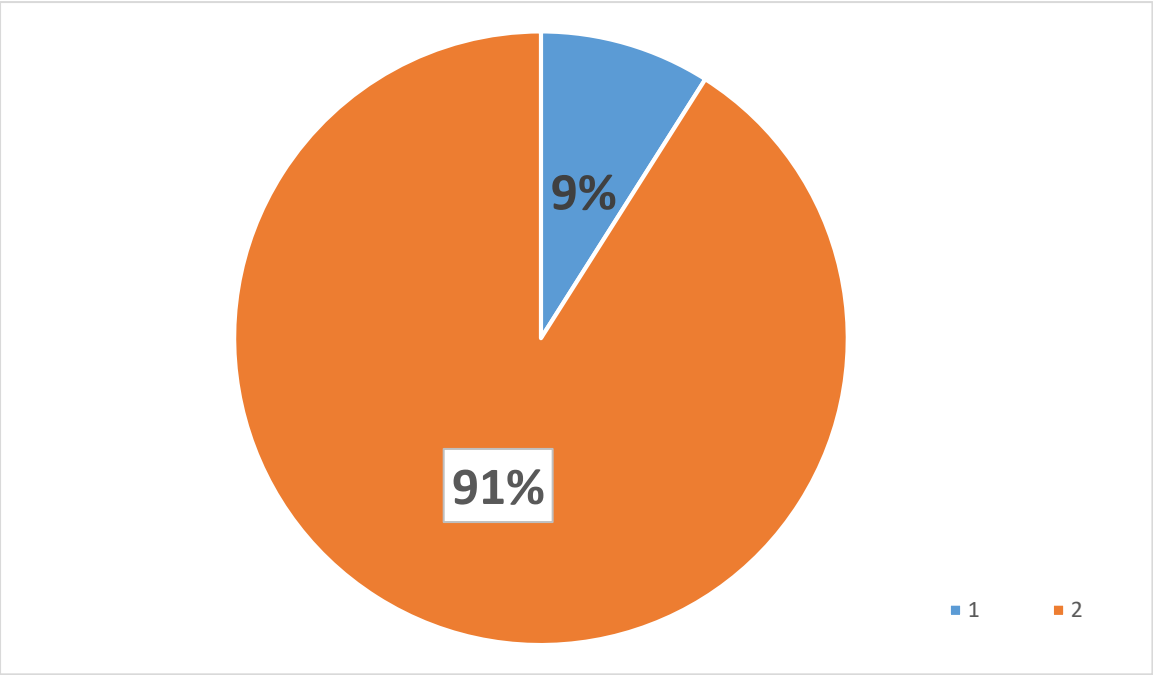
Socio-demographic factors	**Frequency n (Percent)**

Age (in years) Mean(SD)	26.7(6.3)
≤19	3 (13.6)
20-35	16 (72.7)
>35	3 (13.6)
Marital status	
Married	17 (77.3)
Single	5 (22.7)
Divorced and widowed	0(0)
Occupation	
Administrator	1 (4.5)
Business	7 (31.8)
Hotelier	1 (4.5)
Receptionist	1 (4.5)
Student	3 (13.6)
Unemployed	9 (40.9)
Education	
Primary	8 (36.4)
Secondary	9 (40.9)
Tertiary	5 (22.7)

SD- Standard deviation

Bacterial Culture Results

Figure 4: bacteria culture results of amniotic fluid samples from women in spontaneous preterm labour with intact membranes at KNH from April to June 2019.



Key: 1: culture positive amniotic fluid 2: culture negative amniotic fluid

Table 3: Antibiotic sensitivity of bacteria cultured from amniotic fluid from women in spontaneous preterm labour and intact membranes at KNH, April-June 2019.

Bacteria cultured from amniotic fluid	Frequency, n	Antibiotic sensitive to	Resistant to
Enterococcus fecalis	1	Ampicillin, gentamicin, linezolid, teicoplanin, vancomycin	erythromycin
Staphylococcus epidermidis	1	Erythromycin, clindamycin, teicoplanin, vancomycin	Benzyl-penicillin, trimethoprim-sulphurmethoxazole

KNH - Kenyatta National Hospital.

Of the 22 amniotic fluid samples analysed, two grew bacteria. Both of the 2 participants whose amniotic fluid samples had bacterial growth on culture were primigravida. The two were in

active phase of labour at cervical dilatations of 6cm and 8cm at the time of amniocentesis. The bacteria identified in the culture were *Enterococcus feacalis* and *Staphylococcus epidermis*.

The *Enterococcus feacalis* was sensitive to Ampicillin, gentamicin, linezolid, teicoplanin, and vancomycin but resistant to erythromycin and tetracycline. The *Staphylococcus epidermidis* was found to be sensitive to Oxacilin, Erythromycin, Clindamycin, Linezolid, Teicoplanin but resistant to Benzyl-Penicillin, Trimethroprim, Sulphurmethoxazole as shown in table 3

CHAPTER 5:

DISCUSSION

Twenty two participants met the criteria and had their amniotic fluid samples cultured for bacterial. 9% of study participants had their amniotic fluid grow bacteria on culture. The bacterial organisms cultured were *Enterococcus feacalis* and *Staphylococcus epidermidis*.

The prevalence of bacteria in intra-amniotic infections among the study participants was 9%. The study findings may be a true representative of the prevalence of intraamniotic bacterial infections amongst women in spontaneous preterm labour. In the study, participants included were in labour evidenced by characteristic lower abdominal pain with associated cervical changes. We used povidone iodine to ensure skin sterility by reducing risk of contamination by skin flora. Povidone iodine is a broad spectrum microbiocide that destroys microbial proteins and DNA with excellent in-vitro antimicrobial activity. In the study, normal saline was used after one minute following povidone application to clear the povidone from the injection site prior to the actual amniocentesis. This was to minimise contamination of amniotic fluid collected by providon which may have resulted in reduction of culture yield due to its microbiocidal effect. Povidone is often used to sterilise skin during invasive procedures including surgery, collection of blood sample for blood cultures, lumber puncture, ascetic tap and fine needle aspiration for cytology. These efforts strengthen the likelihood that the results are true picture of the intraamniotic cavity of the study participants.

The study prevalence of 9% is comparable to findings by Romero et al (1989) and Gravet et al (1986) who found prevalence of 11% and 9.1% respectively (63, 70). Though an old study, Gravet (1986) recruited participants similar to our study with a few exceptions. The participants

were at <35 weeks gestation while this study had participants at gestational age between 24 to <37weeks. Labour was evidenced by presence of regular painful contractions occurring at least in every 5 minutes associated with cervical dilatation or effacement. This study had labour evidenced by characteristic lower abdominal pains with associated cervical dilatation and or effacement. From his study, Gravet recovered bacteria and candida in 9.1% of the amniotic fluid media cultures. Similarly, Romero found out that participants whose amniotic fluid showed positive cultures were in labour with advanced cervical dilatation and effacement at the time of admission than those whose amniotic fluid were culture negative. This was the similar to our study except for the *Candida albicans* component that he included in his study.

The study finding was higher than the 3% found by Sun Min Kim et al (2012) in Seoul in the Republic of Korea (71). This difference could be because of difference in study population and setting. Sun Min Kim targeted women with uterine contractions with no cervical changes while this study considered cervical changes as part of diagnosis of preterm labour.

The high culture negative amniotic fluid result could be due to the traditional culture method used in the study. Many studies have proven molecular bacterial analysis as a more sensitive method of typing intra-amniotic microorganisms and may have identified more bacteria if present. However from the study, it's evident that amniocentesis to determine intra-amniotic bacterial invasion using the traditional bacteria; culture methods may still be feasible. The use of povidone and subsequent cleaning with sterile normal saline may not have been adequate resulting in microbiocidal effect in the amniotic fluid thus likely reducing the bacterial culture growth.

Amniotic fluid compartment is a sterile environment (8-9). Preterm labour may result from inflammation of fetal membranes arising from intraamniotic space invasion by ascending microbes in up to 48% (11-13). *Enterococcus feacalis* and *Staphylococcus epidermis* are gram

negative and gram positive (respectively) commensals of the perineum and gut. This is partly similar with Mendz et al (2013) findings in his systematic review. Similarly, early neonatal sepsis are commonly caused by microbes similar to those from maternal genitourinary system and lower gut (4, 11, and 31). The bacteria species grown were unique to this study. No other study has isolated these specific bacteria. In KNH setting, labour and its monitoring is done through serial sterile digital vaginal examinations. Our participants had had vaginal examinations at the time of recruitment. Vaginal examinations have been shown to increase risk of ascending infections. This could possibly explain the growth of *Enterococcus fecalis* and *Staphylococcus epidermidis* grown in the study. Vaginal examinations in KNH are done using hibitane antiseptic to clean the vulva and sterile surgical gloves in attempt to minimise infection risk. This if adhered to coupled with limiting the number of vaginal examinations maybe protective. Despite the steps taken to minimise the skin commensal contamination by ensuring sterile technique, commensals inadequately cleared by the povidone iodine could not be ruled out.

The *Enterococcus feacalis* was sensitive to Ampicillin, gentamicin, linezolid, teicoplanin, and vancomycin but resistant to erythromycin and tetracycline. This was comparable to findings by Thomas ER et al and Halgren A et al (2012)(72, 73). Christich et al (2014) in their review of Enterococcus feacalis revealed significant widespread intrinsic resistance and or tolerability to beta lactams, cephalosporin, and macrolides owing to its genetic processes of resistance development. . Vancomycin remained a widely used antibiotic to which the organism showed less susceptibility to develop resistance. In this review, enterococcus species were noted to be successful in developing resistance to any antimicrobial agent put in clinical use(73)

The *Staphylococcus epidermidis* was found to be sensitive to Oxacilin, Erythromycin, Clindamycin, Linezolid, Teicoplanin and vancomycin but resistant to Benzyl-Penicillin, and Trimethroprim, Sulphurmethoxazole. These susceptibility patterns were comparable to studies

by Hamid T (2015) and Pinheiro L et al (2009) (74-75). Haque N et al (2009) in Mymensingh Medical College found multidrug resistance of Staphylococcus epidermis isolated from different clinical specimens and controls. In his study, the microbe was resistant to oxacillin, penicillins, erythromycin, cefuroxime and cefuroximes at various levels. There was however no resistance to vancomycin and rifampicin. (76) The difference could be due to the difference in the settings and the fact that Haque N's study's participants in whom majority of resistance to antibiotics was noted were symptomatic and may have been exposed to different antibiotics.

The varied antimicrobial susceptibility of Enterococcus feacalis and Staphylococcus epidermidis raises significant concern of whether empiric use of antibiotics in spontaneous preterm labour as done in the Oracle 2 study is feasible. It points to the need to isolate the causative organism in IAI among women in spontaneous preterm labour to guide antibiotic choices.

The limitations of the study was the limited number of study participants. This resulted from the strict criteria applied. Sterilisation of the skin using povidone iodine is a strength and a weakness. It was in essence the backbone of the study in minimising risk of contamination of the sample collected as well as preventing iatrogenic introduction of microbes during the procedure. The traditional bacterial culture technique using media may have limited the number and variety of bacteria that could have been identified as being present in the amniotic fluid.

CONCLUSION

This study showed the presence of bacterial intraamniotic infections in spontaneous preterm labour with intact membrane. It was however not possible to determine a causative relationship between identified intraamniotic bacteria and spontaneous preterm labour. Use of antibiotics in women with spontaneous preterm labour with intact should be considered with caution as the prevalence is low. This should be guided by high index of suspicion. Further, there is need to determine the causative microbe prior to initiation of antibiotics as the organisms are varied and have unique antimicrobial susceptibility that may not be predictable.

Given the risks of amniocentesis to fetus and mother, non-invasive methods of identifying intra-aamniotic bacterial invasion in asymptomatic women in spontaneous preterm labour and intact membranes need to be developed especially where there is a high clinical suspicion.

RECOMMENDATIONS

1. The hospital and the Ministry of Health could consider creating protocols on amniocentesis as part of care provided in circumstances where cause of spontaneous preterm labour is not certain especially in cases refractory to tocolytics.
2. Health care providers handling women with spontaneous preterm labour especially Obstetrician and Gyncecologists need to be sensitized on likelihood of intra-amniotic infections as possible cause of spontaneous preterm labour and that prompt diagnosis and management may improve outcomes in preterm births.
3. More robust studies need to be carried out to evaluate the association of intra-amniotic bacterial infection in spontaneous preterm labour with intact membranes preferably using molecular methods.
4. Less invasive ways of diagnosing intra-amniotic infections in spontaneous preterm labour with intact membranes would help avoid the small but significant risk that amniocentesis poses to both the pregnant women and their fetuses.
5. Future studies on intraamniotic infection should include placenta and fetal membranes (culture and histology)

REFERRENCES

1. Moutquin JM. Classification and heterogeneity of preterm birth. In: BJOG: An International Journal of Obstetrics and Gynaecology. 2003. p. 30–3.

2. Mendz GL, Kaakoush NO, Quinlivan JA. Bacterial aetiological agents of intra-amniotic infections and preterm birth in pregnant women. Frontiers in Cellular and Infection Microbiology [Internet]. 2013;3(October):1–7. Available from: http://journal.frontiersin.org/article/10.3389/fcimb.2013.00058/abstract

3. Lettieri L, Vintzileos AM, Rodis JF, Albini SM, Salafia CM. Does idiopathic preterm labor resulting in preterm birth exist? Am J Obstet Gynecol. 1993;168(5):1480–5.

4. Alison F, Barnes a. C, Bonham DG, Harfouche JK, Menon MKK, Merchiers H, et al. World Health Organization. The Prevention of Perinatal Mortality and Morbidity. Report of a WHO Expert Committee. WHO Technical Report Series No. 457. 1970. p. 1–60.

5. WHO. WHO | Preterm birth. WHO World Health Organisation [Internet]. 2016; Available from: http://www.who.int/mediacentre/factsheets/fs363/en/

6. Chawanpaiboon S, Vogel JP[1], Moller AB, Lumbiganon P, Petzold M, Hogan D, Landoulsi S, Jampathong N, Kongwattanakul K, Laopaiboon M, Lewis C, Rattanakanokchai S, Teng DN, Thikhamthrop J, Watananirun K, Zhang J, Zhou W, Gülmezoglu AM. Global, regional and national estimates of levels of preterm birth in 2014: a systematic review and modelling nalysis. Lancet Glob Health. 2018. 30 Oct

7. Impact S, No P. Preterm Labour, Antibiotics, and Cerebral Palsy. 2013;(33).

8. Omero RR, Eo LY, Iranda JM, Haiworapongsa TC. Maternal-fetal conflict in intraamniotic infection. 2:527–36.

9. Jacobsson B. Infectious and inflammatory mechanisms in preterm birth and cerebral palsy. 2003;

10. Wagura 2011. Prevalence and factors associated with preterm births at Kenyatta National Hospital

11. Rowlands S, Danielewski JA, Tabrizi SN, Walker SP, Garland SM. Microbial invasion of the amniotic cavity in midtrimester pregnancies using molecular microbiology. American Journal of Obstetrics and Gynecology [Internet]. 2017 Jul [cited 2017 Sep 25];217(1):71.e1-71.e5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28268197

12. DB. D. Diversity of microbes in amniotic fluid. pubmed [Internet]. 2012;17(1):2–11. Available from: https://doi.org/10.1016/j.siny.2011.10.001

13. Https://www.ncbi.nlm.nih.gov/pubmed/?term=Mendz%20GL%5BAuthor%5D&cauthor=true&cauthor_uid=24137568. Bacterial aetiological agents of intra-amniotic infections and preterm birth in pregnant women. pubmed [Internet]. 2013; Available from: https://www.ncbi.nlm.nih.gov/pubmed/?term=Mendz GL%5BAuthor%5D&cauthor=true&cauthor_uid=24137568

14. Martin JA, Kirmeyer S, Osterman M, Shepherd RA. Born a bit too early: recent trends in late preterm births. NCHS data brief. 2009;(24):1–8.

15. Morken N-H. Preterm birth: new data on a global health priority. Lancet [Internet]. 2012;379(9832):2128–30. Available from: http://www.thelancet.com/journals/a/article/PIIS0140-6736(12)60857-5/fulltext

16. Requejo J, Merialdi M, Althabe F, Keller M, Katz J, Menon R. Born Too Soon: Care during pregnancy and childbirth to reduce preterm deliveries and improve health outcomes of the preterm baby. Vol. 10, Reproductive Health. 2013.

17. Butali A, Ezeaka C, Ekhaguere O, Weathers N, Ladd J, Fajolu I, et al. Characteristics and risk factors of preterm births in a tertiary center in Lagos, Nigeria. Pan African Medical Journal. 2016;24.

18. Committee opinion no 579: Definition of term pregnancy. Vol. 122, Obstetrics and Gynecology. 2013. p. 1139–40.

19. Fleischman AR, Oinuma M, Clark SL. Rethinking the definition of 'term pregnancy'. Vol. 116, Obstetrics and Gynecology. 2010. p. 136–9.

20. Slater DM, Astle S, Bennett PR, Thornton S. Labour is associated with increased expression of type-IIA secretory phospholipase A2 but not type-IV cytosolic phospholipase A2 in human myometrium. Molecular Human Reproduction. 2004;10(11):799–805.

21. Lopez Bernal A, Newman GE, Phizackerley PJ, Bryant-Greenwood G, Keeling JW. Human placental phospholipase A2 activity in term and preterm labour. Eur J Obstet Gynecol Reprod Biol [Internet]. 1992;43(3):185–92. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=1563569

22. Schwarz BE, Schultz FM, MacDonald PC, Johnston JM. Initiation of human parturition. IV. Demonstration of phospholipase A2 in the lysosomes of human fetal membranes. American journal of obstetrics and gynecology. 1976 Aug;125(8):1089–92.

23. Husslein P. [Causes of labor initiation in man: role of oxytocin and prostaglandins]. Zeitschrift fur Geburtshilfe und Perinatologie. 1985;189(3):95–102.

24. Mendelson CR. Minireview: Fetal-Maternal Hormonal Signaling in Pregnancy and Labor. Vol. 23, Molecular Endocrinology. 2009. p. 947–54.

25. Kota SK, Gayatri K, Jammula S, Kota SK, Krishna SVS, Meher LK, et al. Endocrinology of parturition. Vol. 17, Indian Journal of Endocrinology and Metabolism. India; 2013. p. 50–9.

26. Sheller S, Papaconstantinou J, Urrabaz-Garza R, Richardson L, Saade G, Salomon C, et al. Amnion-Epithelial-Cell-Derived Exosomes Demonstrate Physiologic State of Cell under Oxidative Stress. Sun K, editor. PLOS ONE [Internet]. 2016 Jun 22 [cited 2017 Sep 25];11(6):e0157614. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27333275

27. Menon R, Mesiano S, Taylor RN. Programmed Fetal Membrane Senescence and Exosome-Mediated Signaling: A Mechanism Associated With Timing of Human Parturition. Frontiers in Endocrinology [Internet]. 2017 Aug 17 [cited 2017 Sep 25];8:196. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28861041

28. Romero R, Grivel JC, Tarca AL, Chaemsaithong P, Xu Z, Fitzgerald W, et al. Evidence of perturbations of the cytokine network in preterm labor. American Journal of Obstetrics and Gynecology. 2015;213(6):836.e1-836.e18.

29. Gomez-Lopez N, StLouis D, Lehr MA, Sanchez-Rodriguez EN, Arenas-Hernandez M. Immune cells in term and preterm labor. Cellular and Molecular Immunology. 2014;11(6):571–81.

30. Tency I. Inflammatory response in maternal serum during preterm labour. Facts, views & vision in ObGyn [Internet]. 2014;6(1):19–30. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4085999&tool=pmcentrez&rendertype=abstract

31. Edwards RK. Chorioamnionitis and Labor. 2005;32:287–96.

32. Puopolo KM, Beigi R, Silverman NS, El-Sayed YY. ACOG Committee Opinion: Intrapartum Management of Intraamniotic Infection. Obstetrics and gynecology. 2017;130(2):95–101.

33. Galinsky R, Polglase GR, Hooper SB, Black MJ, Moss TJM. The consequences of chorioamnionitis: Preterm birth and effects on development. Journal of Pregnancy. 2013;2013.

34. Miyazaki K, Furuhashi M, Matsuo K, Minami K, Yoshida K, Kuno N, et al. Impact of subclinical chorioamnionitis on maternal and neonatal outcomes. Acta Obstetricia et Gynecologica Scandinavica. 2007;86(2):191–7.

35. Wu HC, Shen CM, Wu YY, Yuh YS, Kua KE. Subclinical Histologic Chorioamnionitis and Related Clinical and Laboratory Parameters in Preterm Deliveries. Pediatrics and Neonatology. 2009;50(5):217–21.

36. Abdel-Malek K, El-Halwagi MA, Hammad BE, Azmy O, Helal O, Eid M, et al. Role of maternal serum ferritin in prediction of preterm labour. Journal of Obstetrics and Gynaecology [Internet]. 2017 Sep 14 [cited 2017 Sep 25];1–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28903602

37. Kagan KO, To M, Tsoi E, Nicolaides KH. Preterm birth: The value of sonographic measurement of cervical length. Vol. 113, BJOG: An International Journal of Obstetrics and Gynaecology. 2006. p. 52–6.

38. Goffinet F. Primary predictors of preterm labour. BJOG : an international journal of obstetrics and gynaecology. 2005;112 Suppl(March):38–47.

39. Mella MT, Berghella V. Prediction of Preterm Birth: Cervical Sonography. Vol. 33, Seminars in Perinatology. 2009. p. 317–24.

40. Malak TM, Sizmur F, Bell SC, Taylor DJ. Fetal fibronectin in cervicovaginal secretions as a predictor of preterm birth. British journal of obstetrics and gynaecology. 1996;103(7):648–53.

41. American Academy of Family Physicians. BA Von Der. American family physician. [Internet]. Vol. 57, American Family Physician. American Academy of Family Physicians; 1970 [cited 2018 Feb 20]. 2457 p. Available from: https://www.aafp.org/afp/1998/0515/p2457.html

42. Preterm labour and birth. 2017;(November 2015).

43. Sociedade Portuguesa de Pediatria, AIDSinfo, Programa Nacional para a Infecção VIH S e T, DGS, Programa Nacional para a Infecção VIH/SIDA, Coordenação Nacional para a Infecção VIH/SIDA, et al. Amniocentesis in pregnant HIV-infected patients. Absence of mother-to-child viral transmission in a series of selected patients. American Journal of

Obstetrics and Gynecology [Internet]. 2014;2013(July 2012):1–102. Available from: http://dx.doi.org/10.1016/j.ajog.2008.08.049%5Cnhttp://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Recomendationses+Portuguesas+para+o+Tratamento+da+Infection+VIH+/+sida#0%5Cnhttp://www.dgs.pt/upload/membro.id/ficheiros/i018596.pdf

44. Akolekar R, Beta J, Picciarelli G, Ogilvie C, D'Antonio F. Procedure-related risk of miscarriage following amniocentesis and chorionic villus sampling: A systematic review and meta-analysis. Ultrasound in Obstetrics and Gynecology. 2015;45(1):16–26.

45. Zalud I, Janas S. Risks of third-trimester amniocentesis. The journal of reproductive medicene. 2008;53(1):8–45.

46. Esplin MS, Elovitz MA, Iams JD, Parker CB, Wapner RJ, Grobman WA, et al. Predictive accuracy of serial transvaginal cervical lengths and quantitative vaginal fetal fibronectin levels for spontaneous preterm birth among nulliparous women. JAMA - Journal of the American Medical Association. 2017;317(10):1047–56.

47. Foster C, Shennan AH. Fetal fibronectin as a biomarker of preterm labor: a review of the literature and advances in its clinical use. Biomarkers in medicine [Internet]. 2014 Apr [cited 2017 Dec 29];8(4):471–84. Available from: http://www.futuremedicine.com/doi/10.2217/bmm.14.28

48. Roos C, Vis JY, Scheepers HCJ, Bloemenkamp KWM, Duvekot HJJ, Van Eyck J, et al. Fetal fibronectin status and cervical length in women with threatened preterm labor and the effectiveness of maintenance tocolysis. Journal of Maternal-Fetal and Neonatal Medicine. 2016;29(10):1556–61.

49. Lockwood CJ, Senyei AE, Dische MR, Casal D, Shah KD, Thung SN, et al. Fetal Fibronectin in Cervical and Vaginal Secretions as a Predictor of Preterm Delivery. New England Journal of Medicine [Internet]. 1991 Sep 5 [cited 2017 Dec 29];325(10):669–74. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1870640

50. van Baaren G-J, Vis JY, Wilms FF, Oudijk MA, Kwee A, Porath MM, et al. Predictive Value of Cervical Length Measurement and Fibronectin Testing in Threatened Preterm Labor. Obstetrics & Gynecology [Internet]. 2014 Jun [cited 2017 Dec 29];123(6):1185–92. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24807328

51. Lockwood CJM, Senyei AEM. Fetal fibronectin in cervical and vaginal secretions as a predictor of preterm delivery. New England Journal of Medicine. 1991;325(10):669–74.

52. Papatsonis D, Vangeijn H, Ader H, Lange F, Bleker O, Dekker G. Nifedipine and ritodrine in the management of preterm labor: A randomized multicenter trial. Obstetrics & Gynecology [Internet]. 1997 Aug [cited 2017 Dec 29];90(2):230–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9241299

53. Conde-Agudelo A, Romero R, Kusanovic JP. Nifedipine in the management of preterm labor: a systematic review and metaanalysis. American Journal of Obstetrics and Gynecology [Internet]. 2011 Feb 1 [cited 2017 Dec 29];204(2):134.e1-134.e20. Available from: https://www.sciencedirect.com/science/article/pii/S0002937810023185

54. Roos C, Spaanderman MEA, Schuit E, Bloemenkamp KWM, Bolte AC, Cornette J, et al. Effect of maintenance tocolysis with nifedipine in threatened preterm labor on perinatal outcomes: A randomized controlled trial. Obstetrical and Gynecological Survey [Internet]. 2013;68(5):342–4. Available from:

http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L368871691%5Cnhttp://dx.doi.org/10.1097/01.ogx.0000430379.07122.ee

55. Roos C, Spaanderman MEA, Schuit E, Bloemenkamp KWM, Bolte AC, Cornette J, et al. Effect of Maintenance Tocolysis With Nifedipine in Threatened Preterm Labor on Perinatal Outcomes. JAMA [Internet]. 2013 Jan 2 [cited 2017 Dec 29];309(1):41. Available from: http://jama.jamanetwork.com/article.aspx?doi=10.1001/jama.2012.153817

56. Tan TC, Devendra K, Tan LK, Tan HK. Tocolytic treatment for the management of preterm labour: a systematic review. Singapore medical journal [Internet]. 2006;47(5):361–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16645683

57. Kenyon SL, Taylor DJ, Tarnow-Mordi W. Broad-spectrum antibiotics for spontaneous preterm labour: The ORACLE II randomised trial. Lancet. 2001;357(9261):989–94.

58. Kenyon S, Brocklehurst P, Jones D, Marlow N, Salt A, Taylor D. MRC ORACLE Children Study. Long term outcomes following prescription of antibiotics to pregnant women with either spontaneous preterm labour or preterm rupture of the membranes. BMC Pregnancy and Childbirth. 2008;8.

59. Mueller-Heubach E, Rubinstein DN, Schwarz SS. Histologic chorioamnionitis and preterm delivery in different patient populations. Vol. 75, Obstetrics and gynecology. 1990. p. 622–6.

60. Lee SM, Park JW, Kim BJ, Park CW, Park JS, Jun JK, et al. Acute histologic chorioamnionitis is a risk factor for adverse neonatal outcome in late preterm birth after preterm premature rupture of membranes. PLoS ONE. 2013;8(12).

61. Pourhoseingholi MA, Vahedi M, Rahimzadeh M. Sample size calculation in medical studies. Gastroenterology and Hepatology from Bed to Bench. 2013;

62. Israel GD. Determining Sample Size 1. 1992;(November):1–5.

63. Gravett MG, Hummel D, Eschenbach DA, Holmes KK. Preterm Labor Associated With Subclinical Amniotic Fluid Infection and With Bacterial Vaginosis. Obstetrics & Gynecology [Internet]. 1986;67(2):229–37. Available from: http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00006250-198602000-00013

64. Kose SA, Akkurt MO, Yavuz, Eris S, Tola EN, Ozkaya MO, et al. Conventional 22- and 20-gauge needle for second trimester amniocentesis: A comparison of short term outcomes. Turk Jinekoloji ve Obstetrik Dernegi Dergisi [Internet]. 2016;13(1):27–30. Available from: http://cms.galenos.com.tr/FileIssue/15/946/article/27-30.pdf%0Ahttp://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=emed18&AN=610111488%250Ahttps://maastrichtuniversity.on.worldcat.org/atoztitles/link?sid=OVID:embase&id=pmid:&id=doi:10.4274%25

65. Shulman LP, Elias S. Amniocentesis and chorionic villus sampling. The Western journal of medicine [Internet]. 1993;159(3):260–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8236967%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC1011338

66. Tchirikov M, Arnold C, Oshovskyy V, Heinrich UR, Thäle V. Three years' experience of using a 29-gauge atraumatic needle for amniocentesis. Journal of Perinatal Medicine. 2012;40(4):413–7.

67. Kong CW, Leung TN, Leung TY, Chan LW, Sahota DS, Fung TY, et al. Risk factors for procedure-related fetal losses after mid-trimester genetic amniocentesis. Prenatal Diagnosis. 2006;26(10):925–30.

68. Eddleman KA, Malone FD, Sullivan L, Dukes K, Berkowitz RL, Kharbutli Y, et al. Pregnancy loss rates after midtrimester amniocentesis. Obstetrics and gynecology [Internet]. 2006;108(5):1067–72. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17077226

69. Seeds JW. Diagnostic mid trimester amniocentesis: How safe? In: American Journal of Obstetrics and Gynecology. 2004. p. 608–16.

70. Romero R[1], Sirtori M, Oyarzun E, Avila C, Mazor M, Callahan R, Sabo V, Athanassiadis AP, Hobbins JC.Infection and labor. V. Prevalence, microbiology, and clinical significance of intraamniotic infection in women with preterm labor and intact membranes.

71. Sun Min KIM, MD, Roberto ROMERO, MD, JoonHo LEE, MD, Seung Mi LEE, MD, Chan-Wook PARK, MD, PhD, Joong Shin PARK, MD, PhD, and Bo Hyun YOON, MD, PhD. The Frequency and Clinical Significance of Intra-amniotic Inflammation in Women with Preterm Uterine Contractility but Without Cervical Change: Do the Diagnostic Criteria for Preterm Labor Need to be changed?

72. Hallgren A, Abednazari H, Ekdahl C, Hanberger H, Nilsson M, Samuelsson A, et al. Antimicrobial susceptibility patterns of enterococci in intensive care units in Sweden evaluated by different MIC breakpoint systems. Journal of Antimicrobial Chemotherapy [Internet]. 2001 Jul 1 [cited 2019 Aug 4];48(1):53–62. Available from: https://academic.oup.com/jac/article-lookup/doi/10.1093/jac/48.1.53

73. Rams TE, Feik D, Mortensen JE, Degener JE, van Winkelhoff AJ. Antibiotic Susceptibility of Periodontal *Enterococcus faecalis*. Journal of Periodontology [Internet]. 2013 Jul [cited 2019 Aug 4];84(7):1026–33. Available from: http://doi.wiley.com/10.1902/jop.2012.120050

74. Hamad T, Hellmark B, Nilsdotter-Augustinsson Å, Söderquist B. Antibiotic susceptibility among *Staphylococcus epidermidis* isolated from prosthetic joint infections, with focus on doxycycline. APMIS [Internet]. 2015 Dec [cited 2019 Aug 5];123(12):1055–60. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26547372

75. Pinheiro L, Brito CI, Pereira VC, Oliveira A, Bartolomeu AR, Camargo CH, et al. Susceptibility Profile of *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* Isolated from Blood Cultures to Vancomycin and Novel Antimicrobial Drugs over a Period of 12 Years. Microbial Drug Resistance [Internet]. 2016 Jun 6 [cited 2019 Aug 5];22(4):283–93. Available from: http://www.liebertpub.com/doi/10.1089/mdr.2015.0064

76. Haque N; Hossain MA; Bilkis L; Musa AK; Mahamud C; Bari MS; Haque N; Muhammad N; Parvin US; Islam MT; Khan SI; Islam MA; Haque S.. Antibiotic susceptibility pattern of Staphylococcus epidermidis. [Internet]. [cited 2019 Aug 5]. Available from: https://reference.medscape.com/medline/abstract/19623137

APPENDICES

1. Questionnaire
2. Dummy tables
3. Consent form

ANNEXES

Annex1: Letter to ERC

Dr. James O. Amenge, (MBCHB)

H58/80616/2015

Jamesyoungky@gmail.com

0724612026

August 3rd, 2018

The Chairperson,

Ethics, Research and Standards Committee,

Kenyatta National Hospital-University of Nairobi,

P.O. Box 20723

Nairobi.

Dear Sir/Madam,

RE: SUBMISSION OF CORRECTIONS FROM ERC ON THESIS PROPOSAL.

I wish to submit my research proposal with the corrections suggested by the KNH/UON ERC on the thesis proposal titled Prevalence of bacteria in subclinical intraamniotic infection in women presenting with spontaneous preterm labour in Kenyatta National Hospital

I am currently a year 3 postgraduate student pursuing a Masters' degree in Obstetrics and Genecology at the University of Nairobi, College of Health Sciences.

Thank you for your kind consideration.

Yours Sincerely,

Dr. James O. Amenge

Resident,

Department of Obstetrics and Gynecology,

College of Health Sciences, University of Nairobi.

Annex 2: Table 1 Time lines

Activity	Up to December 2017	January to June 2018	July 2018	August to December 2018	January to March 2019	March to June 2018	June-Nov 2019
Concept development							
Proposal Development							
Proposal presentation							
Ethical Approval							
Recruitment of study team							
Data Collection							
Data Analysis, Results presentation and Final manuscript preparation.							

Annex 3: Budget

Research stage	**Description**	**Units**	**Unit Cost (KShs)**	**Total (KShs)**
Proposal Development	Printing drafts	5	500	2,500
	Proposal Copies	5	500	2,500
	Statistician in put on methodology	-	10,000	10,000
	ERC approval	-	3,000	3,000
Data Collection	Stationery Packs	-	10,000	10,000
	Training research assistants	4 medical officers 5 Mmed Radiology students	2500 1000 each amniocentesis	22,000
	Research assistants (4)	4	1,000 per questionnaire	22,000
Microscopy, culture and sensitivity per sample	22 samples	22	1000	22,000
Data Analysis	Statistician	-	30,000	30,000
Thesis write up	Computer Services	1	20,000	20,000
	Printing drafts	5	500	2,500
	Printing Thesis	3	1000	3,000
Contingency funds		-	20,000	20,000
Total				**165,000**

Table 2 Budget

Annex 4: Consent form

INFORMED CONSENT EXPLANATION

Introduction and objectives of the study

Dear participant,

My names is Dr. James Amenge, a Master of Medicine (Obstetrics and Gynaecology) postgraduate student in the department of Obstetrics and Gynaecology, University of Nairobi. I am carrying out a study on Prevalence of bacterial subclinical intraamniotic infections in women presenting with spontaneous preterm (before 37weeks) labour in Kenyatta National Hospital.

The purpose of this study is to determine how common intraamniotic bacterial infections without clinical signs and symptoms is, identify the possible bacteria associated with this type of infection and find out the antibiotics to which this infective bacteria responds to best.

Procedure

If you meet the inclusion criteria, my research assistant or I will talk to you about the study. After providing an informed voluntary consent, a questionnaire that will help us collect some information about your health will be administered to you. The procedure that will be done to collect amniotic fluid (the fluid surrounding the baby inside the womb) is as follows:

You will be asked to lie on your back with a small tilt to your left to reduce the pressure the gravid uterus is putting on your vena cava thus making you comfortable. Your abdomen will be exposed to your waistline and the lower body part covered with a linen. A standard obstetric ultrasound will be done to confirm the baby's wellbeing as well as identify the area with amniotic fluid that has no fetal parts. Once identified, the area above the pocket will be sterilized using povidone antiseptic and draped with a sterile towel. The ultrasound probe having gel applied on it will be covered with a sterile glove and under sterile technique a small needle will be introduced through the skin into the amniotic space under continuous ultrasound guidance and caution to avoid any injury to the baby. Once accessed, 5 ml of amniotic fluid will be aspirated and submitted for analysis (microscopy, culture and sensitivity using vitek 2). The needle will be withdrawn under ultrasound guidance as well. Once completed, the discolored (from povidone) skin will be cleaned and you will be asked

to stay rested for at least 30minutes. During the procedure, any untoward outcome will be made known to you and prompt management initiated.

Benefits and Risks

There may be no direct benefits to you the participant. However any infection identified from the study will be treated promptly as per the KNH protocols. The information gathered from this study may be used for better management of your condition as well as form basis for making treatment guidelines for treatment of preterm labour that affects about 12% of pregnant women globally and thus help reduce the associated complications.

There will be a questionnaire to be filled that will ask some questions. Some of the questions may be personal and uncomfortable

During the procedure of collecting amniotic fluid for analysis, the following risks may occur:

You may feel minimal pain during passage of the fine needle through your skin, abdominal wall, and wall of the uterus. This may be sharp pain as the needle go through the skin and a dull distant pain as the needle go through the wall of the uterus. This pain is often minimal and may not require pain medication during and after the procedure.

The procedure is associated with a small risk of starting off labour that may result in delivery of a premature baby. However we will only do the procedure if there is labour that has started by itself therefore removing this fear.

There is a small risk of injury to the baby as the needle gets into where the baby is. This risk has been found to be minimal up to less than 1 in 100 cases. During our study, a doctor well trained on the procedure (amniocentesis) will perform it. Furthermore, the needle will be under direct view using an ultrasound thus ensuring that only pockets that have no body parts are entered to reduce the risk of injury to the baby. There will be a protocol on this and frequent checks will be done to ensure that these safety measures are followed.

Also there may be leakage of water surrounding the baby in the uterus (amniotic fluid). However this is minimal and may not cause any harm.

The procedure of obtaining the amniotic fluid will be done on the abdominal and not any other part of the body. The abdomen will therefore be exposed and the identified area cleaned with an antiseptic (povidone) to reduce risk of introducing new

infections into the uterine cavity. Povidone may discolour the skin red but this is washable.

A maximum of two attempts to obtain amniotic fluid and any failure after that will result in termination of the procedure.

After the procedure, you will be followed up in the ward to ensure you are doing well and no complication occurs and if any occur is promptly treated.

There is a small risk of your baby's blood leaking into the mother's blood system. This may pause a risk in those with Rhesus negative blood as exposing the mother to a baby's blood if the baby has rhesus positive blood, may cause the mother's body to react by forming antibodies against the baby's blood Rhesus antigen. If you have a Rhesus Negative blood such as blood group O Rhesus Negative, you will have a test to check if the babies blood has already entered your blood system during this or past pregnancy and caused your body to form antibodies against the baby's red blood cell antigens (if your baby has rhesus positive blood). This test is called Indirect Coombs test, ICT. A negative ICT implies that your body has not reacted to the baby's blood Rhesus antigens and therefore you require to be given a drug, anti-D to protect your body from reacting to your baby's blood cells which may affect your baby and future babies. This is a standard care for you even if you had not participated in this study. You will therefore meet the cost of anti-D which you will be given immediately after amniocentesis.

Costs involved

As a standard management procedure of preterm labour an obstetric ultrasound scanning is done to assess the baby's wellbeing and to confirm gestational age (intrauterine age). Rhesus negative mothers who are not isoimmunised as part of antenatal care are given anti-D gama globulins 300 micrograms around 28 weeks and within 72 hours after delivery to prevent isoimmunisation and thus protect future fetuses. This is usually paid for by the patient receiving the care either by cash or through National Health Insurance Fund. In this study, the same will apply. The patient will meet the cost of obstetric ultrasound and anti-D for those receiving it after amniocentesis. The investigator will however meet the cost of amniocentesis, the laboratory analysis of the amniotic fluid sample collected and other related costs as this is not a routine part of care given to women with preterm labour.

Voluntary Participation

Participation into the study will be voluntary.

You are free withdraw from the study at any time without losing any benefits you are entitled to this institution.

Confidentiality

Your personal information will kept confidential. Your name will not be used in the study materials. Unique identifiers will be used to hide your identity. Questionnaires will be kept under lock and key and only principal investigator will access it. The questionnaires will be kept for one year and thereafter destroyed as per the institutional policy. Any information given to us by you will remain confidential and will be used for your own benefit.

Contact Information

If you have any questions regarding this study lease contact me , **James Amenge, University of Nairobi P.O Box 19676-00202 Nairobi or on mobile number 0724612026 or my Supervisor, Prof. Omondi Ogutu, the head of department Obstetrics and Gynecology,** University of Nairobi or **the Secretary**, KNH/UON Ethical Research Committee, Tel 726300-9 Ext 44102

INFORMED CONSENT FORM

If you have clearly understood the information provided about the study and you are willing to voluntarily participate, please sign below:

I……..of

…… after reading and being explained to about the study and what it entails, I accept and consent willingly to participate in the study fully aware of the benefits and risks.

I am aware that I can withdraw from this study without loss of any benefit or quality of management/care to which I am entitled.

Participant's Signature/thumb print…….. Date……..

I …… confirm that I have adequately explained the details of the study to the participant and that the participant has given an informed consent without any coercion.

Signature…… Date…….

Annex 5: Questionnaire

Serial No._____

PREVALENCE OF BACTERIAL SUBCLINICAL INTRA-AMNIOTIC INFECTIONS IN WOMEN PRESENTING WITH SPONTANEOUS PRETERM LABOUR IN KENYATTA NATIONAL HOSPITAL

Principal investigator: Dr. James Amenge, H58/80616/2015
Phone no: 0724612026
Supervisor: 1. Prof. Omondi Ogutu

Unique no:

Bio-data

Indicate all times using 24hour clock system and dates in the format of DD/MM/YY

Date:

Time of filling the questionnaire:

Date of admission:

Time of Admission…

Please provide us with the following information about you.

Age …….. Parity ……+ ……. Gravidity……

Gestational age by dates (LNMP----------) …….

Gestation by extrapolation from earliest obstetric ultrasound done ……. (Date when the ultrasound was done…… and gestational age at the time of the ultrasound……)

Gestational age as estimated by 1st ANC attendance……. (Indicate date of 1st ANC- and Fundal height as measured then…

Gestation by quickening: estimated period of quickening.

Marital Status:

Single ☐ Married ☐ Separated ☐ Divorced ☐ Unknown ☐

Occupation……..

Employment status……

Level of Education:

None ☐ Primary ☐ Secondary ☐ Tertiary ☐ Unknown ☐

Current obstetric history

How long have you experienced the following symptoms that have brought to the hospital?

1. Intermittent lower abdominal pains radiating to the back (in hours)
2. Uterine contraction (in hours)

Have you had a urinalysis test done?

If yes, state the results for the following: leucocytes……. Nitrites…….

Have you had any vaginal discharge in the current pregnancy?

If yes, what was the color?

Was it of foul smell?

Antenatal profile:

Have you ever attended antenatal clinic during this current pregnancy? Yes…. No…..

If yes,

1. How many visits? ……
2. At what gestation was your first ANC attendance?.....
3. Were you screened for the following sexually transmitted infections/diseases? If yes, state the results of each screening test below:
   VDRL
   Hepatitis B virus
   Human immunodeficiency Virus

What was your HIV serologic status? Unknown ☐ Negative ☐

Positive ☐

What was your blood group? Blood Group:….. Rhesus factor…….

Have you been diagnosed of or treated for urinary tract infection in the preceding 4 weeks during ANC: Yes---- No------

If yes, state the antibiotics you were given for the illness……..

Have you been diagnosed or treated for sexually transmitted infection? Yes…… No……

If yes, what was the diagnosis? …….

If yes, state the antibiotics you were put on for this illness……

What was the Hemoglobin level?

Below 8g/dl

Above 8 and below 10g/dl

Above 10g/dl

In the current admission, have you been given any antibiotics? Yes--- No----

If yes, name the antibiotic,

days administered,

dosage

Have you been given a drug to make the lungs of the baby mature? Yes---- No---

If yes, name the drug,

dosage and

duration

when was the last time you had penetrative penile-vaginal sexual intercourse?

Date…..

Time……

Past gynecologic and obstetrics history

1. History of preterm labor in current pregnancy 1. Yes….. 2. No……
   If yes, were you admitted 1. Yes…….. 2. No…..
   If admitted, were you given antibiotics? 1. Yes…. 2. No…. 3. Unsure……
2. History of preterm labour in previous pregnancies 1. Yes….. 2. No…..
   If yes, what were the outcomes, a. preterm birth? 1. Yes….. 2. No…..
   b. term delivery? 1. Yes… 2. No…..
   c. preterm rupture of membranes? 1. Yes….. 2. No….

Results (to be filled from the laboratory report)

Indicate the following laboratory results as per the laboratory report:

Culture growth: Positive…….

Negative……

If positive, list the organisms grown

1. ……
2. …….
3. …….
4. ……..

5. …….

Others: ……

For each bacterial isolate above indicate the antibacterial to which the isolate is sensitive to

1. ……
2. ……
3. ……
4. …….
5. …….
6. …….

For each bacterial isolates identified above, indicate the antibacterial to which the isolate is resistant to.

1. ……
2. ……
3. …….
4. …….
5. …….
6. …….

Annex 6: Dummy Tables

Table: Demographic characteristics of patient with preterm labour with intact membranes in Kenyatta National Hospital

Age		**Marital Status**		**Parity**		**Level of Education**	
Category	**%**	**Category**	**%**	**Category**	**%**	**Category**	**%**
< 20 years		Single		Nulliparous		None	
20-25years		Married		Primipara		Primary	
25-30years		Separated		Multiparous		Secondary	
30-35years		Divorced		Above para 5		Tertiary	
35-40years							
Above 40years							

Parity		**Level of Education**	
Category	% identified by bacterial culture	**Category**	% identified by bacterial culture
Nullipara		None	
Primipara		Primary	
Multiparous		Secondary	
Above para 5 Tertiary			

Duration of onset of preterm labour from last penetrative vaginal coitus	
Category	% identified by bacterial culture
< 24hrs	
24-48hrs	
48-72hrs	
3days to 1 week	
>1 week	

Maternal baseline characteristics and subclinical IAI findings

Characteristic	Category	No Subclinical IAI/no. preterm labour with intact membranes identified by gram stain	No subclinical IAI/No. preterm labour with intact membranes identified by bacterial culture
Socio demographics			
Age	<20 20-25 26-30 31-35 >35		
Obstetric History			
Parity	0 1 2 3 4 5		
Preterm labour history	1 2 3 4 >5		
ANC attendance	Yes No		
Facility attended	KNH Other facility		
Number of visits	1 2-4 >4		
Gestational age at first visit	<12weeks 12-24weeks >24weeks		
Hb(g/dl) at 1st visit	**<8** 8-10 >10		
Blood group	A+ B+ AB+ O+ A- B- AB- O-		
VDRL	Positive Negative Unknown		
HIV	Positive		

	Negative Unknown
Medications	Steroids Antibiotics

IAI- intraamniotic infection

Table: bacterial culture results in relation to gestational age

Gestational age	Culture positive (bacterial colonies grown) (No)	Culture negative (no bacterial colonies grown) (No)
32 to <37 weeks		
28 to <32 weeks		
<28 weeks		

Table: list of individual bacterial isolates using Vitek machine

Bacterial isolate grown	No of patients from whom isolate grown	Bacterial classification	Antibacterial sensitivity	Antibacterial resistance/insensitivity