THE INFLUENCE OF TRIMESTERS ON PROGESTERONE AND *Escherichia coli* URINARY TRACT INFECTION IN PREGNANT WOMEN ATTENDING MOI TEACHING AND REFERRAL HOSPITAL ANTENATAL CLINIC

BY

ANNE WANJIKU

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DECLARATION

Declaration by the candidate

This thesis is my original work and has never been presented for a degree award by any other student in any other University. No part of this thesis may be reproduced without the prior written permission of the author and/or University of Eldoret.

Signature: …… Date:……..

Anne Wanjiku

SC/PGB/014/13

Declaration by supervisors

This thesis was submitted for examination with our approval as University Supervisors.

Dr. Lizzy A. Mwamburi

University of Eldoret, Eldoret, Kenya

Signature: …… Date:……

Dr. Moses Ngeiywa

University of Eldoret, Kenya

Signature…… Date…….

DEDICATION

To my Mother, Agnes Wangoi, son Teddy Alvin, Dr. Andrew Obala, Elius Mbogori, MTRH Antinatal Clinic, Lab staff and all my friends who offered their support.

ABSTRACT

Urinary tract infections (UTIs) are common health problem among pregnant women. The UTIs presents as either symptomatic or asymptomatic bacteriuria, which is a major predisposing factor for pyelonephritis that is linked to obstetrical complications including preterm labor and low birth weight of infants. The UTIs are also known to be common cause of maternal and fetal morbidity and mortality. The asymptomatic and symptomatic pregnant women are predisposed to anatomical and physiological changes imposed on the urinary tract by the progesterone hormone leading to urinary stasis, which facilitates bacterial colonization and ascending infection to kidneys. In pregnancy, the maternal immune system is also lowered hence promotes the growth of bacteria in the urinary system. Therefore, the present study aimed at determining the occurrence of *E. coli* in the urinary system and correlate to progesterone hormone levels among pregnant women attending antenatal clinics (ANC) at Moi Teaching and Referral Hospital. A cross-sectional study design and probability sampling method were used to collect 78 blood and urine samples at intervals for 60 working days from pregnant women who met the inclusion criteria and consented to the study. The urine samples were cultured in CLED media while the blood was separated and plasma aliquoted for progesterone estimation using ELISA method. The culture withthebacteriacolony count of more than $10^5$was identifiedusing biochemical tests and gram stain.The cultures showed that the most abundant bacterial organism isolated in urine from the participating women was *E. coli* (9%). There were exponential increases in progesterone levels for the participating pregnant women in trimester three compared to other trimesters, however, these increases seemed to have occurred independently of bacterial infections. The more affected age-group category were women between 30-39years in trimester three, suggesting that the colonization of the genital track occurred more in older women probably due to lower pH in their genital track compared to the genital track of younger women which are more acidic.The levels of progesterone of the pregnant women in the third trimester corresponded withthe highest number of *E.coli* causing UTI.In conclusion,the study showed that progesterone levels increase with trimester and the most prevalent bacteriawas *E. coli*even though age and increase in progesterone levelshad no significant impact on *E. coli* infection.

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LIST OF ABBREVIATION

UTI	:	Urinal Tract infection
CFU	:	Colony Forming Unit
CLED	:	Cystine lactose Electrolyte Deficient Agar
ExPEC	:	Extra-intestinal *Escherichia coli*
EHEC	:	Entero-haemorrhagic*Escherichia coli*
ETEC	:	Enterotoxigenic *Escherichia coli*
MTRH	:	Moi Teaching and Referral Hospital
GnRH	:	gonadotrophin-releasing hormone
IL	:	Interleukin
TH T	:	helper cell
EIEC	:	Entero-invasive *Escherichia coli*
DAEC	:	Diffusely adherent *Escherichia coli*
EAEC	:	Entero aggregative *Escherichia coli*
UPEC	:	Uropathogenic *Escherichia Coli*
TSI	:	Triple iron sugar
EDTA	:	Ethylenediaminetetraacetic acid

DEFINATIONS

Pyelonephritis: It is the inflammation of the kidney, typically due to a bacterial infection.

Detrusor: it is smooth muscle found in the wall of the bladder

Bacteriuria: It is the presence of bacteria in the urine

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CHAPTER ONE

INTRODUCTION

1.1 Background Information

Urinary tract infection (UTI) has been shown to be a common health problem among pregnant women (Izadi *et al*., 2016). Izadi *et al*.,(2016) defined UTI as the presence of uropathogens greater than 10^5CFU/ml in the urine culture. Urinary tract infections can present as either symptomatic or asymptomatic, where symptomatic is when UTI is accompanied by symptoms and asymptomatic shows no symptoms (Masinde *et al*., 2009). Symptoms of UTI are categorized into three; i) during voiding, ii) local and iii) generalized symptoms (Arinzon *et al*., 2012). Symptoms during voiding are accompanied with a painful burning sensation when urinating, whereas local symptoms includes discomfort, the urge to frequently urinate even when the bladder is empty, bloating in the lower abdomen and pain in the pelvic area, finally generalized symptoms involves experiencing fever, abdominal pain, nausea and vomiting.

Asymptomatic (ASB) and symptomatic (SB) bacteriuria have been reported to affect 13% and 17.9% of women respectively (Masinde *et al*., 2009). Asymptomatic bacteriuria is a major predisposing factor for pyelonephritis, which is linked to obstetrical complications, including preterm labor and low birth weight of infants (Masinde *et al*., 2009). The UTIs are also known to be a common cause of maternal and fetal morbidity and mortality (Nanda *et al*.,2009). The bacteriuria asymptomatic and symptomatic pregnant women are predisposed to anatomical and physiological changes imposed on the urinary tract by the pregnancy hormone progesterone, which is the most abundant hormone during pregnancy

(Duarte *et al*, 2008).Lishko *et al*., 2011 reported that progesterone is released by the ovaries and is necessary for implantation of the fertilized egg in the uterus and for maintaining pregnancy. After 8 to 10 weeks of pregnancy, the placenta takes over progesterone production from the ovaries and this change substantially increases progesterone levels.

Progesterone imposes some physiological and anatomical changes in pregnant women including an increase in bladder volume, detrusor tone decreases, and relaxation of ureters smooth muscles. These cause ureters dilatation, leading to urinary stasis, which facilitates bacterial colonization and ascending infection to kidneys (Jennifer *et al*., 2012). These adjustments are driven by progesterone hormones, which increase in concentration during pregnancy (Zen *et al*., 2010).

In pregnancy, the maternal immune system is also loweredto enable immune tolerance towards paternal antigens expressed on fetal cells. The lowered immunity promotes the growth of pathogens including *E. coli* in the urinal system of pregnant women. Increased risk of infection among pregnant women has been attributed to physiological changes, but little emphasis has been made on determining the association between *E. coli* occurrence and the progesterone hormonelevels (Duarte *et al*., 2008). In a study by Ronald, (2003) uropathogenic *E. coli* was shown to be the most abundant isolate comprising 80%, followed by *Staphylococcus saprophyticus* with 10% to 15% among pregnant women with bacteriuria.

Escherichia coli, a normal flora in the gastro-intestinal tract but can cause UTI under certain circumstances like unhygienic conditions tht lead to faecal contamination of the urinary tract. Uropathogenic *E. coli* (UPEC) produces specific virulence factors, which aid

the bacteria to adhere to uroepithelial cells and thus establish UTI. Other factors that contribute to the virulence of the UPEC include toxins, capsules, iron uptake systems and other bacterial products (Oelschlaeger *et al*., 2002). Proper diagnosis and prompt clinical intervention are advocated to curb the vast effects of UTI (Tadesse *et al*., 2014).

Currently urinalysis using the dipstick method is the preferred screening technique, but it does not ascertain the etiology of UTI that is of importance in clinical intervention. A study by Bartges (2004) showed that urine culture is the gold standard for diagnosis of UTI. Urine culture method is important since it allows quantitative enumeration of viable uropathogens in both ASB and SB and also enables identification of etiological causative agents (De Vecchi *et al*., 2013), while the measurement of progesterone in serum using Elisa method is considered to be the most reliable way to assess its rate of production (Wang *et al*., 2014).

1.2 Statement of the Problem

Urinary tract infection is a common problem among pregnant women, characterized by a colony count of 10^5 cfu/ml of the pathogens in urine. Pregnancy is usually accompanied by physiological and anatomical changes mostly due to hormone changes. Progesterone (pregnancy hormone) is a hormone whose levels increase in pregnancy leading to various changes including an increase in bladder volume, detrusor tone decreases, and relaxation of ureteric smooth muscles causing ureters dilatation, which leads to urinary stasis hence promoting bacteriuria, especially *E. coli* (Jennifer *et al.*, 2012).

In pregnancy, immunity is also lowered by progesterone in order to sustain the foetus since it has paternal cells(Zakar *et al*., 2011), and this aids in the colonization of the urinary system by *E. coli,* which is the common etiologic agent of UTI. Ronald(2003) reported that

80% of UTI in pregnant women are caused by *E. coli*.

It is important to educate and properly diagnose both ASB and AB in order to aid in early clinical intervention preventing the life-threatening effects of bacteriuria. There is a much higher risk of advancement to pyelonephritis and probablyincreased the risk of pre-eclampsia, premature birth and low neonatal birth weight (Emiru *et al*., 2013). Currently, urinalysis using the dipstick method is the screening method used but it does not identify the etiology, which is important in clinical intervention. A study by Demilie *et al*.,(2014), showed that urine culture is the gold standard for diagnosis of UTI. Despite the impact of UTIs on health status and quality of life, a limited number of studies have evaluated their aetiology in this population. This study aimed to evaluate the microbial aetiology and progesterone levels among pregnant women.

The determination of the association between *E. coli* and human progesterone hormone levels in trimester one (1) to trimester three (3) has not been established or documented hence the need to determine the association if any exists.

1.3 Justification

Urinary tract infection is a common problem among pregnant women and its consequences for both the mother and the unborn child are severe. That is related to profound structural and functional urinary tract changes typical for pregnancy hence the need to correlate progesterone levels and *E.coli* urinary tract infection among pregnant women attending MTRH antennal clinic.

Proper diagnosis is important in order to aid in suitable treatment. Asymptomatic bacteriuria due to the presence of bacteria in urine cannot be ascertained with the routine dipstick method used for screening pregnant women hence the importance of microbiological analysis.

Routine urine culture of all pregnant women is required to identify the common etiological agents of UTI among pregnant women as well as compare the bacteriological culture results with progesterone hormone levels. The present study investigated the progesterone levels and *E.coli* in the urinary tract of women and also ascertained any relationship between progesterone hormone levels, age and *E. coli* in the urinary system of pregnant women attending antenatal clinics (ANC) at MTRH.

1.4 Objectives

1.4.1 Broad objective

To investigate if there are any associations between the age, trimesters, progesterone hormone levels and *E. coli* prevalence in the urinary system of pregnant women attending Moi Teaching and Referral Hospital antenatal clinic.

1.4.2 Specific objectives

i. To determine the prevalence of *E. coli* in the urine of pregnant women in different trimesters attending the antenatal clinic at MTRH.

ii. To determine the serum progesterone levels of pregnant women in different trimesters attending the antenatal clinic at MTRH.

iii. To evaluate the association of *E coli* and progesterone levels at different trimesters of the pregnant women attending the antenatal clinic at MTRH.

iv. To assess the most affected age group by *E. coli* UTI amongst pregnant women attending the antenatal clinic at MTRH.

1.5 Hypotheses

H_{01}: There is no occurrence of *E. coli*in the urine of different trimester'spregnant women attending the antenatal clinic at MTRH.

H_{02}: The serum progesterone levels of different trimester'spregnant women attending the antenatal clinic at MTRH are low.

H_{03}: There is no significant association between *E.coli* and progesterone levels at different trimesters of the pregnant women attending the antenatal clinic at MTRH.

H_{04}: There is no significant difference in the distribution of *E. coli* UTI among age groups of pregnant women attending the antenatal clinic at MTRH

CHAPTER TWO

LITERATURE REVIEW

2.1: Progesterone hormone

The placenta chiefly produces progesterone hormone during pregnancy (Di Renzo*et al*., 2016). During the preovulatory phase of menstrual cycle progesterone levels are fairly low (>2ng/ml) and tends to escalate after ovulation (>5ng/ml) and are elevated in the luteal phase (Goldstein, 2017). When pregnancy ensues, progesterone is principally produced by the corpus luteum, which is maintained by human chorionic gonadotropin. At 8 weeks luteal-placental shift takes place a process where the placenta takes over production of progesterone from the corpus luteum(Goldstein, 2017). The placenta turning out to be the main source of progesterone and the levels rise from approximately 9-47 ng/mL in the first trimester to 43-300 ng/mL in the third trimester (Winkel *et al*., 1976).

Progesterone plays varied roles during pregnancy. These roles includeinhibiting the uterine smooth muscle contraction and reducing prostaglandin formation, both allowing the foetus to develop with the expanding uterus. It also acts as a precursor to most steroid hormones (Goldstein, 2017). (Halasz *et al*., 2013) progesterone is also important in preparation and maintenance of the endometrium to allow implantation, inhibiting maternal rejection of the trophoblast through suppressing the maternal immunologic response to fetal antigens.

2.2: Association of trimesters and progesterone levels

Pregnancy is a physiological state that involves a significant reduction in uterine vascular

tone and an increase in uterine blood flow, which is mediated in part by steroid hormones, including estrogen and progesterone (Soma *et al*., 2016). The duration of human pregnancy is arbitrarily taken as 280 days (40 weeks). Pregnancy is classified into three trimesters thus first trimester (week 1-week 12) second (week 13-week 28) and third (week 29-week 40) (Baker *et al.,* 2002).However, the foetus is considered to be at high risk once pregnancy exceeds the expected date of confinement (Bhat *et al* ., 2006).

Progesterone levels increase as pregnancy progresses. The progesterone levels are estimated to be as follows; first trimester, 9-47 ng/ml; second trimester, 17-146 ng/ml; and third Trimester, 49-300 ng/ml. In a mice study, serum progesterone concentrations increased from 40 to70 ng/ml at the commencement of pregnancy on days 15 and 16. The last 2 days before parturition, (days 19 and 20) were characterized by a decrease in progesterone concentrations to roughly 30 ng/ml. However, the combination of progesterone concentrations and vaginal smear patterns was found to be regular in only 23.8% of the cases (Nubbemeyer, 1999).

2.3: Progesterone and immunity in pregnancy

During pregnancy, the maternal immune system is altered in order to achieve immune tolerance toward paternal antigen expressed on fetal cells. These changestake place both at the fetal-maternal interface and in the systemic circulation. They are driven by estrogens and progesterone whose blood concentrations escalates during pregnancy**.** The cytokine and hormones receptors can be produced and expressed by immune and endocrine cells. These molecules can either stimulate or suppress the immune and endocrine cells activity by binding to their receptors (Zen *et al*., 2010).

Primary lymphoid organs and peripheral immune cells have binding sites for Sex steroids and gonadotrophin-releasing hormone (GnRH), signifying that they can both affect immune systems by hypothalamic-pituitary-gonadal axis (HPG) activation. The GnRH takes place in thymus maturation and employs a potent immune-stimulatory influence, leading to increased levels of interleukin (IL)-2 receptor (IL-2R) and serum interferon-Gamma (IFN-g), and triggering of helper T (Th) cells(Zen *et al*., 2010).

Progesterone acts as an immunosteroid, by its involvement in establishing a pregnancy protective immune milieu. Progesterone is involved in uterine homing of NK cells and regulates HLA-G gene expression, the ligand for NK inhibitory and activating receptors (Szekeres *et al*., 2009). A protein called progesterone-induced blocking factor (PIBF), mediates the immunological effects of progesterone by inducing a Th_2-dominant cytokine production. The PIBF binds to a novel type of the IL-4 receptor and signals via the Jak/STAT pathway, to induce a number of genes that not only affect the immune response, but also play a role in trophoblast invasiveness (Szekeres *et al*., 2009). The physical and anatomical changes imposed to the urinary system by progesterone hormone makes the system prone to infections.

2.4: Urinary tract infection in pregnancy

Urinary tract infection (UTI) is the presence of more than 10^5cfu/ml uropathogens in early morning mid-stream urine culture.

UTI is characterized by the presence of infectious agents in the genito-urinary tract that cannot be explained by contamination. These agents have the potential to invade the tissues of the urinary tract and adjacent structures. Urinary tract infections (UTIs) are one of the

most common medical hitches of pregnancy (Mittal *et al*., 2005). It is projected that one in three women of childbearing age will have a UTI (Duarte *et al*., 2008). Because of the normal physiologic variations induced by gestation, pregnant women are particularly susceptible to these infections. The infection may be limited to the growth of bacteria in the urine which frequently do not produce symptoms or it can result in several syndromes associated with an inflammatory response to the bacterial invasion. Actually, the term UTI represent a varied range of conditions, including asymptomatic forms of UTIs, urethritis, cystitis, acute pyelonephritis and pyelonephritis with bacteremia or sepsis (Keim *et al*., 2011).

Symptoms of UTI are categorized into three: during voiding, local symptoms, and generalized symptoms (Arinzon *et al*., 2012). Most urinary tract infections begin in the lower urinary tract, which is made up of the urethra and bladder. Bacteria from the bowel live on the skin near the anus or in the vagina. These bacteria can spread and enter the urinary tract through the urethra. If they move up the urethra, they may lead to a bladder infection (called cystitis). Bacteria that have infected the bladder may travel to the upper urinary tract, the ureters and the kidneys. An infection of the kidneys is called pyelonephritis. An upper urinary tract infection may cause a more severe illness than a lower urinary tract infection. Women are more probable to get UTIsthan men because the urethra is shorter in a woman than in a man. In women, the bacteria can reach the bladder more easily. Women's anatomy makes them predisposed to UTIs. The opening of the urethra is in front of the vagina. Infections also can ensue when the bladder does not empty completely. This condition may be due to by blockage (a stone) in the ureters, kidneys, or bladder that prevents the flow of urine through the urinary tract (Tadesse *et al*., 2014).

The microbiological profile is well known and pathogens such as *Escherichia coli* have been present in the vast majority of cases (Celen *et al*., 2011).

2.5: Urinary tract infection etiology in pregnant women

Urinary tract infection is mostly caused by Gram-negative bacteria, which includes; *E. coli (*60-70%), *Klebsiella* species (10%), *Proteus* species (5-10%), *Pseudomonas* species (2-5%), and Gram-positive bacteria, like group B *Streptococcus* and *Staphylococcus* species. These organisms are mostly from the genital tract, external genitalia, vagina, rectum and gastro-intestinal tract (Keim*et al*.,2011). *E.coli* has been reported to be the most prevalent causative agent of UTI among pregnant women (Celen *et al*., 2011).

2.6:*Escherichia coli*

Escherichia coli are gram negative bacilli, a majority of which are normal flora in the human intestine, however they are also known to cause severe human infections (Yang *et al.*,2014). *Escherichia coli* mostly infect individual with reduced immunity including pregnant women, the elderly, children and conditions that lower immunity like diabetes, HIV amongst others (Arinzon *et al*., 2012). Pathogenic strains are classified into two; thus, intestinal pathogens which are responsible for diarrhoea and extra intestinal *Escherichia coli* (ExPEC) which cause a variety of infections in humans and animals including the UTI, meningitis and septicemia (Sorsa *et al*.., 2007). Uropathogenic *Escherichia coli* (UPEC) which are the cause of about 80% of the estimated 130-175 million human UTIs, cause cystitis and pyelonephritis that can lead to urosepsis (Bouzari *et al*., 2012).

The intestinal pathogenic *E. coli* are classified into six serotypes, namely; enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E.*

coli (ETEC), Enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC) and diffusely adherent *E. coli* (DAEC). The UTIs are the most common extra intestinal *E. coli* infections and are caused by uropathogenic *E. coli* (UPEC), (Kaper *et al*., 2004).

Pathogenic *E. coli* strains use a multi-step system of pathogenesis, which includes colonization of mucosal site, evasion of host defenses, multiplication and host damage. Extra-intestinal pathogenic *E. coli* (ExPEC) represent a major subclass of *E. coli* that cause a wide range of diseases in human and animal hosts (Smith *et al*., 2007). One of its external attachment, the fimbriae are the most essential virulence factors of ExPEC strains of *E. coli* (Klemm *et al*., 2010). These long superficial located rod-shaped organelles mediate receptor-specific attachment to host tissue surfaces. Some ExPEC fimbriae have extra functions including promotion of biofilm formation, cell aggregation and adherence to abiotic surfaces for purposes of propagation. Most of the pathogenic *E. coli* strains are extracellular, but EIEC is demonstrated to be intracellular (Klemm *et al*., 2010).

2.7*: Escherichia coli* association with progesterone levels

Progesterone hormone is released by the ovaries and is essential for implantation of the fertilized egg in the uterus and for the conservation of pregnancy(Robeck *et al*., 2012). After 8 to 10 weeks of pregnancy, the placenta proceeds over progesterone production from the ovaries and significantly increases progesterone production to aid pregnancy (Zen *et al*., 2010).

Progesterone is a hormone whose levels increase in pregnancy leading to various changes including, increase in bladder volume, detrusor tone decreases, and relaxation of ureteric smooth muscles causing ureters dilatation, which leads to urinary stasis hence promoting

bacteriuria (Jennifer *et al*., 2012). Urine stasis which is an effect of physiological changes due to increased progesterone levels creates a good medium for bacteriato thrive.

In pregnancy, the immunity is lowered in order to sustain the fetus since it has paternal cells (Zakar *et al*., 2011) and these aids in the pathogenesis of *E. coli* which is the common etiologic agent of UTI (Zakar *et al*., 2011). The maternal immune system is lowered in pregnancy in order to achieve immune tolerance toward paternal antigen expressed on fetal cells. The lowered immunity promotes the growth of *E. coli* in the urinal system of pregnant women. These adjustments are driven by oestrogen and progesterone hormones, which are increased in concentration during pregnancy. Progesterone which is the most abundant hormone, relaxes the uterus during pregnancy, resulting in retention of the fetus (Zakar *et al*., 2011). The pathogenesis of *E. coli* involves colonization of mucosal site, evasion of host defenses, multiplication and host damage (Smith *et al*., 2007) These physiological changes due to progesterone hormones aids in the pathogenesis of *E. coli.*

2.8: Progesterone levels reduction

Progesterone is central to the maintenance of pregnancy, and is thus the ideal target for fertility regulation. Two mechanisms by which progesterone can be targeted are receptor blockage and reduction of progesterone production through enzyme inhibition. Mifepristone, a receptor blocker, is usually given as pretreatment preceding to prostaglandin administration in mid-trimester termination of pregnancy (le Roux *et al*., 2002) Trilostane is a 3beta-hydroxysteroid dehydrogenase inhibitor which reduces progesterone production. Trilostane is thought to be a competitive inhibitor of the 3-hydroxysteroid dehydrogenase (3-HSD), an essential enzyme system for the synthesis of

cortisol, aldosterone and and rostenedione (Sieber-Ruckstuhl *et al*.,2006) . Reduced levels of the progesterone hormone inhibitor can be assessed if they can reduce progesterone levels and maintain the pregnancy to term. A study on rats showed the use of high doses of these inhibitors significantly reduced progesterone levels (le Roux *et al*., 2002).

Enzyme linked Immunosorbent Assay (ELISA) is a calorimetric technique widely used in the clinical, agro-food and environmental fields. By taking advantage of the high binding avidity and selectivity of antigen-antibody binding, these assays allow accurate and sensitive analyte quantification with little or no preanalytical sample treatment (Wang *et al*., 2014). Measurement of progesterone in serum is considered to be the most reliable way to assess its rate of production. The competitive protein binding technique for determination of progesterone has been further simplified for clinical use. A small amount of sample is used for determination of serum progesterone levels. One technician can analyze 20 samples in one day with good precision and accuracy(Wang *et al*., 2014).

Urinary tract infection is one of the main health problems. Urine culture is considered as a gold standard method for the identification of UTI (Shrestha *et al*., 2013). It is the ultimate standard test for the screening of asymptomatic and symptomatic bacteriuria and identification of the etiology**.** Culture is a quantitative method where calibrated loops are used and any growth of 10^5 cfu/ml uropathogens, using a 0.001µl capacity loop is an indication of UTI (Miles *et al.,* 2005). Cysteine lactose electrolyte deficient (CLED) is achromogenic medium, which is reliable, rapid and more economic medium on which to presumptively identify this organism due to the utilization of substrate by strains and

chromogen production (Miles *et al*.,2005).

In Cystein Lactose Electrolyte Deficient (CLED) agar enzymatic digest of casein, enzymatic digest of gelatin, and beef extract provide the nitrogen, vitamins, and carbon. Lactose is the carbohydrate. L- Cysteine is added as a growth supplement for cysteine-dependent coliforms. Organisms capable of fermenting lactose will reduce the pH changing the color of the medium from green to yellow. Bromothymol Blue is the pH indicator in CLED (Miles *et al.,* 2005). Identification of *E. coli* is dependent on various characteristics including indole production and lactose fermentation (Han *et al*.,2011).

CHAPTER THREE

MATERIALS AND METHODS

3.1: Study area

The research was conducted at Moi Teaching and Referral Hospital inUasin Gishu County which is situated in the mid-west of Kenya's Rift Valleyand shares common borders with Trans Nzoia County to the North, ElgeyoMarakwet County to the East, Baringo County to the South East, Kericho County to the South, Nandi County to the South West and Kakamega County to the North West.It lies between Longitudes 340 50‟ East and 350 37‟ West and Latitudes 00 03‟ South and 00 55‟ North.

Moi teaching and referral hospital is a referral hospital facility with a catchment population from western Kenya (Figure 3.1). The facility was purposely chosen because of its elaborate clinic for mother to child infection prevention programme, good patient flow and increased urinary tract infections in pregnant women.

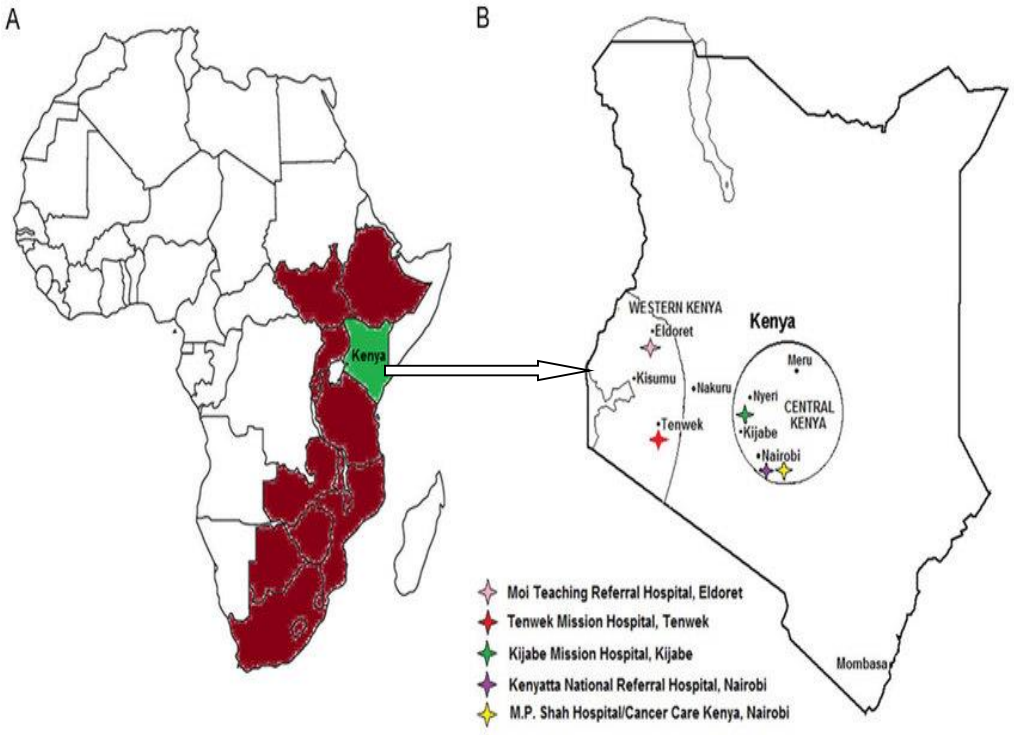


Figure 0.1 Map of the study area (MTRH) where samples were collected (source:research gate.net)

3.2: Study population

The study population for this research comprised of all pregnant women who consented to be included in the study while attending the antenatal clinic at MTRH during the study period of three months

3.3: Study design

A cross sectional study design was used to conduct the study, which involved all pregnant women of any age and trimesters.

3.4: Sample size

The sample size required for a logistic regression, in which the predictor is quantitative is given by (Agresti, 2002, 2007).

$$n = \left(\frac{\left[z_\alpha + z_\beta \exp\left(-\lambda^2 / 4 \right) \right]^2 \left(1 + 2\bar{\pi}\delta \right)}{\bar{\pi}\lambda^2} \right)$$

where

$$\delta = \left(\frac{1 + \left(1 + \lambda^2 \right) \exp\left(5\lambda^2 / 4 \right)}{1 + \exp\left(-\lambda^2 / 4 \right)} \right)$$

$$= \left(\frac{1 + (1 + 1.06) \exp\left(5 \times 1.06 / 4 \right)}{1 + \exp\left(-1.06 / 4 \right)} \right)$$

$$= 5.0$$

Thus

$$n = \left(\frac{\left[1.645 + 0.84 \times \exp\left(-1.06 / 4 \right) \right]^2 \left(1 + 2 \times 0.4 \times 5.0 \right)}{0.4 \times 1.06} \right)$$

$$= 62$$

This sample size was sufficient to model the relationship between the quantitative predictor x, here progesterone levels, and the outcome, presence of *E. coli*. The incidence of *E. coli* from a study conducted atthe University of Nairobi was 40% (Onoh *et al*.,2013). For simplicity and reasons for computing the sample size, it was assumed that these prevalence values were values at an average progesterone level. Higher progesterone levels have been associated with higher incidence of *E coli* (Onoh *et al*., 2013). Using the incidence of 40%

from the study conducted in Nairobi, a sample size that allowed the test to be sensitive to at least a 25% difference in the prevalence of *E coli* was required, that is, to increase to 65% from the population prevalence, at one standard deviation increase in the predictor, progesterone levels. The odds of *E coli* at the mean value of progesterone is 0.40/0.60=0.67, and the odds of *E coli* at a unit standard deviation above the mean are 0.65/0.35=1.9. This means that there is odds ratio hence, the chance of type II error in a test. Thus, the sample size required for one predictor is 62. However, for a multivariate logistic regression model where we have other covariates to adjust for in the model, an assumption was made about the correlation between progesterone and the rest of the covariates. It was assumed that R2 is 0.2 for moderate correlation. To get the sample size that was sufficient to study the relationship between the outcome and the anticipated factors division of the sample size was obtained above by (1-R2), which gave 78 as the participants for the study.

3.5: Sampling frame

The patients flow in MTRH antenatal clinic for 20 working days is approximately two hundred. The sampling frame of three months was used and 78 samples of both urine and blood were collected by appropriate probability sampling method.

3.6: Sampling technique

Probability sampling method wasused and 78 blood and urine samples were collected at Intervals for 60 working days. Approximately 600 samples are collected at antenatal clinic in 60 working days and 78 samples were required for the study, therefore a sample interval of 8 was used. Every 8^{th} pregnant woman who met the inclusion criteria and consented to participate was sampled until 78 participant were sampled.

3.7: Inclusion and exclusion Criteria

3.7.1: Inclusion criteria

- Pregnant women attending MTRH that consented to the study
- Pregnant women who had not used antibiotics during the previous one week.

3.7.2: Exclusion criteria

- Pregnant women attending the MTRHantenatal clinic who did not consent to the study.
- Pregnant women who had used antibiotics during the previous one week

3.8: Ethical considerations

Ethical approval was obtained from the joint Institutional Research and Ethics Committee (IREC) of Moi Teaching and Referral Hospital (MTRH) and Moi University, College of Health Sciences approval number 0001747 (*Appendix 1*). Informed consents (*Appendix 2*) were obtained from study participants before they were recruited into the study. For each confirmed infection, the responsible clinicians were informed and treatment commenced immediately. Information obtained from study participants were kept confidential.

3.9: Specimen Collection and Processing

Seventy-eight (78) blood and urine specimens of pregnant women who consented and fulfilled the inclusion criteria were used in the study. Blood and urine specimens from each study participant were analyzed to determine the association between *Escherichia coli* urinary tract infection (UTI) age and progesterone levels among the study participants.

3.10: Blood Specimen Collection and preparation

A visible vein was identified on the arm and the site first sterilized using 70% methylated

spirit cotton swabs. A venipuncture was made using a needle and 2 ml blood was gently allowed to flow into EDTA vacutainer bottle.The specimens were mixed well, after which plasma was obtained from blood by centrifugation usingan eppendorfcentrifuge model no 5702BN331537,at 3,000 – 5,000 revolutions per minute (RPM) at room temperature. Plasmas were aliquoted into cryovials using micropipettes after which they were used to determine progesterone levels using ELISA technique as per the manufacturer instructions.

3.11: Progesterone levels estimation

The concentration of progesterone was determined from the standard curve. Briefly, the samples were added to the microtiter plates, diluted hormone conjugate added and the mixture was shaken and incubated at room temperature for 1hr, after which the plates were washed to remove unbound materials. The bound hormone conjugates were detected by addition of substrate which generated an optimal color after 30 minutes. Quantitative test results were obtained by measuring and comparing the absorbance reading of the wells of the samples against the standards with a microtiter plate reader (biotek S/N 200136) at 450nm. The extent of color development is inversely proportional to the amount of progesterone in the sample.

3.12: Collection Processing and Analyses of Urine Specimen

Sterile bottles were provided to study participants who were instructed on how to collect mid-stream urine specimensMTRH/LAB/PRC/9001. The urine samples were cultured on cysteine lactose electrolyte deficient (CLED) agar media as described by (Gebrehiwot *et al*., 2012). Any urine specimens that were not processed within 30mins were stored in a refrigerator at 2-8^{0}C,but were not kept for more than six (6) hours before cultures were done.

Briefly, urine specimens were cultured on CLED media using calibrated loops(0.001ml) and incubated aerobically at 37^{O}C for 24hours. To quality control CLED media, every new batch of CLED media went through a sterility andquality check by incubating a plate of CLED at 37^{0}C aerobically for 24 hours and also inoculating standard *Escherichia coli* strain on another plate and processing the growth until the organism was identified before use. Any organism isolated with colony counts of 10^5 CFU/ml of urine was considered significant growth and indicative of a urinary tract infection (Gebrehiwot *et al*., 2012).

For instance, urine specimen inoculated onto the CLED medium using a calibrated wire loop which holds 0.001ml of the specimen and 10 colonies were observed were calculated as follows; colony count x specimen dilution factor (10 x 1000 which equals 10000= 10^4 colony forming units (CFU)/ml. Bacterial identification was done using gram stain and biochemical tests shown in *Appendix 3*.

3.13: Data management

The data were coded and transferred into an electronic format using the double entry approach. The database with the data was encrypted with a password system and accessible to one person to ensure confidentiality. The data were de-identified and coded before entry. Back-up copies of the database were also created. The result slips were kept in safe filing cabinets under lock and key and accessible to the researcher only.

3.14: Data analysis

Data analysis was done using STATA version 13 SE. Descriptive statistics were used to summarize the data and inferential statistics were used to interpret the data. Categorical variables such as age groups, pathogen occurrence and trimesters were summarized as frequencies and the corresponding means/percentages. Continuous variables such as progesterone levels that assumed the Gaussian distribution was summarized as mean and the corresponding standard deviation (SD) while those that violate the Gaussian assumption were summarized as median and the corresponding inter quartile range (IQR). Gaussian assumptions were assessed empirically using the Shapiro-Wilk test and graphically using normal probability plots.

Association between continuous variables were assessed using Pearson product moment correlation coefficient if the two variables assume Gaussian distribution otherwise the non-parametric spearman rank correlation coefficient was used. Association between the binary outcome (presence or absence of *Escherichia coli*) and the predictor variables were modeled using logistic regression model. Odds ratios (OR) and the corresponding 95% confidence limits (95% CL) were reported as well, and the results presented using tables and graphs.

CHAPTER FOUR

RESULTS

4.1: Prevalence of *E. coli* in the urine of pregnant women of different trimesters attending the antenatal clinic at MTRH

Results in Table 4.1 show the occurrence of various bacteria isolated in the urine of pregnant women attending antennal clinics at MTRH. Eleven samples were positive for urinary pathogens. Among the isolates, *E. coli* had the highest percentage of isolation (63.7 %), while the lowest were *Pseudomonas aeruginosa* and *Klebsiella spp* (9.1% each).*Enterococcus fecalis* was isolated at a rate of 18.2%.

Table 4.1: Bacterial pathogens identified in the urine of pregnant women attending antenatal clinics at MTRH

Type of bacteria isolate	Number of isolates	Percentage(%)
Escherichia coli	7	63.7
Enterococcus fecalis	2	18.2
Pseudomonas aeruginosa	1	9.1
Klebsiella spp.	1	9.1
Total	**11**	**100**

4.2: Plasma progesterone levels of pregnant women attending the antenatal clinic at MTRH

The plasma progesterone level estimates among the study participants are presented in Table 4.2.

Generally, significant differences ($p<0.5$) in plasma progesterone levels is shown among samples of participants during the three trimesters. Progesterone levels increased with trimesters. The mean progesterone levels at the first trimester ranged from5.46±1.03 to 47.55±5.54ng/mL. This was within the norm range 9-47ng/ml (Figure 4.1).

Table4. 2: Progesterone estimates (ng/ML) by trimester in pregnant women attending antenatal clinics at Moi Teaching and Referral Hospital.

Serial No.	Trimester One	Trimester Two	Trimester Three
1	44.071±2.31c	58.167±10.12d	104.712±20.13bc
2	8.411±1.18a	52.131±9.64d	60.408±12.57b
3	9.494±1.24a	12.61±2.87b	59.494±11.41b
4	47.552±5.54c	5.895±1.27a	71.383±11.39b
5	9.503±1.27a	39.724±7.98c	329.373±31.97e
6	10.972±3.11a	35.135±9.12c	77.995±11.78b
7	15.987±4.23ab	10.056±2.11b	590.844±27.81g
8	12.383±3.76a	43.625±10.75c	56.937±5.31b
9	9.485±2.44a	80.253±12.97e	157.910±18.95c
10	9.640±2.99a	75.859±13.11e	432.309±24.71f
11	8.955±2.54a	31.634±3.28c	92.013±7.13b

Serial No.	Trimester One	Trimester Two	Trimester Three
13	5.463±1.03a	49.562±9.19c	80.671±9.47b
14	8.814±3.18a	19.975±5.14b	279.059±45.67d
15	9.767±2.12a	105.391±20.76e	393.992±41.34e
16	10.971±3.07a	25.996±3.19c	946.278±57.32g
17	11.498±4.20a	59.027±18.21d	83.759±21.37b
18	12.052±3.56ab	37.852±10.78c	55.759±10.91b
19	11.535±4.06a	39.335±9.13c	301.774±45.31
20	20.713±5.58b	103.564±28.45e	57.657±5.78b
21	9.996±2.96a	37.485±7.11c	8.672±2.43a
22	11.963±3.75a	11.235±2.91b	143.322±29.34c
23	20.218±4.48b	14.084±3.72b	237.497±49.12d
24	10.369±2.94a	15.522±2.88b	704.402±63.43h
25	9.089±1.77a	36.485±7.35c	90.834±17.45b
26	9.885±2.23a	77.318 ±5.57e	34.215±10.23b
F-Value	**9.771**	**2.348**	**473**
P-Value	**0.000****	**0.000****	**0.000****

Normal ranges: (9-47ng/ml) (17-146ng/ml) (43-300ng/ml)

Means followed by different letters within a column are significantly different at p<0.05

During the second trimester, progesterone levels ranged from 5.895±1.27 to 105.391±20.7ng/ml, whichis within the normal range of 17-146ng/ML (Figure 4.1).

Results in Table 4.2 further indicate that progesterone levels during the third trimester ranged from 8.672±2.43 to 946.278±57.32. This range was higher than the normal range of 43-300ng/ML (Figure 4.1)

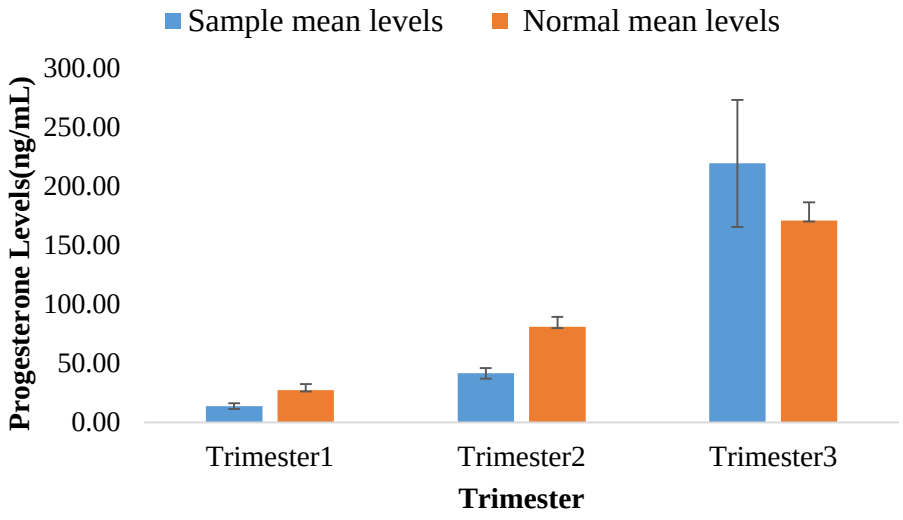


Figure 0.1: Comparison of Progesterone estimates (ng/ML) in pregnant women attending antenatal clinics with normal levels

4.3: Association of *E coli* and progesterone levels at different trimesters of the pregnant women attending the antenatal clinic at MTRH

Results on the distribution of *E. coli* by gestation period are presented in Table 4.3. There was a higher rate of *E. coli*infection in the third trimester (12/%) compared to the second trimester

And the first trimester (8%). However, chi-square analysis showed insignificant differences

(p>0.05) in *E. coli* distribution among the three trimesters.

Table4.3: Distribution of *E. coli* according to the gestation period

Tri-mester	**total samples**	**Cases with *E.coli* n(%)**	**Cases without *E.col* n(%)**	**Chi-square**	**P-Value**
First	26	2(8)	24(92)		
Second	26	2(8)	24(92)		
Third	26	3(12)	23(88)	**49.41**	**0.859**
Total	**78**	**7(9.3)**	**71(90.7)**		

Table4. 4: Association of *E coli* and progesterone levels at different trimesters of the pregnant women attending the antenatal clinic at MTRH

Trimester	**Progestrone(ng/ml)**	**Total no. of samples**	**With *E. coli***	**Without *E.coli***	**Chi-Value**	**P-Value**
	0-9	13	2(15.4)	11(84.6)		
	10 to19	10	0(0)	10(100)	1.53	0.234
First	20-29	2	0(0)	2(100)		
	>30	2	0(0)	2(100)		
	<10	2	0(0)	2(100)		
	11to30	6	0(0)	6(100)	1.32	0.168
Second	31-50	10	1(10)	9(90)		
	51-80	6	1(20)	5(83.3)		
	>81	2	0(0)	2(100)		
	<100	13	1(7.7)	12(92.3)		
	101-300	5	0(0)	5(100)	1.59	0.256
Third	301-500	4	2(50)	2(50)		
	501-700	1	0(0)	1(100)		
	>700	2	0(0)	2(100)		

Findings on the association of *E coli* and progesterone levels at different trimesters of the pregnant women attending the antenatal clinic at MTRH are shown in Table 4.4. The most occurring bacteria is *E coli* (50%) in pregnant women with progesterone between 301-500ng/ML in the third trimester. *E. coli* prevalence of 20% and 18.2% is seen in pregnant women with progesterone between 51-80 ng/ML and 0-9ng/ML in the second and first trimesters respectively. *E. coli* prevalence of only 7.7% is shown in pregnant women with less than 100ng/ML progesterone. Despite these differences in *E. coli* occurrence in relation to progesterone levels and trimesters, Pearson Chi-square results showed insignificant relationship (P>0.05) among the variables as indicated in Table 4.4.

4.4: Assessment the most affected age group by *E. coli* UTI amongst pregnant women attending the antenatal clinic at MTRH

Table 4.5: Prevalence of *E. coli* in pregnant women in relation to age

Age group	Total no. of samples	with *E.coli*	without *E.coli*	Chi	P-Value
15-19	3(4)	0(0)	3(100)		
20-29	46(58.9)	3(6.5)	43(93.5)	2.734	0.434
30-39	25(32.1)	4(16)	21(84)		
40-49	4(5)	0(0)	4(100)		
Total	**78**	**9%**	**91%**		

The prevalence of *E. coli*infection in relation to age isshown in Table 4.5. A majority (58.9%) of participants in this study were pregnant women between age-group 20-29 years. Pregnant women of the age group 30-39 years had the highest incidence of *E. coli* infection (16 %).

Followed by an age group 20-29 years (6.5%). There was no *E. coli* incidence in pregnant women of age groups 15-19 years and 40-49 years. Pearson chi-square results show insignificant association ($p>0.05$) between age and *E. coli* occurrence as indicated in Table 4.5.

CHAPTER FIVE

DISCUSSION

5.1: Prevalence of *E. coli* in the urine of pregnant women attending the antenatal clinic at MTRH

Among the significant isolatesin the urine of pregnant women attending the antenatal clinic at MTRH, were *E. coli* with the highest percentage of isolation (63.7%), while the lowest were *Pseudomonas aeruginosa* and *Kleibsiella spp.* (9.1%) respectively.This could be because uropathogenic *E. coli*expresses a multitude of virulence factors to break the inertia of the mucosal barrier, and can persist within the urinary tract serving as a reservoir for recurrent infections and serious complications. It was also postulated by Zen *et al*., (2010), that tissue receptors CD55 also called DAF, upregulated by progesterone, a hormone that increases with gestational age. Paradoxically, *E. coli* which recognizeCD55 may gain an advantage in the colonization and/or invasion of tissues, a process that is directly proportionalto CD55 receptor density. The findings of the present study are in agreement with the findings of a study (Zen *et al*., 2010;Ronald, 2003) who found that, *E. coli* represents 80.0% of bacterial isolatesin bacteriuria, also consistent with the study at (Hamdan*et al*, 2011;Ezechi *et al*., 2013;Olowe *et al*., 2013). Another study by (Tadesse *et al*., 2014) reported a 10.4% prevalence of *E. coli* among pregnant women.

Pseudomonas spp. was isolated in only five cases (9.1%) in the present study. The very low growth of *Pseudomonas spp*. could be attributed to the fact that all these cases were from *the* outpatient department and *Pseudomonas spp.* is more commonly acquired as a nosocomial infection. Results are in agreement with previous studies (Hamdan *et al*., 2011;Ronald, 2003).

5.2: Plasma progesterone levels of pregnant women attending the antenatal clinic at MTRH

There was an exponential increase in progesterone hormone estimates among study participants from trimesters one (1) to three (3), which is consistent with the observation that progesterone levels increase as pregnancy progresses(Baker *et al*., 2002).The increase was progressive from the first trimester, reaching its peak inthethird trimester of pregnancy. Progesterone during pregnancy is primarily produced by the placenta (Sfakianaki *et al.,* 2006). In women, progesterone levels during the pre-ovulatory phase of the menstrual cycle are relatively low, rises after ovulation and are elevated during the luteal phase (Johansson, 1969).Progesterone levels tend to be <2ng/ml prior to ovulation and >5ng/ml after ovulation. If pregnancy occurs, human chorionic gonadotropin is releasedmaintaining the corpus luteum, allowing it to maintain levels of progesterone. At around 8weeks the placenta begins to produce progesterone in place of the corpus luteum. This process is called the luteal-placental shift (Zen *et al*., 2010).

Progesterone function is to inhibit the smooth muscle in the uterus from contracting and decreasingprostaglandin formation, both of which allow the foetus to grow with the expanding uterus. It also serves as a precursor to most steroid hormones (Demilie *et al*., 2014). The increase in progesterone level at the different stages of pregnancy is as a result of converting the endometrium to its secretory stage to prepare the uterus for implantation (Zen *et al*., 2010). It also affects vaginal epithelium and cervical mucus, making it thick and impenetrable to sperm. During implantation and gestation, it decreases the maternalimmune response to allow for the acceptance of the pregnancy and it also decreases contractility of the uterine smooth muscle (Gebrehiwot *et al*., 2012). Therefore,

the progressive increase in the level of progesterone during the stages of pregnancy shows that progesterone plays a major role in the development of the foetus and it is thus sometimes called the hormone of pregnancy(Onoh *et al*., 2013).

5.3: Association of *E. coli* and progesterone levels at different trimesters of the pregnant women attending the antenatal clinic at MTRH

Results obtained in the present study reported that the frequency of *E. coli* was higher in the third trimester compared to the first and second trimester. This could be attributed to the further weakening of immune systems of the body as pregnancy progresses. These findings are consistent with the report by Jennifer *et al*., (2012) who observed that changes which occur due to pregnancy result in increase in bladder volume, detrusor tone decreases, and relaxation of ureteric smooth muscles causing ureters dilatation, which could lead to urinary stasis hence supporting the colonization of the bladder by bacterial organisms.

The results are also in agreement with (Onoh *et al*., 2013) who reported an increased frequency of *E. coli* in the third trimester compared to the first and second trimester of pregnancy and was also in agreement with the study by Emiru *et al*., (2013) who also reported a high incidence of *E. coli* in pregnant women in their third trimester.

This may be as a result of the pressure effect of a bigger uterus on the ureter at thethird trimester, also the increasing smooth musclerelaxing effect of pregnancy hormones and the pressure on the bladder from the descending presenting part may lead to stasis of urine, which will encourage bacteria multiplication. However, this report does not agree with (Onoh *et al*., 2013), who reported a higher prevalence of *E. coli*infection in the second trimester compared to the thirdtrimester. This difference may be as a result of either change in urinary stasis and vesico ureteral reflux or decrease in urinary progesterones and

oestrogens in the various trimester of pregnancy. Another study by (Emiru *et al*., 2013) reported that women during the 3rd (6th and 7th month) of pregnancy had the higher incidence of *E. coli*, while women in their early pregnancy months had a lower frequency of *E. coli*. Although there is alittle risk of occurrence of an acute incident in early pregnancy,there will be a substantially higher risk (30% to 60%) of occurrence (Tadess *et al*., 2014) during the last trimester. This could be attributed to the pressure of gravid uterus on the ureters causing stasis of urine flow and also attributed to the hormonal& immunological changes during normal pregnancy as well as the great abdominal distention during this stage of pregnancy with the subsequent ease of fecal contamination and the difficult personal hygiene (Hamdan *et al*., 2011).

5.4: Assessment of the most affected age group by *E. coli* UTI amongst pregnant women attending the antenatal clinic at MTRH

A majority (58.9%) of participants in this study were pregnant women between age-group 20-29 years. However, there were fewer participants between age-groups 15-19 years and 40-49 years respectively probably because the former age-group comprise of girls who were pursuing formal education and are less likely to get involved in sexual activities or some used protection and termination while the latter consist of women who have probably obtained the desired number of children, and as a result, are practicing family planning to prevent unwanted pregnancies as showed by the KDHS (2015) data. The data reported were obtained from pregnant women attending antenatal clinics at Moi Teaching and Referral Hospital (MTRH), located in Eldoret Town, which comprise mostly urban and peri-urban catchment population where women would be more interested in the small number of children that they can adequately cater for.

High prevalence of *E. coli* was in pregnant women between 30-39 years was reported in the present study. The reason could be due to the fact that many women within this age group are likely to have had many children before the present pregnancy and it has been reported that multiparity is a risk factor for acquiring *E. coli* in pregnancy (Gebrehiwot *et al*., 2012). Furthermore, certain contraceptive methods are also said to increase the risk and women are mostly sexually active at this age (Emiru *et al*., 2013). Furthermore, this could be due to lower pH compared to the genital tract of younger women whose genital tracts are acidic, which is inimical to the growth of bacterial organisms including *E. coli*. However, the scope of the protocol of this study was limited and did not include the determination of the antigenic structures of the invading *E. coli*, and also omitted was the follow-up of the babies after birth to ascertain the positivity rate of uropathogenic *E. coli* among these neonates in the hospital or at home.In addition, recurrences are common, with nearly half of people getting a second infection within a year. Risk factors include female anatomy, sexual intercourse and family history (Tadesse *et al*., 2014). Similarly, the highest prevalence of *E. coli* was reported among 30-34 years of the age group in Kumasi, Ghana(Tadesse *et al*., 2014). This is inconsistent with the findings of (Ezechi *et al*., 2013).However, the prevalence of UTI did not differ significantly within age groups in this study.

CHAPTER SIX

6.1: CONCLUSIONS AND RECOMMENDATIONS

6.1.1: Conclusions

1. The study therefore concluded that*E. Coli* are prevalent among pregnant women visiting the hospital.

2. There were exponential increases in progesterone levels for the participating pregnant women in trimester three compared to trimester two and one, concluding that progesterone levels increased with trimesters.

3. Increase in progesterone estimates in this study had no impact on *E. coli* infection.

4. Therefore, the study concluded that age has no effect on *E. coli* occurrence in pregnant women

6.1.2: Recommendations

1. Pregnant women to be screened for the presence of *E. coli* during antenatal clinics.
2. Regular studies to determine trends in levels of progesterone hormone among asymptomatic pregnant women.
3. Progesterone levels had no association with *E. coli* occurrence in the present study, therefore further studies to be carried out to determine other possible causes.
4. Determination of antimicrobial susceptibility patterns among pregnant women

REFERENCES

Agresti, A. (2007). A Historical Tour of Categorical Data Analysis. In *An Introduction to Categorical Data Analysis* (pp. 325–331). http://doi.org/10.1002/9780470114759.ch11

Arinzon, Z., Shabat, S., Peisakh, A., & Berner, Y. (2012). Clinical presentation of urinary tract infection (UTI) differs with aging in women. *Archives of Gerontology and Geriatrics*, *55*(1), 145–147. http://doi.org/10.1016/j.archger.2011.07.012

Baker, H., DeAngelis, B., Holland, B., Gittens-Williams, L., & Barrett, T. (2002). Vitamin profile of 563 gravidas during trimesters of pregnancy. *Journal of the American College of Nutrition*, *21*, 33–37.

Bartges, J. W. (2004). Diagnosis of urinary tract infections. *The Veterinary Clinics of North America. Small Animal Practice*, *34*, 923–933, vi. http://doi.org/10.1016/j.cvsm.2004.03.001

Bhat, R. A., & Kushtagi, P. (2006). A re-look at the duration of human pregnancy. *Singapore Medical Journal*, *47*, 1044–1048.

Bouzari, S., Jafari, A., & Aslani, M. M. (2012). Escherichia coli: A brief review of diarrheagenic pathotypes and their role in diarrheal diseases in Iran. *Iranian Journal of Microbiology*.

Celen, S., Oruç, A. S., Karayalçin, R., Saygan, S., Unlü, S., Polat, B., & Danişman, N. (2011). Asymptomatic bacteriuria and antibacterial susceptibility patterns in an obstetric population. *ISRN Obstetrics and Gynecology*, *2011*, 721872. http://doi.org/10.5402/2011/721872

De Vecchi, E., Sitia, S., Romanò, C. L., Ricci, C., Mattina, R., & Drago, L. (2013). Aetiology and antibiotic resistance patterns of urinary tract infections in the elderly: A 6-month study. *Journal of Medical Microbiology*, *62*, 859–863. http://doi.org/10.1099/jmm.0.056945-0

Demilie, T., Beyene, G., Melaku, S., & Tsegaye, W. (2014). Diagnostic accuracy of rapid urine dipstick test to predict urinary tract infection among pregnant women in Felege Hiwot Referral Hospital, Bahir Dar, North West Ethiopia. *BMC Research Notes*, *7*(1), 481. http://doi.org/10.1186/1756-0500-7-481

Duarte, G., Marcolin, A. C., Quintana, S. M., & Cavalli, R. C. (2008). [Urinary tract infection in pregnancy]. *Revista Brasileira de Ginecologia E Obstetrícia : Revista Da Federação Brasileira Das Sociedades de Ginecologia E Obstetrícia*, *30*, 93–100. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/19142482

Emiru, T., Beyene, G., Tsegaye, W., & Melaku, S. (2013). Associated risk factors of urinary tract infection among pregnant women at Felege Hiwot Referral Hospital, Bahir Dar, North West Ethiopia. *BMC Research Notes*, *6*, 292. http://doi.org/10.1186/1756-0500-6-292

Ezechi, O. C., Gab-Okafor, C. V, Oladele, D. A., Kalejaiye, O. O., Oke, B. O., Ekama, S. O., … Ujah, I. A. O. (2013). Prevalence and risk factors of asymptomatic bacteriuria among pregnant Nigerians infected with HIV. *The Journal of Maternal-Fetal & Neonatal Medicine : The Official Journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstetricians*, *26*, 402–6. http://doi.org/10.3109/14767058.2012.733782

Gebrehiwot, A., Lakew, W., Moges, F., Moges, B., Anagaw, B., Yismaw, G., … Kassu, A. (2012). Bacterial profile and drug susceptibility pattern of neonatal sepsis in Gondar university hospital, Gondar northwest Ethiopia. *Der Pharmacia Lettre*. http://doi.org/10.1186/1756-0500-5-197

Goldstein, S. (2017a). Progesterone. *Healthy Women*, 1–7. Retrieved from http://www.healthywomen.org/condition/progesterone

Goldstein, S. (2017b). Progesterone. *Healthy Women*, 1–7.

Halasz, M., & Szekeres-Bartho, J. (2013). The role of progesterone in implantation and trophoblast invasion. *Journal of Reproductive Immunology*, *97*(1), 43–50. http://doi.org/10.1016/j.jri.2012.10.011

Hamdan, H. Z., Ziad, A. H. M., Ali, S. K., & Adam, I. (2011). Epidemiology of urinary tract infections and antibiotics sensitivity among pregnant women at Khartoum North Hospital. *Annals of Clinical Microbiology and Antimicrobials*, *10*, 2. http://doi.org/10.1186/1476-0711-10-2

Han, T. H., Lee, J.-H., Cho, M. H., Wood, T. K., & Lee, J. (2011). Environmental factors affecting indole production in Escherichia coli. *Research in Microbiology*. http://doi.org/10.1016/j.resmic.2010.11.005

Izadi, B., Rostami-Far, Z., Jalilian, N., Khazaei, S., Amiri, A., Hamid Madani, S., & Rostami-Far, M. (2016). Urinary Tract Infection (UTI) as a Risk Factor of Severe Preeclampsia. *Global Journal of Health Science*, *8*(9), 543–564. http://doi.org/10.5539/gjhs.v8n11p77

Jennifer, P., 1, Cyril, R., 2, Piyumi, P., 1, … Microbiology, S. P. of. (2012). Asymptomatic Bacteriuria in Pregnancy: Prevalence, Risk factors and Causative O rganisms, *2*, 42–46.

Johansson, E. D. B. (1969). Progesterone levels in peripheral plasma during the luteal phase of the normal human menstrual cycle measured by a rapid competitive protein binding TECHNIQUE. *European Journal of Endocrinology*, *61*(4), 592–606. http://doi.org/10.1530/acta.0.0610592

Kaper, J. B., Nataro, J. P., & Mobley, H. L. (2004). Pathogenic Escherichia coli. *Nature Reviews. Microbiology*, *2*, 123–140. http://doi.org/10.1038/nrmicro818

Keim, L. S., Torres-Filho, S. R., Silva, P. V., & Teixeira, L. A. (2011). Prevalence, aetiology and antibiotic resistance profiles of coagulase negative staphylococci isolated in a teaching hospital. *Brazilian Journal of Microbiology*, *42*, 248–255. http://doi.org/10.1590/S1517-83822011000100031

Klemm, P., Hancock, V., & Schembri, M. A. (2010). Fimbrial adhesins from extraintestinal Escherichia coli. *Environmental Microbiology Reports*. http://doi.org/10.1111/j.1758-2229.2010.00166.x

le Roux, P. A., Tregoning, S. K., Zinn, P. M., & van der Spuy, Z. M. (2002). Inhibition of progesterone secretion with trilostane for mid-trimester termination of pregnancy: randomized controlled trials. *Human Reproduction (Oxford, England)*, *17*(6), 1483–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12042266

Lishko, P. V., Botchkina, I. L., & Kirichok, Y. (2011). Progesterone activates the principal Ca 2+ channel of human sperm. *Nature*, *471*(7338), 387–392. http://doi.org/10.1038/nature09767

Masinde, A., Gumodoka, B., Kilonzo, A., & Mshana, S. E. (2009). Prevalence of urinary tract infection among pregnant women at Bugando Medical Centre, Mwanza, Tanzania. *Tanzania Journal of Health Research*, *11*, 154–159. http://doi.org/10.4314/thrb.v11i3.47704

Miles, K. I., & Wren, M. W. D. (2005). Evaluation of three commercial agar preparations for the presumptive identification of significant urinary isolates. *British Journal of Biomedical Science*, *62*, 179–181.

Mittal, P., & Wing, D. A. (2005). Urinary tract infections in pregnancy. *Clinics in Perinatology*. http://doi.org/10.1016/j.clp.2005.05.006

Nanda, N., & Juthani-Mehta, M. (2009). Novel biomarkers for the diagnosis of urinary tract infection-a systematic review. *Biomarker Insights*, *4*, 111–121.

Nubbemeyer, R. (1999). Progesterone and testosterone concentrations during oestrous cycle and pregnancy in the common vole (Microtus arvalis Pallas). *Comparative Biochemistry And Physiology A-Molecular And Integrative Physiology*, *122*(4), 437–444.

Oelschlaeger, T. A., Dobrindt, U., & Hacker, J. (2002). Pathogenicity islands of uropathogenic E. coli and the evolution of virulence. In *International Journal of Antimicrobial Agents* (Vol. 19, pp. 517–521). http://doi.org/10.1016/S0924-8579(02)00092-4

Onoh, R. C., Umeora, O. U. J., Egwuatu, V. E., Ezeonu, P. O., & Onoh, T. J. P. (2013). Antibiotic sensitivity pattern of uropathogens from pregnant women with urinary tract infection in Abakaliki, Nigeria. *Infection and Drug Resistance*, *6*, 225–233. http://doi.org/10.2147/IDR.S46002

Robeck, T. R., Gill, C., Doescher, B. M., Sweeney, J., De Laender, P., Van Elk, C. E., & O'Brien, J. K. (2012). Altrenogest and progesterone therapy during pregnancy in bottlenose dolphins (Tursiops truncatus) with progesterone insufficiency. *Journal of Zoo and Wildlife Medicine : Official Publication of the American Association of Zoo Veterinarians*, *43*, 296–308. http://doi.org/10.1638/2011-0166.1

Ronald, A. (2003). The etiology of urinary tract infection: Traditional and emerging pathogens. *Disease-a-Month*. http://doi.org/10.1016/S0011-5029(03)90001-0

Sfakianaki, A. K., & Norwitz, E. R. (2006). Mechanisms of progesterone action in inhibiting prematurity. *The Journal of Maternal-Fetal & Neonatal Medicine : The Official Journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstetricians*, *19*(December), 763–772. http://doi.org/10.1080/14767050600949829

Shrestha, R., Gyawali, N., Gurung, R., Amatya, R., & Bhattacharya, S. K. (2013). Effect of urogenital cleaning with paper soap on bacterial contamination rate while collecting midstream urine specimens. *Journal of Laboratory Physicians*, *5*, 17–20. http://doi.org/10.4103/0974-2727.115910

Sieber-Ruckstuhl, N. S., Boretti, F. S., Wenger, M., Maser-Gluth, C., & Reusch, C. E. (2006). Cortisol, aldosterone, cortisol precursor, androgen and endogenous ACTH concentrations in dogs with pituitary-dependant hyperadrenocorticism treated with trilostane. *Domestic Animal Endocrinology*, *31*, 63–75. http://doi.org/10.1016/j.domaniend.2005.09.004

Smith, J. L., Fratamico, P. M., & Gunther, N. W. (2007). Extraintestinal pathogenic Escherichia coli. *Foodborne Pathogens and Disease*, *4*, 134–163. http://doi.org/10.1089/fpd.2007.0087

Soma-Pillay, P., Nelson-Piercy, C., Tolppanen, H., & Mebazaa, A. (2016). Physiological changes in pregnancy. *Cardiovascular Journal of Africa*, *27*(2), 89–94. http://doi.org/10.5830/CVJA-2016-021

Sorsa, L. J., Feldmann, F., Hildinger, K., Dufke, S., & Schubert, S. (2007). Characterization of four novel genomic regions of uropathogenic Escherichia coli highly associated with the extraintestinal virulent phenotype: A jigsaw puzzle of genetic modules. *International Journal of Medical Microbiology*, *297*, 83–95. http://doi.org/10.1016/j.ijmm.2006.11.007

Szekeres-Bartho, J., Halasz, M., & Palkovics, T. (2009). Progesterone in pregnancy; receptor-ligand interaction and signaling pathways. *Journal of Reproductive Immunology*, *83*, 60–64. http://doi.org/10.1016/j.jri.2009.06.262

Tadesse, E., Teshome, M., Merid, Y., Kibret, B., & Shimelis, T. (2014). Asymptomatic urinary tract infection among pregnant women attending the antenatal clinic of Hawassa Referral Hospital, Southern Ethiopia. *BMC Research Notes*, *7*(1), 155. http://doi.org/10.1186/1756-0500-7-155

Wang, B., Liu, Y., Zhang, X., Feng, T., Xu, W., & Han, S. (2014). A direct competitive enzyme linked immunosorbent assay for progesterone using monoclonal antibody. *Monoclonal Antibodies in Immunodiagnosis and Immunotherapy*, *33*(1), 8–12. http://doi.org/10.1089/mab.2013.0042

Winkel, P., Gaede, P., & Lyngbye, J. (1976). Method for monitoring plasma progesterone concentrations in pregnancy. *Clinical Chemistry*, *22*(4), 422–428.

Yang, K., Pagaling, E., & Yan, T. (2014). Estimating the prevalence of potential enteropathogenic escherichia coli and intimin gene diversity in a human community by monitoring sanitary sewage. *Applied and Environmental Microbiology*, *80*, 119–127. http://doi.org/10.1128/AEM.02747-13

Zakar, T., & Mesiano, S. (2011). How does progesterone relax the uterus in pregnancy? *The New England Journal of Medicine*, *364*, 972–973. http://doi.org/10.1056/NEJMcibr1100071

Zen, M., Ghirardello, A., Iaccarino, L., Tonon, M., Campana, C., Arienti, S., … Doria, A. (2010). Hormones, immune response, and pregnancy in healthy women and SLE patients. *Swiss Medical Weekly*. http://doi.org/smw-12597

APPENDICES

APPENDIX I: COPY OF STUDY APPROVAL LETTER FROM IREC

APPENDIX II: CONSENT FORM

Study Tittle: Association of *Escherichia coli*In Urinary System and Progesterone Hormone in Pregnant Women Attending Moi Teaching and Referral Hospital.

Investigator: Anne Wanjiku

Institution: University of Eldoret

Introduction:

My name is Anne Wanjiku, am performing this study as part of the requirements to complete an MSc programme in Microbiology at the University of Eldoret. The aim of this form is to give you information about the study. Kindly read it carefully and understand then ask me questions about anything that is not clear to you, regarding what is expected of you once you are recruited into the study, the risks and benefits involved and your rights as a volunteer. You can also inquire about anything you wish to know about the study. When all is clear, you can make an informed consent whether or not to participate in the study. If you wish to be informed of your test results, you will be requested to provide your mobile telephone number. Signing or thumb printing on the form is a sign that you have agreed by your choice to participate in the study.

Back ground information

You are being asked to participate in this study because you are pregnant and not on any antibiotics for the past one week. UTI is a common problem in pregnancy which can be caused by infections in the urinary bladder and kidneys called urinary tract infections. Urinary tract infections which are caused by germs called bacteria that can be cured with medications known as antibiotics. UTI could be associated with changes during pregnancy

due to hormones. Progesterone is a hormone whose levels increase as pregnancy progress and it is responsible for pregnancy maintenance. Urinary tract infection if untreated can cause serious complications to the mother and her baby. This study will help you with a free diagnosis of UTI and alsothe determination of progesterone levels. It will also unravel the association between *Escherichia coli* in the urinary system and progesterone levels.

Purpose

This study will find out the association of *Escherichia coli* in the urinary system and progesterone among women attending MTRH. The trimester and age group affected most with *E coli*will also be linked with progesterone levels.

This study will enroll 78 pregnant women attending the MTRH antenatal clinic.

After you are enrolledin the study, I will ask you a few questions concerning you and the pregnancy after which you will be requested to provide blood and urine specimens laboratory testing. The results of the tests will be placed in your file and will be used to treat you in case of any infection. There will be no other visits involved in this study.

Risks or discomfort

There is no expected risk that may arise due to participation in this study. Slight needle pain during venipuncture which is expected. Any discomfort with any procedure of sample collection or questions asked, you are free to decline and withdraw your consent. It will not in any way affect your access to services in this department.

Benefits and compensations

Information gained from this study will be used for treatment if you are found with an infection or abnormally low or high progesterone levels. There is no extra cost to you due to your participation in the study, and no physical injuries are anticipated. There will be no compensations.

Confidentiality

Involvement in this study is voluntary and you can decline your consent without loss of any benefits or any penalties. The participant's information will be de-identified and coded with numbers. Participant's personal information will be treated with confidentially by being accessed only by the principle investigator and will be under key and lock. Any database used will be encrypted with a password by the principle investigator. The investigator can use the data for analysis, quality control purposes or publication but your identity will never be revealed. The urine and blood sample you provide shall only be used for purposes described in this consent form.

Participant signature

The main aim of the study has been explained to me. I have had an opportunity to ask questions and feel contented to make an informed consent to participate in this study.

Participant signature/thumb print: _____ Date: _____

Witness's signature/informer: _____ Date: _____

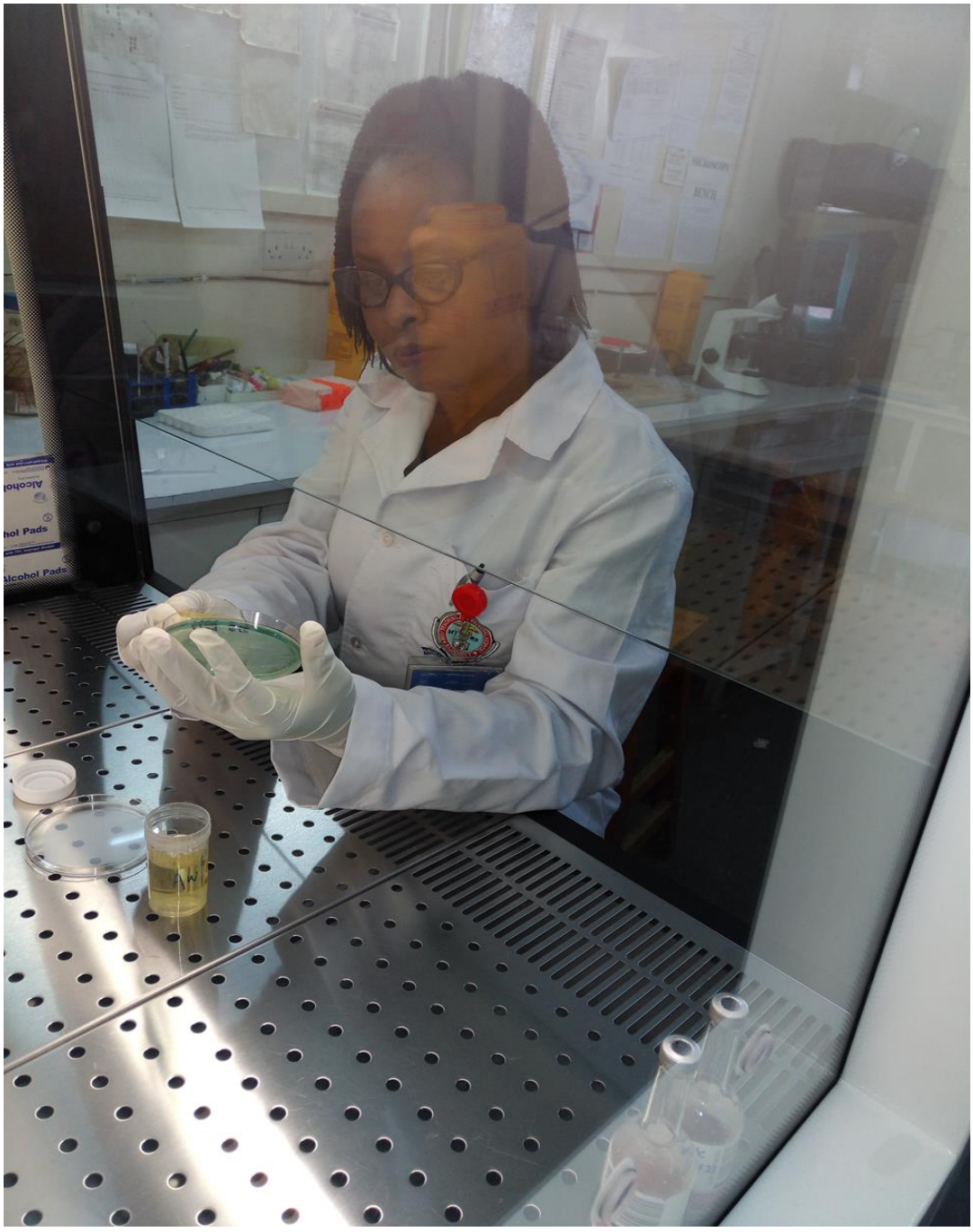
Mobile number (optional): _____

Investigator's signature: _____ Date: _____`

APPENDIX III: FLOWCHART FOR THE IDENTIFICATION OF *Enterobacteriaceae* (Barry *et al*., 1975)

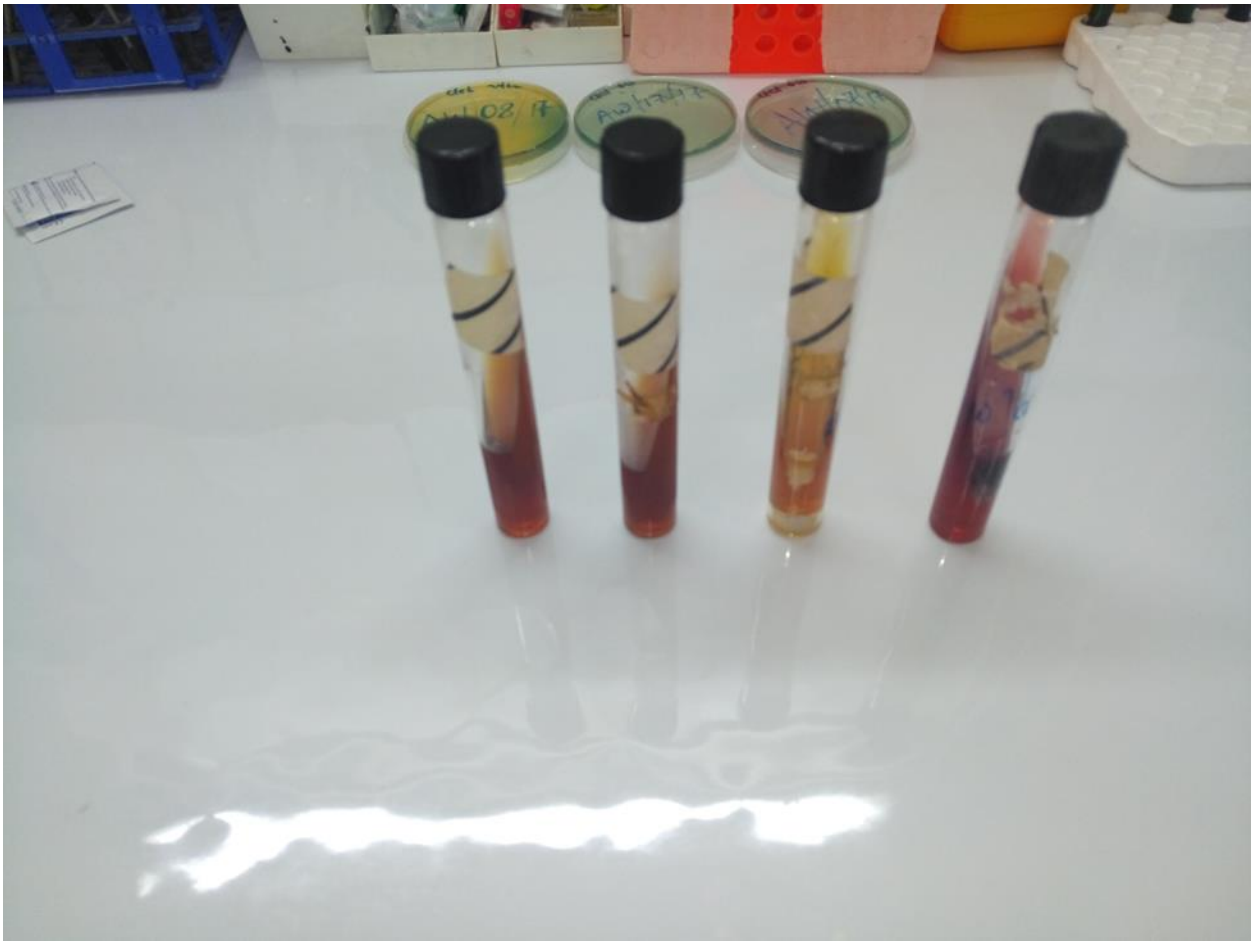
	Reaction	Carbohydrate Fermentation	Suspected Organisms
Slant Butt H_2S Gas	Alkaline (R) Alkaline (R) – –	No carbohydrate fermentation	*Pseudomonas* *Alcaligenes* *Herellea* (see chapter 10)
Slant Butt H_2S Gas	Acid (Y) Acid (Y) – +	Lactose and/or sucrose fermented Glucose fermented	*Escherichia* *Enterobacter-Klebsiella* *Proteus* *Providencia* Intermediate coliforms
Slant Butt H_2S Gas	Acid (Y) Acid (Y) + +	Lactose and/or sucrose fermented Glucose fermented	*Citrobacter* *Arizona*
Slant Butt H_2S Gas	Alkaline (R) Acid (Y) – –	Lactose and sucrose not fermented Glucose fermented	*Providencia* *Proteus* *Serratia* *Shigella* *Salmonella*
Slant Butt H_2S Gas	Alkaline (R) Acid (Y) + +	Lactose and sucrose not fermented Glucose fermented	*Proteus* *Citrobacter* (certain types) *Salmonella* *Arizona* (certain types) *Edwardsiella*

APPENDIX IV: INOCULATING URINE ON CLED AGAR.



APPENDIX V: URINE CULTURE ON CLED MEDIA

APPENDIX VI: TRIPPLE IRON SUGAR BIOCHEMICAL TEST



APPENDIX VII: TSI SHOWING ACID/ACID WITH GAS PRODUCTION

APPENDIX VIII: SIMON CITRATE BIOCHEMICAL TEST

APPENDIX IX: PROGESTERONE MICROTITER