

**PREVALENCE OF ASYMPTOMATIC BACTERIURIA AND ANTIMICROBIAL
SUSCEPTIBILITY AMONG WOMEN ATTENDING ANTENATAL CLINIC OF
GATUNDU HOSPITAL IN KIAMBU COUNTY, KENYA**

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**A thesis submitted in partial fulfillment of the requirements for the award of the degree of
Master of Science in Infectious Diseases (bacteriology) in the School of Medicine of
Kenyatta University**

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DECLARATION

This is my original work and has not been presented for a degree in any other university or any other award.

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DEDICATION

To my family for their love, support and encouragement.

To my dear late father, Mr. Naftaly Muturi, who insisted that education does not end, hence, the challenge to undertake this course.

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ABBREVIATIONS AND ACRONYMS

ANC	Antenatal Care
ASB	Asymptomatic bacteriuria
ATTC	American type culture collection
CFU	Colony-forming Units
CLED	Cystine Lactose Electrolyte Deficient
MSU	Midstream Urine
NACOSTI	National Council for Science and Technology
NICE	National Institute for Health and Clinical Excellence
QA	Quality Assurance
SPSS	Statistical Package for Social Science
UPEC	Uropathogenic <i>Escherichia coli</i> .
UTI	Urinary Tract Infection
WHO	World Health Organization

OPERATIONAL DEFINITIONS

Bacteriuria	The presence of bacteria in urine.
Antibiotic	Antimicrobial drugs used in the treatment and prevention of bacterial infections.
Cervicitis	Inflammation of the cervix.
Commensal	Living on or within another organism and deriving benefit without harming or benefiting the host.
Cystitis	An inflammatory process of the urinary bladder, typically caused by bacterial infection.
Interstitial cystitis	Chronic pain in the bladder.
Multigravidae	A woman who has been pregnant for more than once.
Multiparous	Having given birth two or more times.
Nulliparous	Women who has never given birth.
Parity	The number of pregnancy.
Pathogen	Biological agent that causes disease or illness to its host.
Primigravidae	A woman who is pregnant for the first time.
Prostatitis	Inflammation of the prostate gland.
Pyelonephritis	A urinary tract infection involving the kidney
Pyuria	The presence of white blood cells in the urine and is a marker of inflammation in response to bacterial infection.
Trimester	A period of three months during a pregnancy in which there is specific fetal development.
Urethritis	An inflammation or infection of the urethra.
UTI	Includes the inflammatory response and the associated signs and symptoms that result from the presence of the bacteria
Vaginitis	Inflammation of the vagina.

ABSTRACT

Asymptomatic bacteriuria in pregnancy is associated with risk of preterm birth and pyelonephritis if untreated. The apparent decline in immunity of pregnant women appears to promote the growth of both commensal and non-commensal microorganisms. Women with asymptomatic bacteria in the early pregnancy develop symptomatic bacteriuria later in pregnancy. The incidence of antibiotic resistance has been steadily increasing over the past few years resulting in limitation of therapeutic options. The aim of this study was to identify the prevalence of asymptomatic bacteriuria, the most common causative microorganisms and the antibacterial susceptibilities of the isolated microorganisms in pregnant women attending ante-natal clinic at Gatundu Level Four hospital. An analytical cross sectional study design was applied to women with asymptomatic bacteriuria who attended the antenatal clinic and utilized the laboratory services. Quantitative data was analyzed using a computer package IBM SPSS statistics 20.0 by invoking the analysis of descriptive statistics and focusing on frequencies and percentages. Pearson Chi square test was carried out where $p < 0.05$ values were considered significant. A total number of 120 asymptomatic pregnant women were screened for ASB by urine culture using a semi quantitative culture method. Midstream urine was collected and aerobically incubated at 37°C on CLED and MacConkey Agar. Growth of $\geq 1 \times 10^5$ CFU/mL was taken as significant bacteriuria. Gram-negative bacteria were identified and confirmed by biochemical tests. In this study, the prevalence of bacteriuria among the pregnant women was 10.8 %. The prevalences of isolated bacteria from urines of pregnant women were: *Escherichia coli* 69.2 %, *Proteus species* 23.1 % and *Klebsiella species* 7.7 %. Among cases which showed positive cultures, 10.0 % were nulliparous and 11.4 % were multiparous. The highest incidence (33.3 %) of asymptomatic bacteriuria was found in the maternal age category age group of 31-35 years. Majority of the isolates in the study were susceptible to Cefataxime (100 %) Ofloxacin (88.9 %) and Gentamycin (44.4 %). Findings of the study provide empirical data on the prevalence of asymptomatic bacteriuria within pregnant women. The prevalence of asymptomatic bacteriuria is high among this study population, therefore all pregnant women should be screened during their first visit to ante-natal clinic by urine culture to detect and treat ASB to avoid complications that may ensue in both mother and fetus. Establishment of antibiotic policy to guide against the emergence of resistant organisms that cause asymptomatic bacteriuria should be effected.

CHAPTER ONE

INTRODUCTION

1.1 Background to the study

Asymptomatic bacteriuria (ASB) is a condition in which urine culture reveals a significant growth of pathogens that is greater than 10^5 bacteria/ml of urine, but without the patient showing symptoms of urinary tract infection. The single most important risk factor for the development of urinary tract infections (UTIs) is the history of infection prior to pregnancy or at an early stage of pregnancy, (Schnarr and Smaill, 2008). Other risk factors include: high level of sexual activity, multiparity, low socioeconomic status, advanced maternal age, diabetes, and anomalies and defects of the urinary tract (Matuszkiewicz-Rowińska *et al.*, 2015). The infection can occur at different points in the urinary tract, including bladder, kidneys, ureters and urethra. Urinary tract infection is the most common bacterial infection during pregnancy and it is not only common but the range of clinical effect varies from asymptomatic bacteriuria (ASB) to acute pyelonephritis (Ebie *et al.*, 2001). The incidence of UTI varies depending on the local prevalence of asymptomatic bacteriuria (Newal and Senani, 2011).

Asymptomatic bacteriuria (ASB) is a major risk factor for the development of UTIs during pregnancy (Kalawole *et al.*, 2009). Specific subpopulations at increased risk of UTI include infants, pregnant women, the elderly, patients with spinal cord injuries and catheters, patients with diabetes or multiple sclerosis, patients with Acquired Immunodeficiency Disease Syndrome /Human Immunodeficiency Virus, and patients with underlying urologic abnormalities (Newal and Senani, 2011). Asymptomatic bacteriuria requires medical treatment during pregnancy (MacLean, 2001). Asymptomatic bacteriuria affects 2-10 % of pregnant women, depending on

the patient population. If left untreated, as many as 30-50 % would develop symptomatic UTI, often in the form of pyelonephritis (Smaill and Vazquez, 2009). The hormonal and mechanical changes of the upper and lower urinary tract during pregnancy contribute to development of bacteriuria and UTI (Krcmery *et al.*, 2001).

There is insufficient local data on asymptomatic bacteriuria among pregnant women in Kenya (Masinde *et al.*, 2009). This lack of data on state of asymptomatic bacteriuria in pregnant women in Kenya calls for action as ASB is a common infection in pregnancy and possible cause of poor obstetric outcome. Untreated ASB elevates the risk of pyelonephritis, premature delivery, and fetal mortality among pregnant women, and is associated with impaired renal function and end-stage renal disease among pediatric patients (Mobasheri *et al.*, 2012). Asymptomatic bacteriuria which leads to urinary tract infections among pregnant women need to be addressed by all countries in order to prevent bad obstetric outcomes (Coulthard *et al.*, 2010). It would also help control the enormous health and financial burdens that accompany the ASB. The best way to achieve this goal is to include culturing of urine in the screening of all pregnant women attending antenatal clinics. (Masinde *et al.*, 2009).

Infections in the urinary system are often classified by the anatomical site or organ involved, namely the bladder, urethra and the nephrons, although the entire urinary tract may be affected (Girishbuabu *et al.*, 2011). Infection of the upper urinary tract is known as pyelonephritis, a condition which presents with fever, tachycardia, painful urination and abdominal pain. Pyelonephritis is a serious condition that can lead to septic shock if untreated. Pyelonephritis can lead to medically indicated preterm, hospital stays (mean 3.5 days), anemia (23 %) blood transfusion (1 %), renal dysfunction (2 %) and respiratory insufficiency (7 %) (Smaill and Vazquez, 2009).

It is thought that pyelonephritis may occur because of the mechanical effect of urinary tract compression or smooth muscle relaxation in pregnancy together leading to either symptomatic or asymptomatic UTI. Isolated bacterial urethritis is rare in women (Masinde *et al.*, 2009). Vaginitis and cervicitis, often related to sexually transmitted organisms, may also cause symptoms attributed to cystitis or urethritis (Masinde *et al.*, 2009). Recurrent UTIs involve re-infection from the source outside the urinary tract or from bacterial persistence within it. In each case, the infections may be caused by the same or different organisms.

The bacteria that cause urinary tract infections typically enter the bladder via the urethra. However, the infections may also occur via the blood or lymph (Hazhir, 2007). It is believed that the bacteria are usually transmitted to the urethra from the bowel, with females at greater risk, the result of several clinical factors including anatomical differences and hormonal effects. After gaining entry to the bladder, the bacteria are able to attach to the bladder wall and form a bio-film that resists the body's immune response (Demmille *et al.*, 2012). The clinical manifestations of UTI depend on the portion of the urinary tract involved, the etiologic organisms, the severity of the infection and the patient's ability to mount an immune response to it. Signs and symptoms may include fever, chills, dysuria, urinary urgency, frequency, suprapubic discomfort, pyuria and cloudy urine, (Sibi, 2011). Lower UTIs typically resolve after a course of antibiotic therapy. Recurring or persistent symptoms suggest improper antibiotic selection or the presence of unsuspected upper UTI, which requires a longer course of antibiotic therapy or a complication may be present (Chang *et al.*, 2001).

Asymptomatic bacteriuria may be caused by a variety of different organisms, most commonly normal flora of the host which usually originate from the bowel, vagina, or skin. The most frequent bacterial cause of ASB in pregnant women is *Escherichia coli*, which is part of the

normal gut flora, (Ojiegbe and Nworie, 2009). This organism accounts for approximately 85 % of community acquired urinary tract infections and 50 % of hospital acquired urinary tract infections (Smaill and Vazquez, 2009). Other common organisms include *Enterococcus faecalis*, *Klebsiella pneumoniae*, and *Staphylococcus saprophyticus* and *Proteus* species. Nosocomial infections and those associated with foreign bodies may involve more aggressive organisms such as *Pseudomonas aeruginosa*, *Serratia*, *Enterobacter* and *Citrobacter* species which may cause ASB in pregnant women. Non-bacterial infections are also common and tend to occur more often in second and third trimesters in pregnant women or those with diabetes mellitus (Smaill and Vazquez, 2009). Fungal infections with *Candida* species are the most common non-bacterial infections which occur as co-infections with asymptomatic bacteriuria (Hazhir, 2007). Other rare causes of urinary tract pathogens include *Mycobacterium tuberculosis*, *Salmonella* species, *Chlamydia trachomatis* and a variety of anaerobic organisms. Anaerobes may be especially dangerous in immunocompromised pregnant women due to an increased risk of severe infections such as emphysematous pyelonephritis or cystitis (Smaill and Vazquez, 2009).

Hygiene habits, such as voiding before and after sexual intercourse and wiping from anterior to posterior, are often advocated to decrease the risk of ASB. Alternative means of contraceptive other than certain spermicides or diaphragm should be adopted by women who are at risk to urinary tract infections (Trussell *et al.*, 2011). This study was aimed at determining the causative agents associated with asymptomatic bacteriuria among pregnant women attending the antenatal clinic of Gatundu Level Four Hospital. It also finds out the prevalence and contributing factors of asymptomatic bacteriuria among pregnant mothers attending the antenatal clinic and also identifies the causative bacteria. Antimicrobial susceptibility patterns of the bacteria obtained would lead to the right prescription of the effective drug. These contributions would be of

interest to health care givers as well as practicing managers in healthcare in the management of pregnant women and would directly impact on the management of maternal child welfare.

The study adds more data to the limited information regarding routine urine culture among asymptomatic antenatal clinic attendees in Kenya. Its findings would be used to reduce the overall rate of asymptomatic bacteriuria among pregnant women that is clinically diagnosed. The study would also help control the enormous health and financial burdens that accompany the disease.

1.2 Statement of the problem

Despite the antenatal care accorded to the pregnant women, asymptomatic urinary tract infections are on the increase globally. Regular screening and treatment of bacteriuria would lower the incidence of untreated pregnant mothers developing acute pyelonephritis, cystitis, preterm births and underweight babies. Maternal child health has been a major issue in terms of family and community health however, data on the causative microorganisms, drug sensitivity and prevalence of ASB is very limited. Modern, updated and accurate methods of diagnosing asymptomatic urinary tract infections and produce antimicrobial susceptibility patterns are not routinely used in limited resource antenatal laboratory settings which may contribute to rise in the infections among pregnant mothers.

There is need to evaluate factors such as maternal age, parity and gestational age in trimesters and ascertain if they predispose pregnant mothers to ASB (Hazhir, 2007). In addition the incidence of bacterial resistance to antibiotics is of great concern in the management of pregnant mothers because immune system is compromised by the hormonal changes accompanying pregnancy. Rapid emergence of antibiotic resistance warrants continuous monitoring of the

susceptibility patterns of bacterial isolates. Resistance to erythromycin (58.6%), co-trimoxazole (57.4%) and ciprofloxacin (50.1%) was observed in a study done on pregnancy-associated asymptomatic bacteriuria and drug resistance in India. This led to continuous local monitoring of resistance patterns to determine the appropriate empirical antimicrobial therapy (Khan *et al.*, 2015). Therefore antimicrobial susceptibility analysis should be carried out and used in determining therapy as inappropriate treatment has been responsible for recurrences of asymptomatic bacteriuria with development of acute pyelonephritis later (Gilstrap and Ramin, 2001). Thus, regular screening of pregnant women may provide means to reduce the cases of asymptomatic bacteriuria.

1.3 Justification

Asymptomatic bacteriuria is a condition that has the potential to affect pregnancy outcome if not addressed early enough (Alvarez *et al.*, 2010). Globally asymptomatic bacteriuria affects 2 to 10% of all pregnant women, and approximately 30% of these will develop pyelonephritis if not properly diagnosed and treated (Alvarez *et al.*, 2010). The practice of targeting pregnant women in high-risk groups for screening, such as those with a past history of urinary tract infection, immunosuppression and diabetes instead of screening all pregnant women has increased the incidence and prevalence of UTI and its associated complications later in pregnancy. In Kenya, not much research has been done on asymptomatic bacteriuria in pregnant women as only one research was found on asymptomatic bacteriuria in catheterized patients (Theresa, 2011). Only scanty information is available about the prevalence of asymptomatic bacteriuria in different trimesters, almost all the evidence in the literature was collected in the first trimester (Wamalwa *et al.*, 2013). Failure to identify asymptomatic bacteriuria during pregnancy is observed among health personnel managing pregnant women (Kehinde *et al.*, 2011). There is limited information

regarding routine urine culture among asymptomatic antenatal clinic attendees in Kenya, hence the need for this study. It is expected that findings from this study will be used to reduce the overall rate of asymptomatic bacteriuria among pregnant women that progress to clinically detectable levels. It will also help control the enormous health and financial burdens that accompany the disease.

1.4 Research questions

1. What is the prevalence of asymptomatic bacteriuria and which bacteria cause the infection among pregnant women in Gatundu Level Four Hospital Kiambu County?
2. What is the antimicrobial susceptibility pattern of bacteria isolated among pregnant women in Gatundu Level Four Hospital Kiambu County?
3. Does the maternal age, parity and gestational age in trimesters correlate with variation in occurrence of bacteriuria?

1.5 Objectives

1.5.1 General objective

To determine the prevalence of ASB and antibiotic susceptibility profile among pregnant women attending the antenatal clinic of Gatundu Level Four Hospital Kiambu County.

1.5.2 Specific objectives

1. To isolate and identify the bacteria causing asymptomatic bacteriuria among the pregnant women in Gatundu Level Four Hospital Kiambu County.
2. To determine the antimicrobial susceptibility pattern of bacteria isolated from pregnant women in Gatundu Level Four Hospital Kiambu County.

3. To determine the prevalence and variations arising from maternal age, parity and gestational age in trimester on the bacteriuria among pregnant women in Gatundu Level Four Hospital Kiambu County.

1.6 Conceptual framework

This study was based on the conceptual framework maternal age, parity and gestational age as independent variables while the dependent variable was asymptomatic bacteriuria. In maternal age, the study finds out whether there is a variation in incidences of ASB between different age groups of pregnant women in the study. The study also finds out whether incidences of ASB are significant in different gestational ages and parity.

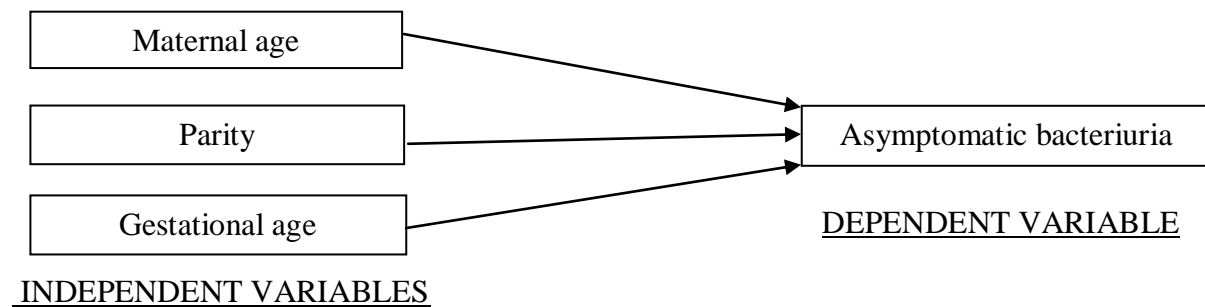


Figure 1.1 Conceptual framework.

CHAPTER TWO

LITERATURE REVIEW

2.1 Background

During pregnancy physiological changes that affect the normal functioning of the body occur which are caused by the pregnancy in its different trimesters (Mobasheri *et al.*, 2012). Hormonal and mechanical changes increase the risk of urinary stasis and backward flow of urine from the bladder into the kidneys. These changes, along with an already short urethra (approximately 3-4 cm in females) and difficulty with hygiene due to a distended pregnant belly, increase the frequency of urinary tract infections (UTIs) in pregnant women. Indeed, UTIs are among the most common bacterial infections during pregnancy (Celen *et al.*, 2011). In general, pregnant patients are considered immunocompromised UTI hosts because of the physiologic changes associated with pregnancy. These changes increase the risk of serious infectious complications from asymptomatic urinary infections even in healthy pregnant women (Mobasheri *et al.*, 2012). Oral antibiotics are the treatment of choice for asymptomatic bacteriuria and cystitis. The standard course of treatment for pyelonephritis is hospital admission and intravenous antibiotics. Antibiotic prophylaxis is indicated in some cases. Patients treated for symptomatic UTI during pregnancy should be continued on daily prophylactic antibiotics for the duration of their pregnancy (Beerepoot *et al.*, 2012)

2.2 Global picture of asymptomatic bacteriuria

The urinary tract is a common site of infection in humans. During pregnancy, UTI is associated with increased risks of maternal and neonatal morbidity and mortality, even when the infection is asymptomatic (Gilbert *et al.*, 2013). By mapping available rates of ASB in pregnancy across

different populations, it is noted that this is a problem of global significance (Gilbert *et al.*, 2013). Globally, prevalence of ASB in pregnant women is generally reported to range from 2.5 % to over 10 %, although there are certain variations in different countries and geographical regions. For example, ASB screening in pregnant women in Turkey has suggested incidence of 8.5 % (Celen S. *et al.*, 2011), 10.7 % in Nigeria (Kehinde *et al.*, 2011), 12.3 % in Brazil (Darze *et al.*, 2011) 13.2 % in India (Rajaratnam *et al.*, 2014) and 10.4 % in North-west Ethiopia (Alemu *et al.*, 2012). In Bangladesh, prevalence of 12% was obtained (Ullah, *et al.*, 2007) and 7.3% in Kumasi, Ghana (Turpin *et al.*, 2007). Masinde *et al.*, (2009) observed prevalence of 2-13% in Tanzania. In a study conducted in West Bengal, a prevalence of 11% among pregnant women was recorded (Rajshkehar and Umashanker, 2013). Many countries with high rates of preterm birth and neonatal mortality also have rates of ASB in pregnancy that exceed rates seen in more developed countries.

A global analysis of the etiologies of urinary tract infection revealed familiar culprits as well as emerging threats (Gilbert *et al.*, 2013). Screening and treatment of ASB have improved birth outcomes in several more developed countries and would likely improve maternal and neonatal health worldwide. However, challenges of implementation in resource-poor settings must be overcome (Gilbert *et al.*, 2013). Prevalence of ASB in pregnant women in Asian countries such as Pakistan, Bangladesh, and India was reported to be 6–10.2 % by Khattak *et al.*, (2006), Jubaida *et al.*, (2015) and Urmila *et al.*, (2012). The highest age-specific prevalence was found in the 26-30 years, and the lowest in the 46-48 years in Saudi Arabia, *Streptococcus agalactiae* was the most predominant organism closely followed by *Escherichia coli*, (Nawal and Senani, 2011).

A large population-based study of nearly 200,000 pregnant Israeli women demonstrated a 2.5 % rate of asymptomatic bacteriuria (Versi *et al.*, 1997) and a 2.3 % rate of symptomatic UTI

(Sheiner *et al.*, 2009). In this population, asymptomatic bacteriuria was found to have an association with multiple pregnancy complications, including hypertension, diabetes, intrauterine growth retardation, prolonged hospitalization, and preterm labor. The authors suggested that these findings may be a marker for intensity of prenatal care which would reduce urinary infections (Nawal and Senani, 2011). Additionally, their follow-up study examining women with asymptomatic urinary tract infection showed a clear association between asymptomatic bacteriuria and low birth weight and preterm delivery, a finding consistent with those of multiple previous investigations (Sheiner *et al.*, 2009).

2.3 Prevalence of asymptomatic bacteriuria in pregnant women in Africa

Prospective hospital-based study carried out in Ghana found a prevalence of asymptomatic bacteriuria of 9.5 % (Obirikorang *et al.*, 2012). Pregnant women in their second trimester from the study had the highest prevalence of significant bacteriuria with the age ranges between 30-34 years having the highest prevalence, (Obirikorang *et al.*, 2012). Another study conducted in Ibadan city, Nigeria reported a prevalence rate as high as 47.5 % (Okonko *et al.*, 2010). This high incidence of ASB may be attributed to factors such as poor housing, poor drainage systems, lack of proper personal and environmental hygiene, genuine population susceptibility since it is factors such as low socio-economic status, sexual intercourse, and pregnancy among others that are found among Nigerian women (Kolawole *et al.*, 2009). In studies done in the East African region, the prevalence of UTIs among pregnant women was found to be 15.5 % in Tanzania (Masinde *et al.*, 2009), 13.3% in Uganda (Andabati and Byamugisha, 2010) and 14 % in Sudan (Hamdan *et al.*, 2011). This was however lower than a study in Kenya (Ayoyi *et al.*, 2017) that found prevalence at 21.5 %.

2.4 Risk factors for UTI

2.4.1 Host factors

Pregnancy causes numerous changes in the woman's body. Hormonal and mechanical changes increase the risk of urinary stasis and the retrograde flow of urine from the bladder to the kidneys (Alvarez *et al.*, 2010). These changes, along with an already short urethra and difficulty with hygiene due to a distended pregnant belly, increase the frequency of urinary tract infections (UTIs) in pregnant women. In general, pregnant patients are considered immunocompromised UTI hosts because of the physiologic changes associated with pregnancy. These changes increase the risk of serious infectious complications from symptomatic and asymptomatic urinary infections (Alvarez *et al.*, 2010).

Bacteria can reach the bladder more easily in pregnant women. Host factors such as changes in normal vaginal flora due to pregnancy may also affect the risk of UTI (Kolawole *et al.*, 2009). Contamination of the lower third of the urethra with pathogens from the vagina and the rectum, incomplete emptying of their bladders and exposure of the female urogenital system to bacteria during sexual intercourse (Hamdan *et al.*, 2011).

Several patient social demographic factors are associated with an increased frequency of bacteriuria during pregnancy. Compared with high social-economic status patients, patients with low socio-economic status have a 5-fold increased incidence of asymptomatic bacteriuria (Hamdan *et al.*, 2011). Other risk factors for asymptomatic bacteriuria include diabetes mellitus, (Alvarez *et al.*, 2010), neurogenic bladder retention, history of backward flow of urine from the bladder into the kidneys (treated or untreated), previous renal transplantation, (Ghafari *et al.*, 2008) and a history of previous urinary tract infections.

Infections result from ascending colonization of the urinary tract, primarily by existing vaginal, perineal, and fecal flora (Celen *et al.*, 2011). Various maternal physiologic and anatomic factors predispose to ascending infection. Such factors include urinary retention caused by the weight of the enlarging uterus and urinary stasis due to progesterone-induced ureteral smooth muscle relaxation. Blood-volume expansion is accompanied by increases in the glomerular filtration rate and urinary output (Celen *et al.*, 2011).

Loss of ureteral tone in pregnant women combined with increased urinary tract volume results in urinary stasis, which can lead to dilatation of the ureters, renal pelvis, and calyces. Urinary stasis and the presence of backward flow of urine from the bladder into the kidneys predispose some pregnant women to upper urinary tract infections and acute nephritis (Coulthard *et al.*, 2010). Although the influence of progesterone causes relative dilatation of the ureters, ureteral tone progressively increases above the edge of the pelvic inlet during pregnancy. Glycosuria and an increase in levels of urinary amino acids during pregnancy are additional factors that lead to asymptomatic urinary tract infections. In many cases, glucose excretion increases during pregnancy over non-pregnant values of 100 mg/day. Glycosuria is due to impaired reabsorption by the collecting tubule and loop of Henle of the 5 % of the filtered glucose, which escapes proximal convoluted tubular reabsorption (Celen *et al.*, 2011).

2.4.2 Pathogenesis of ASB

Infections result from ascending colonization of the urinary tract, primarily by existing fecal flora due to contamination (Celen *et al.*, 2011). Various maternal physiologic and anatomic factors predispose to ascending infection by the bacteria, such factors include urinary retention caused by the weight of the enlarging uterus and urinary stasis due to progesterone-induced ureteral smooth muscle relaxation. The infecting bacteria remain in the urinary tract because the

organisms are able to adhere to the cells at the sites and avoid being eliminated by the flushing action of voided urine. These organisms possess highly specialized adhesins like colonization factor antigens, aggregative adherence fimbriae and pili that enable them to attach to host cells. Bacteria attached to the cells lining the urethra then travels to the bladder. Once in the bladder the pathogen absorbs food from the cells and multiplies and inflammation begins leading to cystitis, a primary complication in asymptomatic bacteriuria. The bacteria then invades the ureters.

Loss of ureteral tone in pregnant women combined with increased urinary tract volume result in urinary stasis, which can lead to dilatation of the ureters (Coulthard *et al.*, 2010). Urinary stasis and the presence of backward flow of urine from the bladder into the kidneys makes it easy for the bacteria to colonize the upper urinary tract and the kidneys which leads to acute nephritis. Primary morbidity is then caused by advanced pyelonephritis (Gupta *et al.*, 2011). Endotoxins that alter alveolar-capillary membrane permeability are produced; subsequently, pulmonary edema and acute respiratory distress syndrome develop (Hazhir, 2007). Other complications may include the following; perinephric cellulitis and abscess, septic shock, and renal dysfunction. Premature delivery leading to increased infant morbidity and mortality is common in pregnant mothers with ASB (Colgan *et al.*, 2006).

Untreated ASB is associated with low birth weight, prematurity, premature labour, hypertension, preeclampsia, maternal anemia, and amnionitis (Hill *et al.*, 2005). A retrospective population-based study by Mazor-Dray *et al* showed that asymptomatic urinary tract infection during pregnancy is independently associated with intrauterine growth restriction, preeclampsia, preterm delivery, and cesarean delivery (Mazor-Dray *et al.*, 2009)

2.4.3 Sexual intercourse

Sexually active women are at a greater risk of UTI than women who do not engage in sexual intercourse. Pregnant women who evidently engage in sexual activity increase the chances of bacterial contamination which leads to asymptomatic urinary tract infections. Having sexual intercourse may also cause asymptomatic UTIs in women because bacteria can be pushed into the urethra (Olaitan, 2006).

2.4.4 Past history of UTI

Two strongest predictors of ASB at prenatal care are identified to be antepartum UTI prior to prenatal care and a past urinary tract infection during pregnancy (Bérard *et al.*, 2011). *E. coli* is the most common cause of urinary tract ASB, accounting for approximately 80-90% of cases. It originates from fecal flora colonizing the periurethral area, causing an ascending infection. Other pathogens also encountered include urea-splitting bacteria, including *Proteus* and *Klebsiella* species (Nicolle, 2005).

2.4.4.1 *Escherichia coli*

Escherichia coli is a Gram-negative, facultative anaerobic and non-spore-forming bacterium. Cells are typically rod-shaped, and are about 2.0 micrometers (μm) long and 0.25–1.0 μm in diameter, with a cell volume of 0.6–0.7 μm^3 (Cheesbrough, 2006). *E. coli* stains Gram-negative because its cell wall is composed of a thin peptidoglycan layer and an outer membrane. During the staining process, *E. coli* picks up the color of the counterstain safranin and stains pink. The outer membrane surrounding the cell wall provides a barrier to certain antibiotics such that *E. coli* is not damaged by penicillin (Cowan *et al.*, 2004). Strains that possess flagella are motile. The flagella have a peritrichous arrangement (Cheesbrough, 2006).

Uropathogenic *E. coli* (UPEC) is one of the main causes of urinary tract infections. It is part of the normal flora in the gut and can be introduced in many ways. In particular for females, the direction of wiping after defecation (wiping back to front) can lead to fecal contamination of the urogenital orifices. Anal intercourse can also introduce this bacterium into the male urethra, and in switching from anal to vaginal intercourse, the male can also introduce UPEC to the female urogenital system (Frances *et al.*, 2007). Optimum growth of *E. coli* occurs at 37 °C, but some laboratory strains can multiply at temperatures of up to 49 °C (Celen *et al.*, 2011). Growth can be driven by aerobic or anaerobic respiration, using a large variety of redox pairs, including the oxidation of pyruvic acid, formic acid, hydrogen and amino acids and the reduction of substrates such as oxygen and nitrates. *E. coli* is classified as a facultative anaerobe. It uses oxygen when it is present and available. It can however, continue to grow in the absence of oxygen using fermentation or anaerobic respiration. The ability to be able to continue growing in the absence of oxygen is an advantage to bacteria because their survival is increased in environments where water predominates (Celen *et al.*, 2011).

2.4.4.2 *Klebsiella* species

Klebsiellae are non-motile, rod-shaped, gram-negative bacteria with a prominent polysaccharide capsule. This capsule encases the entire cell surface, accounts for the large appearance of the organism on gram stain, and provides resistance against many host defense mechanisms. The species is known to cause urinary tract infections (Cheesbrough, 2006).

2.4.4.3 *Proteus* species

Proteus is a genus of Gram-negative bacteria. *Proteus* bacilli are widely distributed in nature as saprophytes, being found in decomposing animal matter, sewage, manure soil, and human and

animal feces. They are opportunistic pathogens, commonly responsible for urinary asymptomatic infections.

2.4.5 Preeclampsia

The development of preeclampsia is associated with maternal UTI (asymptomatic bacteriuria or symptomatic infection) during pregnancy. A recent case-control study demonstrated an increased odds (1.22-fold) of preeclampsia in women with any UTI during pregnancy versus those without UTI (Minassian *et al.*, 2013). Furthermore, a retrospective review of the perinatal database at a major tertiary center revealed a UTI rate of 16.2 % in normotensive patients, but this increased to 27.3 % in women with mild preeclampsia and 35.9 % in women with severe preeclampsia. The underlying renal damage weakens patients' systemic defense mechanisms against ascending infection (Gupta and Trautner, 2013).

2.4.6 Menopause

The most common age range at which women experience menopause is 48-55 years while the average age of women at the time is 51 years. (Jitendra, 2011). Post-menopausal women are at higher risk of UTI than younger women (Griebing, 2007). This is because they lack estrogen, which is essential to maintain the normal acidity of vaginal fluid (Hazhir, 2007). This acidity is critical to permit the growth of *lactobacillus* in the normal vaginal flora, which acts as a natural host defense mechanism against symptomatic UTI. Restoration of the normal hormonal milieu in the vagina is not effective treatment for active urinary tract infections, but it may be useful for prevention (Griebing, 2007). Other urologic factors potentially associated with an increased risk of UTI in post-menopausal women include urinary incontinence and elevated volumes of post void residual urine. Antecedent antimicrobials used to treat other ailments 15 to 28 days before a

UTI may alter urogenital normal flora in favour of pathogen-dominated flora (Dethlefsen *et al.*, 2007).

2.4.7 Contraceptives

Contraceptive use affects the rate of UTI, which appears to be greater in women who use certain types of spermicides or diaphragm (Gupta and Trautner, 2013). Certain types of contraceptives can also increase the risk of UTIs. In particular, women who use diaphragms tend to develop UTIs. The spring-rim of the diaphragm can bruise the area near the bladder, making it susceptible to bacteria (Hazhir, 2007). Diaphragms push against the urethra and make it more difficult to completely empty the bladder. The urine that stays in the bladder is more likely to allow growth of bacteria and cause infections (Hooton and Thomas, 2010). Spermicidal foam or gel used with diaphragms, and spermicidal-coated condoms, also increase susceptibility to UTIs. Most spermicides contain nonoxynol-9, a chemical whose side effects are associated with increased UTI risk (Gupta and Trautner, 2013).

2.4.8 Urinary obstruction

A foreign body in the urinary system may act as an infection and may be associated with a current active infection (Ann and Kieran, 2010). Common examples include urinary calculi and indwelling catheters. Indwelling urinary catheters are associated with chronic bacterial colonization, which occurs in almost all patients after five to seven days (Jacobsen *et al.*, 2008). This colonization significantly increases the risk for symptomatic UTI. Catheter modifications with antibiotic and silver impregnation have been developed in an effort to decrease the rate of infection in patients with indwelling catheters (Johnson *et al.*, 2006).

2.5 Effect of Trimesters on UTI

A study done in Ghana by Obirikorang *et al.*, (2012) on 200 asymptomatic pregnant women showed that 73 (36.5 %) subjects sampled within the first trimester of pregnancy had 4 testing positive for significant bacteriuria which was (5.5 %) prevalence rate, 65 (32.5 %) who were in their second trimester of pregnancy had 10 testing positive for significant bacteriuria which was (15.4 %) prevalence rate and 62 (31.0 %) who were in their third trimester of pregnancy had 5 testing positive for significant bacteriuria which was (8.1 %) prevalence rate (Obirikorang *et al.*, 2012). The study further observed that pregnant women in the second trimester of pregnancy had the highest prevalence of asymptomatic bacteriuria followed by pregnant women in the third trimester of pregnancy (Obirikorang *et al.*, 2012). The prevalence of ASB in the trimesters in this study also agrees with (Okonko *et al.*, 2010) who also reported the higher prevalence of bacteriuria in the second trimester.

2.6 Low birth weight and pre-term delivery

Women with asymptomatic bacteriuria during pregnancy are more likely to deliver premature or low-birth-weight infants. These pregnant women also have a 20 to 30-fold increased risk of developing pyelonephritis; compared with women without bacteriuria (Hooton and Thomas, 2010). The odds ratio of having a medically indicated preterm delivery with asymptomatic bacteriuria compared to no bacteriuria was found to be 2.03 (Catherine, 2010). This association between asymptomatic bacteriuria and low birth weight and preterm birth is confounded by low socio-economic status (Smaill and Vazquez, 2009).

2.7 Diagnosis of UTI and treatment

Multiple bacilli between white blood cells in urinary microscopy are indicative of a urinary tract infection. In straightforward cases, a diagnosis may be made and treatment given based on symptoms alone without further laboratory confirmation (Cheesbrough, 2006). In complicated or questionable cases, it is useful to confirm the diagnosis via urinalysis, looking for the presence of urinary nitrites, proteins, white blood cells or leukocyte esterase (Mohammad *et al.*, 2002). Further identification of the bacteria isolates is carried out by biochemical tests which include; Indole test, Methyl Red test, Voges Proskauer test and Citrate utilization test. Urease test is also incorporated to identify the isolates. Each isolate gives a characteristic reaction which lead to its identification (Cheesbrough, 2006).

Urine culture is deemed positive if it shows a bacterial colony count of greater than or equal to 10^5 Cfu/ mL of a typical urinary tract organism (Coulthard *et al.*, 2010). Antibiotic sensitivity can also be tested with these cultures, making them useful in the selection of antibiotic treatment. Pregnant women are generally offered routine screening for asymptomatic bacteriuria by midstream urine culture early in pregnancy (Coulthard *et al.*, 2010). Differential diagnosis could also be considered in women with cervicitis, vaginitis and UTI symptoms, to detect other causes of infection e.g. *Chlamydia trachomatis*, *Neisseria gonorrhoea* and yeast (Sheiner *et al.*, 2009).

Asymptomatic bacteriuria is treated with oral antibiotics, particularly those that affect *E.coli* as this is the most common bacteria found in asymptomatic bacteriuria. A recent systematic review showed little conclusive proof of the superiority of any one antibiotic compared to another or longer versus shorter duration of treatment, (Krcmery *et al.*, 2001). Antibiotic therapy is rarely explored through randomized trials as antibiotic sensitivity for each patient is established at the

same time as culture is performed. The recommended antibiotics for pyelonephritis are a broad spectrum cephalosporin or a quinolone for 10-14 days (Kallen *et al.*, 2006). Cephalosporins are not known to be harmful during pregnancy but quinolones are not recommended in pregnancy since they have been reported to cause musculoskeletal problem, cartilage and bone damages in the growing foetus (Gupta *et al.*, 2011). If pyelonephritis is the first indication of infection, treatment, would be started before antibiotic susceptibility has been established. There is a possibility that potential damage from pyelonephritis could be prevented if antibiotics were given for asymptomatic bacteriuria rather than waiting for pyelonephritis to occur (Catherine, 2010). For those with recurrent infections, a prolonged course of daily antibiotics is effective (Krcmery *et al.*, 2001). In post-menopausal women, topical vaginal estrogen has been found to reduce recurrence (Raz, 2011).

2.8 Previous cases of UTI

The presentation of UTI varies according to whether the patient has asymptomatic bacteriuria, a lower urinary tract infection (cystitis) or an upper UTI (pyelonephritis) (Cai *et al.*, 2012). Burning with urination (dysuria) is the most significant symptom in pregnant women with symptomatic cystitis (Colgan and William, 2011). This burning is not experienced in asymptomatic bacteriuria therefore enabling undetected disease progression. Other symptoms include frequency and urgency of passing urine, suprapubic pain, and hematuria in the absence of systemic symptoms. The usual complaints of increased frequency, nocturia, and suprapubic pressure are not particularly helpful in diagnosis because most pregnant women experience these as a result of increased pressure from the growing uterus, expanding blood volume, increased glomerular filtration rate, and increased renal blood flow (Colgan and William, 2011). Pyelonephritis symptoms on presentation vary. They often include fever ($>38^{\circ}\text{C}$), shaking chills,

cost vertebral angle tenderness, anorexia, nausea, and vomiting. Right-side flank pain is more common than left-side or bilateral flank pain. Patients may also present with hypothermia (as low as 34°C). Lower UTI symptoms are common but not universal (Gupta *et al.*, 2011).

2.9 Clinical management

Clinical management for the routine care of healthy pregnant women is covered by NICE Guideline CG62, 10 antenatal visits are recommended for nulliparous and 7 for multiparous women. The first appointment should be fairly close to 10 weeks. The guideline states that “women should be offered routine screening for asymptomatic bacteriuria by midstream urine culture early in pregnancy”. Identification and treatment of asymptomatic bacteriuria reduces the risk of pyelonephritis (Lumbiganon *et al.*, 2010).

Intervention for patients identified with asymptomatic bacteriuria through early detection, is treatment with oral antibiotics (Gupta *et al.*, 2011). In this study, antibiotics assayed included cefuroxime, gentamycin, ofloxacin, nitrofurantion, amikacin, norfloxacin, aztreonam, cefotaxime, ceftriaxone, nalidixic acid, ciprofloxacin, cefixime and cefdinir. Their mode of action is best on Gram negative bacteria and targets enterobacteriaceae group of bacteria. The antibiotics range that were assayed included third generation cepharosporins (cefdinir, cefotaxime, cefixime and ceftriaxone) second generation cephalosporin (cefuroxime), quinolones (ciprofloxacin and norfloxacin), nitrofurantoin, aminoglycoside (gentamycin) and monobactam (aztreonan). The most appropriate antimicrobial agents for treating the organisms isolated from the urine were carefully selected from the antimicrobial susceptibility results.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study location

The study was conducted at Gatundu Level Four Hospital located in Kiambu County 40 km North- East of Nairobi, Kenya. The target area had a well-represented demographic profile of central Kenya population. It is a 107 bed hospital that serves as a referral center for all the dispensaries and health centers in the sub-county and neighboring areas, targeting a human population of 244,000 that include rural and urban habitants, hence a good representative of Kiambu County.



Figure 3.1: Map of Gatundu sub-county, Kiambu County

3.2 Study design

An analytical cross-sectional study was conducted at Gatundu Level Four Hospital between January and March 2016 with 150 asymptomatic pregnant women in the aged between 18-50 years. The age bracket included the older mothers who would experience late menopause due to varying dietary lifestyles and nutrition in the rural areas.

3.3 Target population

The target population was all pregnant women attending Gatundu Level Four Hospital between January and March 2016, with a requisition on antenatal care.

3.3.1 Inclusion criteria

All pregnant women without diabetes, pyrexia, history of UTI symptoms (dysuria, frequency and urgency) and history of antibiotic therapy in the previous two weeks attending Gatundu Level Four Hospital between January and March 2016, irrespective of parity and gestational age in the age group between 18-50 years were included in the study.

3.3.2 Exclusion criteria

Pregnant women with diabetes, pyrexia, history of UTI symptoms (dysuria, frequency and urgency), history of antibiotic therapy in the previous two weeks, or those who refuse to consent to the study were excluded from the study. Diabetes impair some parts of the immune response which lead to patients with diabetes getting complications like cystitis and pyelonephritis (Cruz *et al.*, 2017)

3.4 Sampling method

To randomize, one participant was picked out of every five that were serially registered and were in attendance of the clinic. Pregnant women in all the three trimesters within the age group of 18-50 were selected. A structured questionnaire was used to gather information related to UTI. Those who met the criteria were screened for bacteriuria in the laboratory.

3.5 Sample size

The number of patients that were recruited in the study were determined using Fisher's formula 1998.

$$N = \frac{Z^2 PQ}{d^2}$$

Where, N = estimated sample size.

Z = z-score, Z = 1.96 at 95% confidence interval.

P = 8 % Average prevalence from previous studies (Celen *et al.*, 2011).

Q = 1-P (1-0.08 = 0.92)

d = Degree of precision set at 0.05

$$N = \frac{(1.96)^2 \times (0.09)(0.91)}{0.05^2} = 125$$

Substituting the values in the above formula, a sample size of at least 125 asymptomatic pregnant women was recruited in the study. To take care of any confounding factors that could have led to disqualification of some members the sample size of 150 asymptomatic was selected.

3.6 Study variables

3.6.1 Dependent variable

Asymptomatic bacteriuria.

3.6.2 Independent variables

Maternal age, parity and gestational period.

3.7 Laboratory procedures

3.7.1 Sample collection

Urine was collected from consenting clients who met the criteria for the study. The urine samples were collected by standard mid-stream “clean catch” method whereby clients were instructed to wash their hands with soap and warm water and dry with sterile paper towel. A sterile wet sanitary towel was provided to clean the vaginal area as described by Karlowsky *et al.*, (2006). They were instructed to spread the labia with one hand, urinate in the toilet and then hold the urine flow, then collect the mid-stream into a wide mouthed sterile urine container which was provided. The urine sample was transported to the laboratory immediately.

3.7.2 Urine processing and culture

3.7.2.1 Urine microscopy

Urine microscopy was carried out to detect pus cells, bacteria both motile and non-motile, casts and epithelial cells. The urine samples were adequately mixed and 10mls centrifuged at 1000 rpm for 5 minutes. The supernatant fluid was poured completely by inverting the tube into a second one. The sediment was remixed by tapping the bottom of the tube. A drop of the well mixed sediment was transferred to a slide and covered with a cover glass. Examination of the preparation was done microscopically using both 10x (low power field) and 40x (high power field) objectives. Up to 10 white blood cells (WBC) per high power field (HPF), were reported as

few. A count of 11-40 WBC/HPF were reported as moderate and more than 40WBC/HPF were reported as many. Samples with 10 WBC or more per high power field were regarded as significant. Motile and non-motile bacteria, casts and epithelial cells were also reported (Cheesbrough, 2006).

3.7.2.2 Urine analysis

A dip stick test was carried out on uncentrifuged urine. A standard urine test strip from Uriscan was used. This stick comprised of different chemical pads or reagents which react by changing color when immersed in, and then removed from, a urine sample. The test was read within 60 to 120 seconds after dipping. The analysis included testing for the presence of proteins, nitrites and leucocyte esterase (Cheesbrough, 2006). In protein detection, the test area was impregnated with tetrabromophenol blue and buffered to an acid pH. In the presence of protein there was change in the colour of the indicator from light yellow to green-blue that indicated presence of albumin. Testing for nitrites depended on the conversion of nitrate to nitrite by action of Gram-negative bacteria in the urine. In the reaction pad, nitrite in urine reacts with p-arsanilic acid to form a diazonium compound. Diazonium compound in turn couple with 1, 2, 3, 4-tetrahydroquinolin-3-ol to produce a pink colour that is an indication of presence of bacteria in the urine. Test for leucocytes reveals the presence of granulocyte esterases. The esterases cleave a derivatized pyrazole amino acid ester to liberate derivertized hydrozyl pyrazol. The pyrazol then reacts with a diazonium salt to produce a purple colour which indicates presence of leucocytes in the urine (Henry, 2001).

3.7.2.3 Urine culture

In cases of positive nitrites, proteins and leucocytes, the urine was cultured aerobically on dried

plates of MacConkey's agar and Cystine Lactose Electrolyte Deficient (CLED) agar. The freshly collected clean catch specimen was mixed by rotating the container. A calibrated wireloop was used to inoculate 0.01ml of urine on agar. The inoculated agar plate was then incubated aerobically at 37° C overnight. Culture results were interpreted as being significant and insignificant, according to the standard criteria of colony count. Colonies were counted on CLED and multiplied by the loop volume to give bacteria numbers. A count of less than 10⁵ CFU/ mL was considered not significant while that of greater than or equal to 10⁵ CFU/ mL was reported as significant bacteriuria (Coulthard *et al.*, 2010).

3.7.3 Microbial identification

The organisms were identified by their growth characteristics, Gram staining of isolated bacteria and biochemical tests. The morphological characteristics observed colour, size and opaqueness of the colonies. On CLED agar, yellow (lactose fermenting) opaque colonies often with slightly deeper colored center indicated *E.coli*. Large mucoid yellow or yellow-white colonies was characteristic of *Klebsiella* species while translucent blue-grey colonies indicated *Proteus* species (Cheesbrough, 2006). Gram staining results indicated bacteria was Gram negative and showed spore forming and capsulated bacteria. The four major reactions in the biochemical testing included Indole test, Methyl Red test, Voges Proskauer test and Citrate utilization test. Urease test incorporated to identify the isolates. Each isolate gave a characteristic reaction which led to its identification (Cheesbrough, 2006).

3.7.3.1 Gram stain

The Gram staining reaction is used to help identify pathogens in cultures by their Gram reaction (Gram positive or Gram negative) and morphology. Dried smears were made on clean glass from colonies growing on the media. The smear was heat fixed by passing it over a flame. The

fixed smear was covered with crystal violet stain for 30-60 seconds and rapidly washed with clean water. The smear was covered with Lugol's iodine for 30-60 seconds and then washed off with clean water. The smear was decolorized rapidly with acetone and washed immediately. Neutral red stain was applied for 2 minutes and washed off with clean water. The smear was air dried and examined under a microscope (Cheesbrough, 2006). Gram positive bacteria stain dark purple with crystal violet and are not decolorized by acetone. Gram negative bacteria stain red because after staining with crystal violet they are decolorized by acetone and then take up the red counterstain by neutral red stain (Cowan *et al.*, 2004).

3.7.3.2 Biochemical tests

The indole, methyl red, Voges-Proskauer and citrate tests were carried out for differentiating the bacteria isolates alongside the urease test.

3.7.3.2.1 Indole test

The indole test was performed on bacterial isolates to determine the ability of the organism to convert tryptophan into the indole. This division is performed by a chain of a number of different intracellular enzymes. A positive result is shown by the presence of a red or red-violet color in the surface alcohol layer of the broth. A negative result appears yellow (Frances and Marshall, 2007).

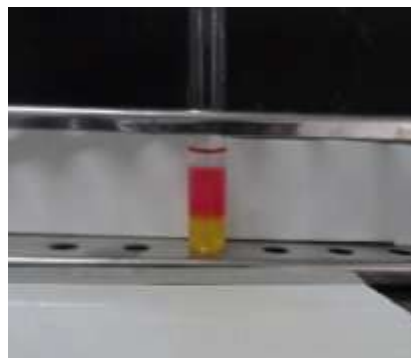


Plate 3.1: Indole production.

3.7.3.2.2 Methyl red test

The Methyl Red (MR) test was done to determine whether the microbe performed mixed acids fermentation when supplied with glucose. This was performed to differentiate various genera of enteric bacteria. When the culture medium turns red after addition of methyl red, because of a pH at or below 4.4 from the fermentation of glucose the test is positive. When the culture medium remains yellow, which occurs when less acid is produced from the fermentation of glucose, the test is negative (Cheesbrough, 2006).

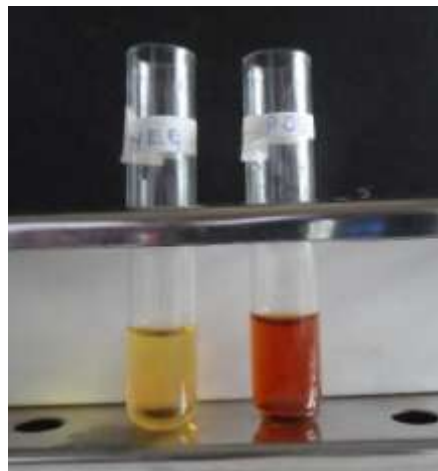


Plate 3.2: Methyl red test

3.7.3.2.3 Voges-Proskauer test

Voges - Proskeur test was performed on the isolates to detect pathways that result in the production of acetyl methyl carbinol, a neutral-reacting end product. A cherry red color indicates a positive result, while a yellow-brown color indicates a negative result (Cheesbrough, 2006).



Plate 3.3: Voges – Proskeur test.

3.7.3.2.4 Citrate utilization test

Citrate agar was used to test an organism's ability to utilize citrate as a source of energy. The medium contains citrate as the sole carbon source and inorganic ammonium salts as the sole source of nitrogen. When the bacteria metabolize citrate, the ammonium salts are broken down to ammonia, which increases alkalinity. The shift in pH turns the bromthymol blue indicator in the medium from green to blue (Cheesbrough, 2006).



Plate 3.4: Citrate utilization test.

3.7.3.2.5 Urease test

Urease test was performed on the isolates and the ones that possessed urease enzyme were identified. Urea is hydrolyzed with the release of ammonia and carbon dioxide. Some bacteria have a urease enzyme which is able to split urea in the presence of water to release ammonia and carbon dioxide. The ammonia combines with carbon dioxide and water to form ammonium carbonate which turns the medium alkaline, turning the indicator phenol red from its original orange yellow color to bright pink (Cheesbrough, 2006).

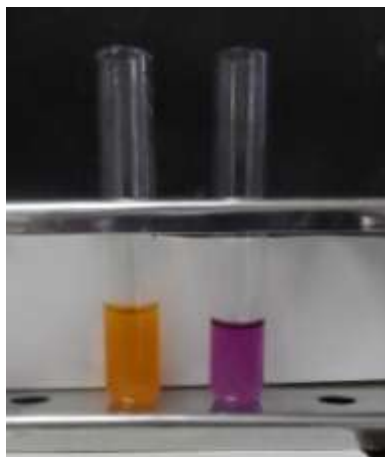


Plate 3.5: Urease test.

3.7.4 Antimicrobial susceptibility testing

Antimicrobial susceptibility was tested for the isolates by the modified Kirby-Bauer sensitivity testing technique on Mueller-Hinton agar (Cowan *et al.*, 2004). Using a sterile loop, a bacterial suspension of 1.0×10^8 CFU/ml was prepared by picking 3 to 5 colonies and emulsifying in 3-4 millilitres of sterile physiological saline. McFarland standard suspension was for comparison in preparing the recommended suspension. A sterile swab was then used to inoculate a plate of Mueller- Hinton Agar by streaking the surface of the medium (Cheesbrough, 2006). Appropriate antimicrobial multi-discs commercially available from CLAIRO[®] COMBI were placed on the medium using sterile forceps. The choice of antimicrobials depended largely on their availability in the local market and the type of the specimen. The plate was then inverted with the lid on and incubated aerobically at 37° C overnight (Cheesbrough, 2006). The isolates were tested against antibiotics available locally against Gram negative bacteria. The antibiotics used in the research were: cefuroxime (30 µg), gentamycin (10 µg), ofloxacin (5 µg), nitrofurantoin (300 µg), amikacin (30 µg), norfloxacin (10 µg), aztreonam (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), cefixime (5 µg), and cefdinir (5 µg) (Bell *et al.*, 2013).

3.7.5 Quality control

Culture media were sterilized by autoclaving at 150° C for 15 minutes. Autoclaving indicator tape was used and change in its colour indicated successful sterilization. Sterility testing on the media was carried out by incubating a sample of the culture media batch at 37° C overnight and checked for any growth of contaminants. Any media which showed growth of contaminants was discarded. The standard reference strains used for testing quality of culture media were

Staphylococcus aureus (ATCC 25923), *Escherichia coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853). McFarland standard comparable to a bacterial suspension of 1.0×10^8 CFU/ml was used as a reference to adjust the turbidity of bacterial suspensions inoculated on media to obtain a standardized antibiotic susceptibility testing. Degree of growth, size of colonies and other characteristics were examined for every control strain. Antibiotic susceptibility was conducted with antimicrobial paper discs that are commercially available. A calibrated ruler was used for the direct measurements in millimeters of diameters of zones of inhibition of bacterial growths on agar plates. The zone sizes of each control strains were within the zone limits of 20 millimeters or more as provided by the interpretative chart of Kirby- Bauer technique (Cheesbrough, 2006).

3.8 Ethical consideration

Permission to conduct the research was obtained from the medical superintendent Gatundu Level Four Hospital (Appendix VII) and the Kenyatta University Ethical Review Committee (Appendix IV) and a permit was granted by National Commission for Science, Technology and Innovation (Appendix VI).

3.9 Data processing and analysis

Data was organized in Microsoft Excel 2013 and processed using SPSS 20 statistical tool by invoking the analysis of descriptive statistics and focusing on frequencies. Pearson Chi-Square was carried out and p values calculated where $p > 0.05$ was considered statistically insignificant and $p < 0.05$ was considered significant.

CHAPTER FOUR

RESULTS

4.1 Demographic characteristics

A total of 120 consenting pregnant women who met the inclusion criteria were studied. Their age ranged between 18 and 50 years. Most of the women, (90.0 %) were married, while 10 % were single. The table also shows that majority (48.3 %) of the women had secondary education while 36.7 % and 15 % had primary and tertiary education respectively. The parity of the women ranged between zero and four, 32.5 % women were nulliparous, while 67.5 % belonged to the para 1-2 group. In gestational age in trimesters, forty nine (40.8 %) of the women fell in the 13-27 weeks' range. Thirty (30 %) had a gestational age between 1 and 12 weeks, while 29.2 % had gestational age between 28 to 42 weeks.

Table 4.1.Socio-demographic characteristics of study subjects.

Characteristics	Number (%)
Age (years)	
< 21	8(6.7)
21-25	86(71.7)
26-30	23(19.1)
31-35	3(2.5)
Marital status	
Single	12(10)
Married	108(90)
Education level	
Primary	44(36.7)
Secondary	58(48.3)
Tertiary	18(15.0)
Parity	
0	39(32.5)
1-2	75(62.5)
3-4	6(5.0)
Gestational age	
1-12 weeks	36(30.0)
13-27weeks	49(40.8)
28-42 weeks	35(29.2)

Of the 120 urine samples that were sampled in the study, 13 (10.8 %) had colony counts of 10^5 colonies/mL or more which was significant bacteriuria. Urine samples with less than colony counts of 10^5 colonies/mL were 14 (11.7 %) and were considered to have non-significant bacteriuria. A total of 93 (77.5 %) urine samples had no bacteria growth and were considered sterile.

4.2. Aetiological agents and prevalence

Result of culture	Number of cases	Prevalence (%)
Significant bacteriuria	13	10.8
Non – significant	14	11.7
Sterile	93	77.5
Total	120	100

Table 4.2. Number of samples analyzed.

Yellow opaque colonies which fermented lactose and had a slightly deeper center were obtained on CLED medium as shown in plate 4.1.



Plate 4.1: Bacteria growth on CLED medium.

Clear zones are shown in plate 4.2 indicating sensitivity of the drugs impregnated in the multi-discs for Gram- negative bacteria on Muller Hinton medium. Different antibiotic strengths are shown in micrograms.



Plate 4.2: Gram negative antibiotic multidisc used in this study.



Plate 4.3: Researcher carrying out antimicrobial susceptibility testing in this study.

Heavy growth of bacteria is shown on MacConkey agar after 24 hours aerobic incubation at 37⁰ C MacConkey agar is a selective and differential culture medium for bacteria designed to selectively isolate Gram-negative and enteric (normally found in the intestinal tract) bacilli and differentiate them based on lactose fermentation. Sodium chloride is added to MacConkey medium to allow spreading or swarming of *Proteus* species. The pink colonies growing on the medium are lactose fermenters.



Plate 4.4: Heavy bacterial growth on MacConkey medium.

E.coli stained Gram negative while its reaction to biochemical tests, Methyl red and indole were positive. Vorges- Proskeur, citrate and urease tests results all turned negative. *Proteus* species stained Gram positive while biochemical reactions, Methyl red, citrate, urease and indole tests were positive. Vorges- Proskeur test result was negative. *Klebsiella* species stained Gram negative while biochemical reactions, Citrate and urease tests' results were positive. Vorges- Proskeur, methyl red and indole tests results were negative.

Table 4.3: Biochemical characteristics and Gram-staining reaction of the isolates.

Characteristics	Results		
	<i>E.coli</i>	<i>Proteus</i> species	<i>Klebsiella</i> species
Gram stain	Gram –ve rods	Gram –ve rods	Gram –ve rods
Indole test	+	+	–
Methyl red test	+	+	–
V-P test	–	–	–
Citrate test	–	+	+
Urease test	–	+	+

4.2.1 Bacterial isolates from the infected women

The prevalence of asymptomatic bacteriuria amongst the study participants based on colony count from urine culture was 10.8 %. Out of the 120 asymptomatic pregnant women who were sampled, 13 (10.8 %) had colony counts of 10^5 colonies/mL or more and were considered positive while a total of 14 (11.7 %) pregnant women had colony counts less than 10^5 colonies/mL which was non-significant bacteriuria. Those who had no bacteria growth in their urine samples were 93 (77.5 %).

4.2.2 Asymptomatic bacteriuria based on isolates

Based on the bacteria species, *E. coli* was most prevalent isolated organism at 69.2 % followed by *Proteus* species 23.1 % and *Klebsiella* species 7.7 %.

Table 4.4: Incidences of ASB in pregnant women sampled based on the isolates.

Isolate	Frequency	Percentage
<i>Escherichia coli</i>	9	69.2
<i>Proteus</i> species	3	23.1
<i>Klebsiella</i> species	1	7.7
Total (n)	13	100

4.3 Antimicrobial profiles

Most bacterial isolates (38.5 %) were sensitive to two drugs and three drugs while 23.1 % were sensitive to four drugs.

Table 4.5: Sensitivity of combined isolates to drugs.

Susceptibility	Frequency	Percentage (%)
Sensitive any 2 drugs	5	38.5
Sensitive any 3 drugs	5	38.5
Sensitive any 4 drugs	3	23.1
Total (n)	13	100

Sensitivity to *E. coli*, the most prevalent isolate was highest (100 %) to Ceftriaxone and Cefotaxime followed by Ofloxacin (88.9 %) and Cefuroxime (55.6 %). Ampicillin and Erythromycin. Resistance by *Escherichia coli* isolates to antimicrobials was highest (100 %) to Amikacin, Cefdinir and Cefixime followed by Ceftazidime, Aztreonam and Ciprofloxacin all at 88.9 %.

Table 4.6: Antimicrobial susceptibility of *E. coli* species.

Antimicrobial	Sensitive isolates		Resistant isolates	
	No.	Percentage	No.	Percentage
Norfloxacin	2	22.2	7	77.8
Ceftazidime	1	11.1	8	88.9
Amikacin	0	0	9	100
Nitrofurantoin	2	22.2	7	77.8
Cefotaxime	9	100	0	0

	Sensitive isolates		Resistant isolates	
Antimicrobial	No.	Percentage	No.	Percentage
Cefdinir	0	0	9	100
Ofloxacin	8	88.9	1	11.1
Gentamycin	4	44.4	5	55.6
Norfloxacin	2	66.7	1	33.3
Nalidixic Acid	0	0	9	100
Ceftazidime	1	33.3	2	66.7
Aztreonam	1	11.1	8	88.9
Amikacin	0	0	3	100
Cefixime	0	0	9	100
Nitrofurantoin	2	66.7	1	33.3
Ciprofloxacin	1	11.1	8	88.9
Cefotaxime	3	100	0	0

Sensitivity to *Proteus* species, the second most prevalent isolate was highest (100 %) to Aztreonam, Cefotaxime, Cefixime followed by Norfloxacin and Ofloxacin (66.7 %) and Gentamycin (33.3 %). Resistance by *Proteus* isolates to antimicrobials was highest (100 %) to Amikacin and Nalidixic acid.

Table 4.7: Antimicrobial susceptibility of *Proteus* species.

Cefdinir	2	66.7	1	33.3
Ofloxacin	2	66.7	1	33.3
Gentamycin	1	33.3	2	66.7
Nalidixic Acid	0	0	3	100
Aztreonam	3	100	0	0
Ceftazidime	3	100	0	0
Cefixime	3	100	0	0
Amikacin	0	0	1	100
Ciprofloxacin	2	66.7	1	33.3
Nitrofurantoin	1	100	0	0

Sensitivity of the assayed antimicrobials to the two *Klebsiella* isolates was highest (100 %) to nitrofurantoin, cefotaxime, cefdinir, ofloxacin, and gentamycin. Resistance by *Klebsiella* isolates to antimicrobials was highest (100 %) to norfloxacin, ceftazidime, amikacin, nalidixic acid and aztreonam.

Table 4.8: Antimicrobial susceptibility of *Klebsiella* species.

Cefotaxime	1	100	0	0
Cefdinir	1	100	0	0
Ofloxacin	1	100	0	0
Gentamycin	1	100	0	0
NalidixicAcid	0	0	1	100
Aztreonam	0	0	1	100
Cefixime	0	0	1	100
Ciprofloxacin	0	0	1	100

4.4 Risk factors associated with ASB

4.4.1 Maternal age in years

Among the thirteen pregnant women who had significant bacteriuria, the highest incidence was observed within 31-35 (33.3 %) years' age group followed by the 26-30 (27.3 %) years' age group. Under 20 (25.0 %) years' age group followed and lastly 21-25 (4.6 %) years' age group. Maternal age ($p= 0.005$) was therefore significantly associated with bacterial count $\geq 1 \times 10^5$ CFU/ml of urine.

Table 4.9: Incidences of ASB in the study population based on maternal age.

Maternal age-group in years	No. of Pregnant women (%)	No. with bacterial count $\geq 1 \times 10^5$ CFU/ml of urine. (%)	No. without bacterial count $\geq 1 \times 10^5$ CFU/ml of urine. (%)	p value
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≤20	8 (6.7)	2 (25.0)	6(75.0)	
21-25	87 (72.5)	4 (4.6)	83 (95.4)	0.005
26-30	22 (18.3)	6 (27.3)	16 (72.7)	
31-35	3 (2.5)	1 (33.3)	2 (66.7)	
Total(n)	120 (100.0)	13(10.8)	107 (89.2)	

4.4.2 Incidence of ASB based on parity

The pregnant women were classified by their number of pregnancy. Out of the 120 pregnant women 39 (32.5 %) were in their first pregnancy and 2 (1.7 %) testing positive for bacteriuria. In their second pregnancy were 43 (35.8 %) women and 3 (7.0 %) tested positive bacteriuria. Out of 32 (26.7 %) pregnant women in their third pregnancy and 6 (5.0 %) in their fourth, 5 (4.2 %) and 3 (2.5 %) tested positive for significant bacteriuria. Parity (p= 0.007) was significantly associated with bacterial count $\geq 1 \times 10^5$ CFU/ml of urine.

Table 4.10: Incidences of ASB in the study population based on parity.

Maternal age-group	No. of Pregnant	No. with bacterial count $\geq 1 \times 10^5$ CFU/ml of urine.	No. without bacterial count $\geq 1 \times 10^5$ CFU/ml	p value
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in years	women (%)	(%)	of urine. (%)	
1	39(32.5)	2(1.7)	37 (30.8)	
2	43 (35.8)	3(2.5)	40 (33.3)	0.007
3	32(26.7)	5 (4.2)	27 (22.5)	
4	6 (5.0)	3(2.5)	3(2.5)	
Total (n)	120(100)	13(10.8)	107(89.2)	

4.4.3 Gestational age in trimesters

Amongst the 120 asymptomatic pregnant women sampled, 36 (30.0 %) were within the first trimester of pregnancy with 1 (2.8 %) positive for significant bacteriuria as shown in Table 13. In the second trimester of pregnancy there were 49 (40.8 %) respondents and 5 (10.2 %) tested positive for significant bacteriuria while 35 (29.2 %) were in the third trimester of pregnancy with 7 (20.0 %) testing positive for significant bacteriuria. Gestational age in trimesters ($p=0.286$) was not significantly associated with bacterial count $\geq 1 \times 10^5$ CFU/ml of urine.

Table 4.11: Incidences of ASB in the study population based on trimester.

	No. with bacterial count	No. without bacterial count
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Trimester	No. of pregnant women (%)	$\geq 1 \times 10^5$ CFU/ml of urine. (%)	$\geq 1 \times 10^5$ CFU/ml of urine. (%)	p value
1	36(30.0)	1 (2.8)	35(97.2)	0.286
2	49(40.8)	5 (10.2)	44(89.8)	
3	35(29.2)	7 (20.0)	28(80.0)	
Total (n)	120(100.0)	13(10.8)	107(89.2)	

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

In this study, the most common bacterial isolates from midstream urine samples of asymptomatic pregnant women were *E. coli* (69.2 %) followed by *Proteus* species (23.1 %) and lastly *Klebsiella* species (7.7 %). In studies done elsewhere, Amala and Nwokah, (2015) and Obirikorang *et al.* (2012) in separate studies also reported *E. coli* as being the commonest pathogen responsible for bacteriuria which is consistent with the findings of this study. Different studies done by Delzell and Lefevre, (2000), Colgan *et al.*, (2006), Turpin *et al.*, (2007), Hernandez *et al.*, (2007) and Hazhir (2007) have all shown *E. coli* as the most common isolate.

High risk of acquiring *E. coli* UTI could be due to the anatomical and the physiological changes a woman that occur during pregnancy and the fact that in the rectal and vaginal area, *E. coli* is the most common micro-organism as also reported by Mohammad *et al.* (2002). Shanson, (2009) and Delzell and Lefevre, (2000) reported that this significant finding could be due to the fact urinary stasis is common in pregnancy and since most *E. coli* strains and other bacteria prefer that environment, they are able to persist and cause UTI. Akram *et al.*, (2007) reported that the anatomical proximity of the anal and urogenital opening in females makes it possible for fecal contamination of the urinary tract from commensals of the bowel of which *E. coli* is a typical example. In this study , prevalence of *Proteus* species (23.1 %) and lastly *Klebsiella* species (7.7 %) was similar to other reports which suggest that other Gram- negative bacteria other than *E. coli* ,are achieving more prominence as aetiological agents of asymptomatic UTI than previously reported (Akinola *et al.*, 2012 and Kolawole *et al.*, 2009).

The antibiotic susceptibility patterns from this study showed that isolate strains of *Escherichia coli*, *Proteus* and *Klebsiella* species were all susceptible to Cefataxime and Ofloxacin. The

choice of antibiotic for treatment should however be based on urine culture, stage of gestation and the characteristics of the antibiotic (Celen *et al.*, 2011). In this study, antibiotic resistance to *E. coli* was observed in cefdinir and amikacin antibiotics. Resistance to *Proteus* species was observed in amikacin and nalidixic acid while in *Klebsiella* species resistance was to norfloxacin, ceftazidime and amikacin. Studies on the antibiotic sensitivity of microbial agents in cases of ASB in pregnancy report considerable prevalence of isolates resistant to two or more antibiotics, which is particularly so in Gram-negative isolates. Such resistance patterns are typical in *Enterobacteriaceae*, and *E. coli*, *Klebsiella* and *Proteus* species belong to this group (Sojatha and Nawani, 2014). It is possible that this set of antibiotics are available from chemist shops even without a doctor's prescription and that may cause antibiotic resistance. Thus, the antibiotic sensitivity patterns should be used in determining therapy as inappropriate therapy has been responsible for recurrences of asymptomatic bacteriuria with development of acute pyelonephritis later (Gilstrap and Ramin, 2001).

The prevalence of asymptomatic bacteriuria among the pregnant women in this study was 10.8 %. Ayoyi *et al.*, (2017) reported a prevalence of 21.5 % in Kenya which is higher than what is reported this study. In comparison varying prevalence rates of asymptomatic bacteriuria in pregnant women have been reported elsewhere. In Ghana, Turpin *et al.*, (2007) reported a prevalence of 7.3 % while Hazhir (2007) reported a prevalence rate of 6.1 % in Teheran. Hernandez *et al.*, (2007) reported a prevalence of 8.4 % in Mexico and Tadesse *et al.* (2007) reported a prevalence of 9.8 % in Ethiopia. As low as 3.3 % prevalence rates (Iraqian and Moghadas, 2009) and 3.7 % (Mobasheri *et al.*, 2002) have been reported in Jordan. Rates as high as 40 % (Akinola *et al.*, 2012) have also been reported. There is therefore an implication that about 10.8 % of pregnant women from this study are at risk of developing acute episodes and

complications of UTI during pregnancy if they are not properly treated.

In this study, the highest prevalence rate (33.3 %) was found among subjects aged 31 to 35 years. This was in contrast to the findings of Turpin *et al.*, (2007) whose highest rate of 13 % was reported in the age group 35 to 39 years. This may be due to hormonal and mechanical changes in this age group which increase the risk of urinary stasis and the retrograde flow of urine from the bladder to the kidneys (Alvarez *et al.*, 2010). These changes, along with an already short urethra and difficulty with hygiene due to a distended pregnant belly, may have increased the frequency of urinary tract infections (UTIs) in this age group. Mothers aged 21-25 had a significantly lower incidence of ASB at 4.6 % compared to other group categories. Turpin *et al.*, (2007) reported a high prevalence of asymptomatic bacteriuria in pregnant women aged 35 to 39 years. Alghalibi *et al.*, (2007) reported a higher prevalence of UTI in pregnant women aged 21 to 25 years in their study. The observed trend of bacteriuria in this study and reports from other studies by Hamdan *et al.*, (2011) on asymptomatic pregnant women in Sudan, Masinde *et al.*, (2009) on asymptomatic pregnant women in Tanzania and Turpin *et al.*, (2007) among asymptomatic pregnant women in Ghana, show the age range of 26 to 35 years serving as a risk group for developing ASB in pregnant women. This may be due to sexual activity which could increase the chances of bacterial contamination of female urethra (Colgan *et al.*, 2006). From findings of this study, maternal age ($p=0.005$) was significantly associated bacterial count $\geq 1 \times 10^5$ CFU/ml of urine.

In this study, pregnant women with at least an existing child (multiparous) had 9.2 % prevalence rate of asymptomatic bacteriuria compared to nulliparous women who had a 1.7 % prevalence

rate. Parity ($p=0.007$) was significantly associated with bacterial count $\geq 1 \times 10^5$ CFU/ml of urine which

is in agreement to the findings of El Sheikh *et al.* (2005) in their study on pregnant women attending a clinic in a teaching hospital in Sudan which reported increased incidence of asymptomatic bacteriuria in multigravidae than in primigravidae. This finding, they suggested, to be attributable to the trauma caused during birth hence the increased prevalence of bacteriuria multigravidae study of Olusanya *et al.*, (2003).

This study further observed that pregnant women in the third trimester of pregnancy had the highest prevalence of asymptomatic bacteriuria followed by pregnant women in the second trimester of pregnancy which is in agreement with the findings of Alghalibi *et al.*, (2007) who in a study on bacterial urinary tract infection among pregnant women in Yemen reported the second and third trimesters of pregnancy as being associated with the highest prevalence of UTI. It is however contrary to the findings of Turpin *et al.*, (2007) who reported a high percentage of asymptomatic bacteriuria in the first and early second trimesters of pregnancy and attributed it to pregnant women reporting late at the antenatal clinic for booking during these periods. The high prevalence of ASB among mothers in the third trimester in this study could be attributed to mothers starting their ANC clinic late and diagnosis of ASB not carried out early, as well as urine stasis and ureteral dilatation. However, results in this study shows gestational age in trimesters ($p=0.286$) is not significantly associated with bacterial count $\geq 1 \times 10^5$ CFU/ml of urine. These results are however concordant with results reported elsewhere (Shahira *et al.*, 2007, Masinde *et al.*, 2009 and Shanson, 2009).

5.2 Conclusions

- The isolated causative agents for the ASB were *E. coli*, *Proteus* and *Klebsiella* species with *E. coli* having the highest frequency of isolation followed by *Proteus* then *Klebsiella*.
- All the bacterial isolates were sensitive to cefotaxime, and had varying sensitivity to the other drugs. However, they were 100 % resistant to amikacin and nalidixic acid.
- From this study, the prevalence of ASB among pregnant women in Gatundu Level Four hospital was 10.8 %. Maternal age and parity lead to variation in occurrence of bacteriuria. Gestational age in trimesters do not lead to variation in occurrence of bacteriuria.

5.3 Recommendations

- Expectant mothers should be screened for asymptomatic bacteriuria periodically in every trimester of the gestation period.
- Routine urine culture tests should be carried out for all antenatal women to detect asymptomatic bacteriuria in every trimester.
- In view of changing patterns of bacterial resistance to common drugs, the importance of educating physicians on use of antibiotics accordingly to provide empirical therapy, is important.

5.4 Recommendation for future work

It would be important to conduct studies to come up with universal antibiogram that can show which antibiotics the isolates in the country are sensitive to bearing in mind the pregnancy status and safety to the mother and unborn child to facilitate management of this condition. A larger sample size is recommended to have a larger representation.

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APPENDICES

Appendix I: Questionnaire

Screening for Asymptomatic Bacteriuria among Pregnant Women

Dear respondent,

This questionnaire is constructed for assessing information relevant in screening for asymptomatic bacteriuria in pregnant women at Gatundu Level Four Hospital Laboratory.

Information obtained will be used for statistical purposes only and will be confidential.

Laboratory Number Date of data collection

PART I: Socio-demographic factors

Please tick the appropriate answer in the box.

1. What is your age?

a) 21-25 b) 26-30 c) 31-35 d) 36-40

2. What is your marital status?

a) Single b) Married c) Divorced/separated d) Widowed

3. What is the highest level of education attainment?

a) Primary education b) secondary education c) Higher education

d) No education e) others (please specify).....

4. What is your occupation?

a) Civil servant b) Private sector c) self-employed

d) Un-employed e) others (please specify).....

PART II: Awareness

Please provide short answers to the following questions

6. What do you know about urinary infection?

.....

7. Where have you learned about urinary infection?

.....

8. What do you think causes urinary infections?

.....

9. What symptoms do you think urinary infections cause?

.....

10. Who do you think urinary infection affects?

.....

11. What would you do if you had a urinary infection?

.....

12. What effect do you think it has on pregnant women?

.....

13. What would worry you about having a urinary infection?

.....

14. How could the population become more educated about urinary infection?

.....

PART III: Healthcare

Please tick the appropriate answer in the box

15. What is the trimester of your pregnancy (trimester: period of three months)?
- a) First trimester (1-3months) b) Second trimester (4-6 months)
- c) Third trimester (7-9 months)
16. What is the number of your pregnancy?
- a) 1st pregnancy b) 2nd pregnancy c) 3rd and above
17. Have you ever had a urinary infection before?
- a) Yes b) No
18. Are you currently experiencing the following symptoms; a strong, persistent urge to urinate, a burning sensation when urinating, passing frequent, small amounts of urine, vaginal discharge and itchy sensation?
- a) Yes b) No
19. Have you taken any antibiotics in the last one week?
- a) Yes b) No
20. Have you ever been diagnosed with diabetes?
- a) Yes b) No
21. Have you ever been diagnosed with high blood pressure?
- a) Yes b) No

Appendix II: Research Consent Form

Informed consent

My name is Ephantus Kiama Muturi. I am a MSc. student from Kenyatta University. I am conducting a study on **“Prevalence of Asymptomatic Bacteriuria and Antimicrobial Susceptibility Profile among Women Attending Antenatal Clinic of Gatundu Hospital in Kiambu County, Kenya.”** The information will be used by the Ministry of Medical Services and Ministry of Public Health and Sanitation to improve access and quality for screening of asymptomatic bacteriuria among pregnant women in this hospital as well as other regions of Kenya

Procedures to be followed

Participation in this study will require that I ask you some questions and collect information about you, including information about age, gravidity, parity and age of gestation, previous antibiotic intake, previous history of urinary tract infection, personal history of hypertension and diabetes. I will record information from you in a questionnaire.

You will also be asked to provide a sample specimen of clean catch urine 5ml after being given instruction on how to collect the urine in a sterile, wide mouthed container. The sample will be taken during your scheduled clinic visit. The results of the study of your sample shall be used for research purpose. In addition you will be able to know whether you have an asymptomatic bacterial infection and get advised accordingly.

You have the right to refuse participation in this study. You will get the same care and medical treatment whether you agree to join the study or not and the decision will not change the care you will receive from the clinic today or that you will get from any other clinic at any time. Please remember that participation in the study is voluntary. You may ask questions related to the study at any time.

You may refuse to respond to any questions and you may stop an interview at any time. You may also stop being in the study at any time without any consequences to the services you receive from this clinic or any other organization now and in future.

Discomforts and risks

Some of the questions you will be asked are on intimate subject and may be embarrassing or make you uncomfortable. If this happens, you may refuse to answer these questions if you choose. You may also stop the interview at any time. The interview may add approximately half an hour to the time you wait before you receive your routine services. There will be no risks involved in this study.

Benefits

If you participate in this study you will help us learn how to provide effective screening services that can improve the health of women and reduce the risk of preterm deliveries. You will also benefit for being screened for asymptomatic bacterial infections.

Reward

You will not be paid participate in this study.

Confidentiality

The interview will be conducted in a private setting within the clinic. Your name will be recorded on the questionnaire. Questionnaires will be kept in a locked cabinet for safe keeping at Kenyatta University. Everything will be kept private.

Contact information.

If you have any questions you may contact Dr. Scholastica G. Mathenge on 0722936884 or Dr. Wachuka G. Njoroge on 0722737669 or the Kenyatta University Ethical Review Committee Secretariat on chairman.kuerc@ku.ac.ke, secretary.kuerc@ku.ac.ke, ercku2008@gmail.com.

Participant's Statement.

The above information regarding my participation in the study is clear to me. I have been given to ask questions and my questions have been answered to my satisfaction. My participation in this study is entirely voluntary. I understand that my records will be kept private and that I can leave the study any time. I understand that I will get the same care and medical treatment whether I decide to leave the study or not and any decision will not change the care I will receive from the clinic today or that I will get from any other clinic at any other time.

Code of Participant

 / /2016

Signature or Thumbprint

Date

Investigator's statement

I, the undersigned, have explained to the volunteer in a language she understands, the procedures to be followed in the study and the risks and benefits involved

Name of interviewer.....

____/____/2016

Interviewer's signature

Date

Appendix III: Kenyatta University Graduate School research approval



KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: kubps@yahoo.com
dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 810901 Ext. 57530

Internal Memo

FROM: Dean, Graduate School **DATE:** 28th September
TO: Mr. Ephantus K. Muturi **REF:** P150/CE/28083/13
C/o Medical Laboratory Science Dept.
Kenya University
SUBJECT: APPROVAL OF RESEARCH PROPOSAL

We acknowledge the receipt of your revised Research Proposal as per recommendations raised by the Graduate School Board of 16th September, 2015 entitled “**Prevalence of Asymptomatic Bacteria and Antimicrobial Susceptibility among Pregnant Women Attending Antenatal Clinic of Gatundu Level Four Hospital in Kiambu County**”.

You may now proceed with your Data collection, subject to clearance with the Director General, National Commission for Science, Technology & Innovation.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed supervision Tracking Forms per semester. The form has been developed to replace the progress Report Forms. The Supervision Tracking Forms are available at the University’s Website under Graduate School webpage downloads.

Thank you.

REUBEN MURIUKI
FOR: DEAN, GRADUATE SCHOOL
c.c. Chairman, Medical Laboratory Science Dept.

Supervisor

1. Dr. Scholastica Mathenge
Medical Laboratory Science Dept.
Kenya University
2. Dr. Wachuka G. Njoroge
Medical Laboratory Science Dept.
Kenya University

RM/cao

Committed to Creativity, Excellence & Self-Reliance

Appendix IV: Kenyatta University Ethics Review Committee Approval



KENYATTA UNIVERSITY
ETHICS REVIEW COMMITTEE

Email: chairman.kuerc@ku.ac.ke
secretary.kuerc@ku.ac.ke
erc@ku.ac.ke
Website: www.ku.ac.ke

P. O. Box 43844 - 00100 Nairobi
Tel: 8710901/12
Fax: 8711242/8711575

Our Ref: KU/R/COMM/51/601

Date: 18th January, 2016

Ephantus Kiama Muturi,
Kenyatta University,
P.O Box 43844,
Nairobi

Dear Kiama,

RE APPLICATION NUMBER PKU/423/1 392 – “PREVALENCE OF ASYMPTOMATIC BACTERIA AND ANTIMICROBIAL SUSCEPTIBILITY AMONG PREGNANT WOMEN ATTENDING ANTENATAL CLINIC OF GATUNDU LEVEL FOUR HOSPITAL IN KIAMBU COUNTY, KENYA”.

1. IDENTIFICATION OF PROTOCOL

The application before the committee is with a research topic “Prevalence of asymptomatic bacteria and antimicrobial susceptibility among pregnant women attending antenatal clinic of Gatundu level Four Hospital in Kiambu County, Kenya” received on 2nd October, 2015.

2. APPLICANT

Ephantus Kiama Muturi, Department of Medical laboratory Sciences

3. STUDY SITE

Gatundu Level Four Hospital in Kiambu County, Kenya.

4. DECISION

The committee has considered the research protocol in accordance with the Kenyatta University Research Policy (section 7.2.1.3) and the Kenyatta University Ethics Review Committee Guidelines AND APPROVED that the research may proceed for a period of ONE year from 18th January, 2016.

5. ADVICE/CONDITIONS

- i. Progress reports are submitted to the KU-ERC every six months and a full report is submitted at the end of the study.
- ii. Serious and unexpected adverse events related to the conduct of the study are reported to this board immediately they occur.
- iii. Notify the Kenyatta University Ethics Committee of any amendments to the protocol.
- iv. Submit an electronic copy of the protocol to KUERC.

When replying, kindly quote the application number above.

If you accept the decision reached and advice and conditions given please sign in the space provided below and return to KU-ERC a copy of the letter.



DR. TITUS KAHIGA
CHAIRMAN ETHICS REVIEW COMMITTEE

I EPHANTUS KIAMA MUTURI accept the advice given and will fulfill the conditions therein.

Signature: [Signature] Dated this day of 01/02/ 2016.
cc. Vice-Chancellor
DVC-Research Innovation and outreach

Appendix V: Research authorization by NACOSTI



**NATIONAL COMMISSION FOR SCIENCE,
TECHNOLOGY AND INNOVATION**

Telephone: +254-20-2213471,
2241349, 310571, 2219420
Fax: +254-20-318245, 318249
Email: secretary@nacosti.go.ke
Website: www.nacosti.go.ke
When replying please quote

9th Floor, Utalii House
Uhuru Highway
P.O. Box 30623-00100
NAIROBI-KENYA

Ref. No. **NACOSTI/P/16/74770/9597**

Date:

4th April, 2016

Ephantus Kiama Mutuiru
Kenyatta University
P.O. Box 43844-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on "*Prevalence of asymptomatic bacteriuria and antimicrobial susceptibility among pregnant women attending antenatal clinic of Gatundu Level Four Hospital in Kiambu County,*" I am pleased to inform you that you have been authorized to undertake research in **Kiambu County** for a period ending **1st April, 2017.**

You are advised to report to **the County Commissioner, the County Director of Education and the County Coordinator of Health, Kiambu County** before embarking on the research project.

On completion of the research, you are expected to submit **two hard copies and one soft copy in pdf** of the research report/thesis to our office.


BONIFACE WANYAMA
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Kiambu County.

The County Director of Education
Kiambu County.




National Commission for Science, Technology and Innovation is ISO 9001:2008 Certified


Appendix VI: Research permit by NACOSTI

THIS IS TO CERTIFY THAT:
MR. EPHANTUS KIAMA MUTUIRI
of KENYATTA UNIVERSITY, 0-200
Nairobi, has been permitted to conduct
research in Kiambu County


on the topic: PREVALENCE OF
ASYMPTOMATIC BACTERIURIA AND
ANTIMICROBIAL SUSCEPTIBILITY AMONG
PREGNANT WOMEN ATTENDING
ANTENATAL CLINIC OF GATUNDU LEVEL
FOUR HOSPITAL IN KIAMBU COUNTY

for the period ending:
1st April, 2017


Applicant's
Signature



Permit No : NACOSTI/P/16/74770/9597
Date Of Issue : 4th April, 2016
Fee Received :ksh 1000


Director General
National Commission for Science,
Technology & Innovation

Appendix VII: Approval by Gatundu Level Four Hospital



MINISTRY OF HEALTH

Telegram: "MEDICAL" Gatundu
Telephone: Thika 067-74024
When replying please quote
Email Address :

GATUNDU LEVEL IV HOSPITAL
P.O BOX 84 - 01030
GATUNDU
gatundul4h@gmail.com

Ref:GTD/GEN/37/VOL.1/62

17th March 2016

EPHANTUS KIAMA MUTURI
P150/CE/28083/2013
KENYATTA UNIVERSITY
DEP. OF MEDICAL LABORATORY SCIENCES

RE: COLLECTION OF DATA

Your application to conduct research on "*Prevalence of Asymptomatic Bacteriuria and Antimicrobial Susceptibility among Pregnant Women attending Antenatal Clinic of Gatundu Level IV Hospital in Kiambu County*" in this institution has been granted.

During the entire period of your research, you will be reporting to the MCH in Charge. He will support you access any information that may be relevant for the successful undertaking of the research.

Finally, you are expected to adhere to all the regulations relating to confidentiality of patient information, ethics in research as well as all norms regarding conduct in a Public Health Institution.

Wishing you a successful research.

KARIUKI J.G.
F: MEDICAL SUPERINTENDENT
GATUNDU LEVEL 4 HOSPITAL

