PREVALENCE, ANTIMICROBIAL SUSCEPTIBILITY PROFILES AND GENOTYPIC CHARACTERIZATION OF ISOLATES OBTAINED FROM URINE SAMPLES OF PREGNANT WOMEN ATTENDING ANTENATAL CLINIC AT PUMWANI MATERNITY HOSPITAL, KENYA

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Prevalence, Antimicrobial Susceptibility Profiles and Genotypic Characterization of Isolates obtained from Urine Samples of Pregnant Women Attending Antenatal Clinic at Pumwani Maternity Hospital, Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University

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This thesis has been submitted for examination with our approval as the university supervisors

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DEDICATION

To my children

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OPERATIONAL DEFINITIONS

Bacteriuria	Presence of bacteria in urine
Cervicitis	Inflammation of the cervix
Cystitis	An inflammatory process of the urinary bladder, typically caused by bacterial infection
Pyelonephritis	Urinary tract infection involving the kidney
Pyuria	Presence of white blood cells in urine
Urethritis	Inflammation of the urethra
UTI	Urinary Tract Infection - the inflammatory response of urothelium to bacterial invasion
Vaginitis	Inflammation of the vagina

LIST OF ABBREVIATIONS

AMC	Amoxicillin-clavulanic acid
AMP	Ampicillin
ANC	Antenatal care
ASB	Asymptomatic Bacteriuria
ATCC	American Type Culture Collection
bla	Beta lactamase gene
CAZ	Ceftazidime
CFU	Colony Forming Units.
CHL	Chloramphenicol
CI	Confidence Interval
CLSI	Clinical and Laboratory Standards Institute
CIP	Ciprofloxacin
CMR	Center for Microbiology Research

CMTs	Complex mutant TEMs
CRO	Ceftriaxone
CTX	Cefotaxime
CTX-M	CefoTaXimases ‘Munich’
CLED	Cystein Lactose Electrolyte Deficient Agar.
DNA	Deoxyribonucleic Acid
DOX	Doxycycline
E. coli	Escherichia coli
ERY	Erythromycin
ESBL	Extended spectrum Beta Lactamases.
ERIC	Enterobacterial repetitive intergenic consensus
FEP	Cefepime
FOX	Cefoxitin
GBS	Group B streptococci.
GEN	Gentamicin

IPM	Imipinem
IUGR	Intrauterine Growth Retardation.
IRT	Inhibitor-resistant TEM
Kb	Kilobase
KEMRI	Kenya Medical Research Institute.
LBW	Low Birth Weight.
LNZ	Linezolid
MDR	Multidrug Resistance
MSU	Mid-stream urine
MRSA	Methicillin Resistant *Staphylococcus aureus*
NSBLs	Narrow spectrum β-lactamases
NAL	Nalidixic acid
NCCLS	National Committee for Clinical Laboratory Standards
NIT	Nitrofurantoin
OFX	Ofloxacin

PAmpC	Plasmid-mediated ampicillin β-lactamases
PCR	Polymerase Chain Reaction
PET	Pre-eclamptic Toxaaemia
PFGE	Pulsed-Field Gel Electrophoresis
PMNs	Polymorphonuclear cells
PTL	Preterm Labour
PROM	Premature Rapture of Membrane
QDA	Quinupristin
RAPD	Random amplified polymorphic DNA
REP	Repetitive extragenic palindromic
RFLP	Restriction fragment length polymorphism
S.E.R.U	Scientific and Ethics Review Unit
SHV	Sulfhydryl Variable Enzymes
STR	Streptomycin
SXT	Sulfamethoxazole - Trimethoprim

spp	Species.
TEM	Temoneira Enzymes
TBE	Tris-Borate - EDTA
UTI	Urinary tract infection.
WHO	World Health Organization

ABSTRACT

Urinary Tract Infections (UTIs) during pregnancy are among the most common infections worldwide and can lead to poor perinatal and maternal outcomes. A cross-sectional study was conducted among 210 pregnant women attending antenatal clinic at Pumwani Maternity hospital. Ethical clearance was granted by KEMRI/ Scientific and Ethics Review Unit and Administrators of Pumwani Maternity Hospital. Their social-demographic profiles were obtained using a structured questionnaire. Cultures were done from midstream urine and antimicrobial susceptibility testing determined using the disc diffusion test. The genetic diversity of the isolates was determined using molecular techniques. The overall prevalence of UTI was 15.7%. *E. coli* was the most predominant UTI organism at (44.5%). Barrier methods of contraceptives, material of undergarment and frequency of changing the undergarments were found to contribute significantly to the acquisition of UTI ($P<0.05$). Almost a half (over 49%) of all Gram-negative organisms showed resistance prevalence against third generation Cephalosporins, Fluoroquinolones, Sulfamethoxazole-Trimethoprim, Cefoxitin, Nitrofurantoin and Amoxicillin-clavulanic acid. Gram-positive strains were susceptible to Amoxicillin-clavulanic acid, Nitrofurantoin, Linezolid and Ofloxacin. The bla_{TEM} gene was the most prevalent at 58.1%, $bla_{\text{CTX-M}}$ at 45.2% bla_{SHV} at 29%, and bla_{OXA} gene at 22.6%. Genetic analysis revealed diverse genotypic characteristics of the isolates clustered in small groupings of highly similar strains. In conclusion, UTIs are still a burden to maternal and child health and can therefore vastly contribute to poor perinatal and maternal outcomes. There is a need to educate women, on factors that could predispose them to UTIs especially during pregnancy. Our data suggest a serious resistance trend among UTI strains and more research should be done to slow down this trend.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Urinary tract infection (UTI) is the inflammatory response of urothelium to bacterial invasion, usually associated with bacteriuria and pyuria (Hooton *et al.,* 2010). In developing countries, UTIs are among the most common health problems affecting many women in their reproductive ages. Pregnant women are more susceptible to UTIs due to a combination of hormonal, mechanical and physiological changes contributing to significant changes in urinary tract creating a profound impact on the acquisition and natural history of bacteriuria in pregnancy (Aseel *et al.,* 2011). Factors such as history of recurrent urinary tract infection, diabetes, low social economic status, increasing maternal age, multiparity, level of education, occupation and anatomical abnormalities of the urinary tract have also been associated with a two fold increase in bacteriuria during pregnancy (Schnarr *et al*., 2008), but the risk factors associated with UTIs in Africa remains poorly investigated.

Globally, the prevalence of UTIs in pregnancy ranges between 13%-33% with symptomatic bacteriuria occurring in 1% -18% while asymptomatic cases are noted in 2%-10% of women (Agersew *et al.,* 2012). The prevalence has remained constant and most of the recent studies, including those from developing countries, report almost similar rates (Schnarr *et al*.,, 2008). The most common agent implicated in symptomatic and asymptomatic bacteriuria is *Escherichia coli* that is responsible for 70–80% of the infections (Masinde *et al*., 2009). Other microorganisms include *Staphylococcus* spp, *Klebsiella pneumoniae*, *Proteus* spp, *Pseudomonas aeruginosa*, *Enterococcus* spp and *Acinetobater* (Delzell *et al*., 2000). Previous studies indicate that UTI among pregnant mothers in Kenya range from 10%-19% (Gilbert *et al.,* 2013). However, most of these studies focus on selected bacterial pathogens and the larger spectrum of bacterial

etiologic agents remains unknown. Furthermore, the socio-demographic and lifestyle factors associated with UTIs among pregnant women in Kenya remain uninvestigated.

Despite screening for and treatment of UTI becoming a standard of obstetric care, there are challenges in prompt diagnosis. In many hospitals in developing countries such as Kenya, routine urine culture is not carried out even for antenatal mothers. Currently, most patients are treated empirically without culture and antimicrobial susceptibility testing (AST) and treatment is therefore based on empiric guidelines that are rarely updated (Kose *et al.,* 2007). Even where UTI tests are done, only dipstick analysis and direct wet microscopy of urine are used. The tests are important for their rapidity and low costs but they have poor positive and negative predictive values to detect bacteriuria particularly in asymptomatic persons (Nicolle *et al.,* 2005). The overreliance on these methods and absence of culture and susceptibility testing have partially led to under-diagnosis of UTIs and this may be fueling the rising cases of treatment failure. In standard urine culture, a colony count of 10^5 CFU/ml is usually considered significant for infection (Nicolle *et al.,* 2005), but there's missing data regarding the phenotypic characteristics of isolates whose counts fall below this threshold. Currently, such counts are regarded as contaminants. It is therefore not known if such counts represent a receding infection or an infection that is establishing. Little is known about their identity, their genotypic and phenotypic characteristics. In this study, we compared the results of antimicrobial resistance phenotypes and genotypes from UTI cases and those normally regarded as contaminants.

Increasing rates of resistance among bacterial uropathogens has caused growing concern in both developed and developing countries over the last decade (Dromigny *et al.,* 2005). Extended spectrum β-lactamase (ESBL) producing bacteria are among the most problematic multi-drug resistanct (MDR) bacteria world wide and are increasingly causing UTIs both in hospitals and outpatients making infections difficult to treat (Romero *et al.,* 2005). Delay in the detection and reporting of ESBL production by Gram-negative bacteria is associated with prolonged hospital stay, increased morbidity,

mortality and healthcare costs (Kollef, 2003). In Kenya, there is lack of enforcement in antibiotic policy and issuance of prescriptions without culture and susceptibility data. It's therefore important to have routine monitoring of ESBL producing clinical isolates by the microbiology laboratory as infections caused by these isolates are not efficiently treated by most antimicrobials.

Results of this study provides critical data to care givers and health planners regarding diagnosis, common etiological agents, their genetic reletadness and probable treatment options with regard to antimicrobial resistance. This study also identified associated risk factors for UTI amongst pregnant women attending antenatal clinic at Pumwani Maternity Hospital (the largest antenatal clinic in Kenya).

1.2 Problem Statement

Many pregnant women living within Nairobi County and its environs seek essential maternal health-care from Pumwani. Despite being the largest obstetric referral hospital in East Africa, the prevalence of UTIs and the larger spectrum of bacterial aetiologic agents remains largely unknown. Furthermore, the socio-demographic, clinical and lifestyle factors associated with UTIs among pregnant women in Kenya remain poorly investigated. The over-reliance on dipstick analysis and direct urine microscopic examination for screening and diagnosis of UTI has partially led to under- diagnosis of UTIs. The antimicrobial resistance prevalence and trends are not known since culture is rarely done, and this may be fueling the rising cases of treatment failure. Globally, the menace of antimicrobial resistance is one of the world's most crucial public health problems. The emergence and spread of ESBL producing bacteria among uropathogens has complicated treatment options for UTI, yet the ESBL prevalence and genetic diversity of UTI isolates from pregnant women in Pumwani is not known UTI poses a threat to pregnancy and there's need to screen all pregnant women by urine culture and treatment should be guided by sensitivity reports.

1.3 Justification

Urinary tract infection is a condition that has the potential to affect pregnancy outcome if not addressed early enough. UTIs complicate upto 30% of pregnancies and also accounts for the majority of the antepartum admissions to the obstetric wards. The economic, clinical and social burden associated with these infections is significant.

While an extensive published literature regarding UTIs during pregnancy is available from other African countries such as Tanzania, Uganda, Ethiopia and Ghana (Masinde *et al*., 2009; Andabati *et al*., 2010; Agersew *et al*., 2012; Obirikorang *et al*., 2012), there is limited published data regarding this disease among pregnant women in Kenya. Results of this study provide critical data to care givers and health planners in Pumwani maternity hospital regarding diagnosis, common aetiological agents and probable treatment options with regard to antimicrobial resistance. The data generated reveals some of the social-demographic, clinical and lifestyle factors associated with UTIs as well as the genetic diversity of the associated isolates.

1.4 Objectives

1.4.1 General objective.

To determine the prevalence, antimicrobial susceptibility profiles and genotypic characterization of isolates obtained from urine samples of pregnant women attending antenatal clinic at Pumwani maternity hospital, Kenya.

1.4.2 Specific Objectives

1. To determine the prevalence of UTI among pregnant women in Pumwani maternity hospital.
2. To determine the association between social-demographic factors and UTIs in pregnancy in the study population.

3. To determine the antimicrobial susceptibility profiles among UTIs strains from this study population.
4. To determine the prevalence of selected β-lactamase phenotypes and resistance markers that pose the greatest clinical and chemotherapeutic challenges among isolates obtained from this study population.
5. To determine the genetic diversity of bacterial pathogens recovered from UTI cases.
6. To determine whether isolates whose counts fall below the UTI threshold are related to isolates from confirmed UTI cases based on resistance phenotypes and genetic clustering.

1.5 Research Questions

1. What is the prevalence of UTI among pregnant women in Pumwani maternity hospital
2. What is the association between social-demographic factors and UTIs in pregnancy in the study population?
3. What antimicrobial susceptibility profiles are common among UTIs strains from this population?
4. What is the prevalence of selected β-lactamase phenotypes and resistance markers that pose the greatest clinical and chemotherapeutic challenges among isolates obtained from this population
5. What is the genetic diversity of bacterial pathogens recovered from UTI cases?
6. Are isolates recovered from urine that don't meet the threshold for UTI related to those from confirmed UTI cases based on resistance phenotypes and genetic clustering?

CHAPTER TWO

LITERATURE REVIEW

2.1 Urinary Tract Infection.

Urinary tract infection (UTI) is an infection caused by the presence and growth of microorganisms within the urinary tract. It is perhaps the most common bacterial infection of mankind (Ebie *et al*., 2001). The prevalence and incidence of urinary tract infection is higher in women than in men, possibly due to several clinical factors including anatomic differences, hormonal effects, and behavior patterns (Griebling, 2007). UTIs can either be asymptomatic or symptomatic. Asymptomatic bacteriuria is the presence of actively multiplying bacteria within the urinary tract without the symptoms of an acute urinary infection. Symptomatic UTIs are divided into lower tract (acute cystitis) or upper tract (acute pyelonephritis) infections. Cystitis is significant bacteriuria with associated bladder mucosal invasion, while pyelonephritis is significant bacteriuria associated with inflammation of the renal parenchyma, pelvis and calices (Connolly *et al*., 1999).

UTIs result from the interaction between the uropathogens and the host. Infection is determined in part by the bacterial virulence factors, size of the inoculum, and the inadequacy of host defense mechanisms. Pathogenesis of UTIs begins when uropathogens colonizes site outside urinary tract then spread up the urinary tract to the bladder. The bacteria attach to the mucosa using adhesins such as P fimbriae and colonizes the bladder overcoming the host defense mechanisms. The pathogen establishes a population of $>10^5$ bacteria/ml and subsequently the urine flow is affected (Momtaz *et al.,* 2013). The established bacteria produce hemolysin which in turn lyses the cells of urinary tract and later invades the superficial cells forming intracellular bacterial communities (IBCs). These bacteria further produce capsular polysaccharide that contributes to biofilm formation (Naveen *et al.,* 2005). The virulence factors

possessed by these bacteria not only localize the site of infection but also stimulate inflammatory response as indicated in **Figure 2.1.**

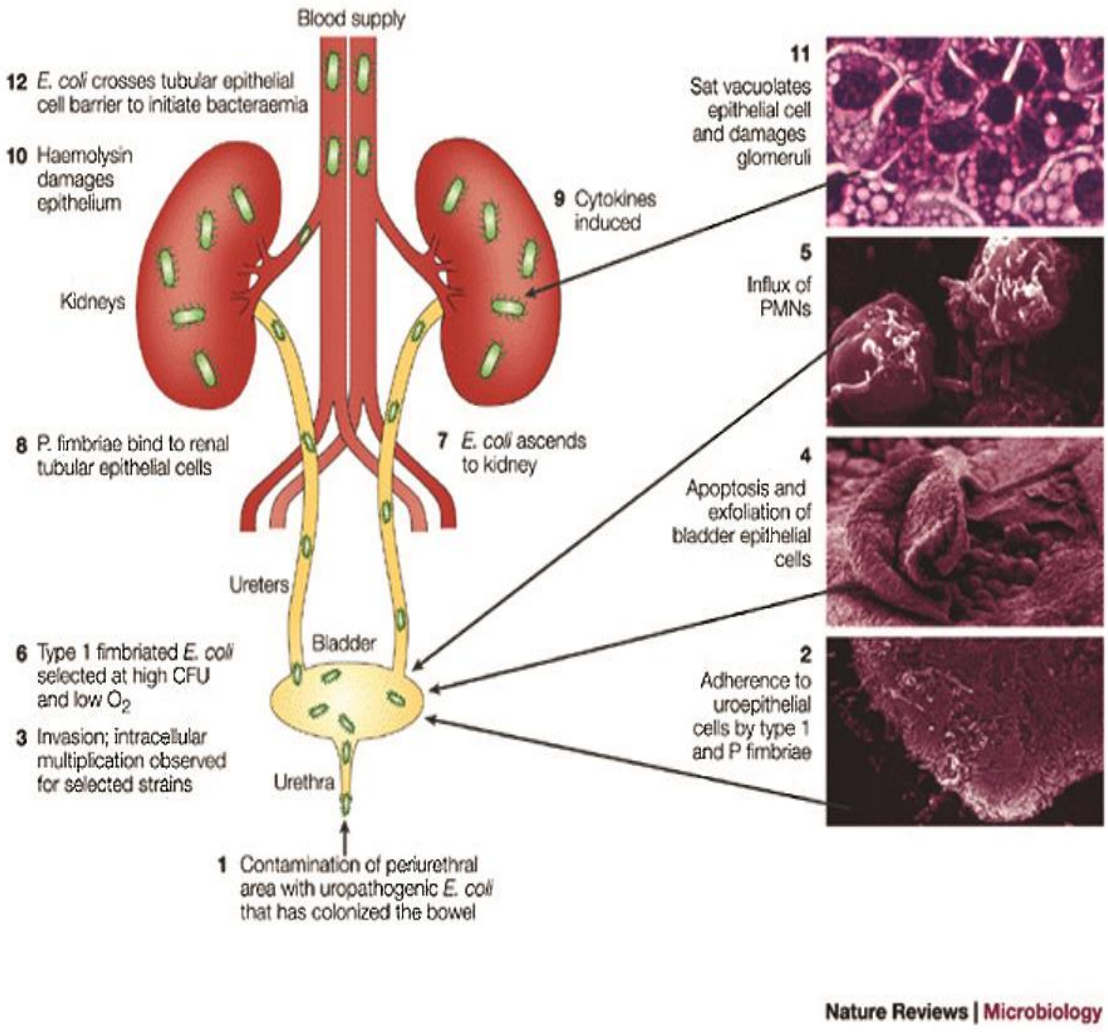

Figure 2.1: Schematic diagram illustrating pathogenesis stages of UTIs (Kaper et al., 2004)

2.2 Pregnancy and Urinary Tract Infections

It is estimated that one in three women of child-bearing age will have a UTI (Duarte *et al.*, 2008). Even though the incidence of bacteriuria in pregnant women is similar to that in non-pregnant women, the incidence of acute pyelonephritis in pregnant women with bacteriuria is significantly increased (Schnarr *et al*., 2008). Therefore, screening for and treatment of UTIs has become a standard of obstetric care and most antenatal guidelines include routine screening for asymptomatic bacteriuria (Nicolle *et al.*, 2005; MOH, 2007). Pregnant women are at an increased risk of UTIs beginning from the 6^{th} week and peaking during weeks 22 to 24 (Delzell *et al*., 2000) with the incidence of kidney infection increasing during the third trimester of pregnancy (Dafnis *et al.*, 1992). The economic and social burden of UTIs during pregnancy is significant, not to mention the stigma associated with these infections, (Griebling, 2011). The healthcare direct and indirect costs associated with UTIs in terms of bed occupation, staff and supply are also large and include substantial out-of-pocket expenses for the patients (Griebling, 2011).

Several factors tend to increase the risk of UTI during pregnancy. The renal pelvis and the ureters dilate as early as the eighth week of pregnancy opening up the route for bacteria to ascend up (Jeyabalan *et al*., 2007) displacing the bladder superiorly and anteriorly. The enlarging uterus causes mechanical compression affecting the bladder volume as well as blockage of the free urine drainage. Due to abdominal distension, it's difficult for pregnant women to clean their genitalia well and expel urine as quickly and easily as she does normally contributing to occurrence of UTIs during pregnancy (Dafnis *et al*., 1992). Smooth muscle relaxation due to progesterone results in reduced peristalsis of the ureters and decreased bladder tone increasing urinary stasis that is associated with the infection (Masinde *et al.*, 2009; Jeyabalan *et al*., 2007)

Hormonal effects particularly during pregnancy and post-menopausal period intensifies the risk for UTIs due to lack of estrogen. Estrogen loss thins the walls of the urinary tract and decreases its ability to resist bacteria. It also reduces certain immune factors in

the vagina that help block uropathogens from adhering to vaginal cells (Harvey, 2009). *Lactobacillus* a normal flora, is essential in maintaining the normal acidity of vaginal fluid thus acting as a natural host defense mechanism against symptomatic UTIs (Griebling, 2011). Differences in urine osmolarity and pH in addition to pregnancy-induced glycosuria and aminoaciduria may facilitate bacterial growth (Jeyabalan *et al*., 2007; Schnarr *et al*., 2008).

Several studies have associated UTIs during pregnancy with the risk of adverse perinatal and maternal outcomes. However, other studies failed to prove such associations (Gilstrap III *et al.*, 1985;Reddy *et al*., 1985). Inconsistencies in these results could be due to selection bias, low statistical power and inadequate control for potential confounders. Despite the inconsistencies, the general consensus is that UTIs can lead to complications, such as low-birth-weight infants, premature delivery and sometimes stillbirth (Lee *et al*., 2008).

2.2.1 UTIs and perinatal outcomes

The relationship between perinatal outcomes and UTIs has been studied for many years (Mittal *et al*., 2005, Duarte *et al*., 2008). From a global health perspective, UTIs are one of the most common and potentially preventable causes of early preterm births. Intrauterine infections are thought to be accountable for up to 50% of preterm births of not more than 28 weeks of gestation, with both neonatal mortality and morbidity being high (Simmons *et al*., 2010). Other recognized perinatal complications of UTIs, include premature rupture of membranes, intrauterine growth retardation, cerebral palsy, mental retardation and perinatal deaths (Polivka *et al.,* 1997; McDermott *et al.*, 2001; Duarte *et al.,* 2008). Other cases involving periventricular leukomalacia and fetal septicemia have been reported resulting from transplacental transfer of cytokines originating from maternal UTIs (Spinillo *et al*., 1998;Oda *et al*., 2008). Study by Turiani. (2009) estimated that 27% of preterm deliveries are associated with pre-existing UTIs and that women with pyelonephritis had prevalence for low birth weight (birth weight less than

2500g) infants of 15%. Microbiological analysis of the genital-urinary tract of women with premature labor or preterm rupture of membranes, showed that UTI is a risk factor for perinatal morbidity and fetal death. Data originating from 52 cases of neonatal sepsis showed that UTIs were present in 63% of the cases (Ananthakrishnan *et al*., 2009).

There has also been a hypothesis suggesting that UTI during pregnancy is linked to child developmental delay and mental retardation (Broman, 1987). One study found a 30% increase in the risk for cognitive delay in children whose mothers had UTI during gestation, as compared to children whose mothers were not infected (McDermott *et al.*, 2001). Thus, these results support the association between UTI during pregnancy and cognitive delay and emphasize the importance of the rapid diagnosis and treatment. However, the multifactorial nature of these outcomes makes the determination of etiology difficult, and no firm consensus has been reached on this matter (Mittal *et al*., 2005).

2.2.2 UTIs and maternal outcomes

The maternal complications of UTI results from tissue damage caused by bacterial endotoxins, more so in pyelonephritis (Neal, 2008). The most dramatic maternal complication associated with UTIs is bacteremia and septic shock, induced by persistent pyelonephritis (Mittal *et al*,, 2005). Endotoxin-mediated damage involves reduced peripheral vascular resistance and changes in cardiovascular output. When endotoxins are released into the maternal circulation, a cascade response of pro-inflammatory cytokines, histamine, and bradykinins is triggered, leading to the more serious complications such as septic shock, disseminated intravascular coagulation (DIC), respiratory insufficiency, and adult respiratory distress syndrome (Galajdova, 2010). Other maternal complications attributed to UTIs during pregnancy are hypertension and preeclampsia (Conde-Agudelo *et al*., 2008; Rustvelt *et al*., 2008), chorioamnionitis and endometritis (Delzell *et al*., 2000). The association between UTIs during pregnancy and

preeclampsia is consistent throughout studies performed over the last years, and is equally present in diverse settings worldwide (Conde-Agudelo *et al*., 2008).

2.3 Prevalence of UTIs in Pregnancy

Globally, the prevalence of UTI in pregnancy ranges from 13%-33% with symptomatic bacteriuria occurring in 1%-18% and asymptomatic accounting for 2%-10% (Agersew *et al*., 2012), **Figure 2.2**. The prevalence of asymptomatic bacteriuria has remained constant and most of the recent studies, including those from developing countries reporting similar rates (Schnarr *et al*., 2008; Duarte *et al.,* 2008; Bahadi *et al.,* 2010). In Africa, studies reported the prevalence of UTIs to be 9.5% in Ghana (Obirikorang *et al.,* 2012), 15.5 % in Tanzania (Masinde *et al.,* 2009), 13.3% in Uganda (Andabati *et al*., 2010), 18.8% in Ethiopia (Tadesse *et al*., 2014). In Kenya, studies by Wamalwa *et al.* (2013) reported a prevalence of 14.2% while studies by Nabbugodi *et al.* (2015) reported a prevalence of 26.7%.

The prevalence of pyelonephritis during pregnancy ranges from 0·5 to 2% but was reported to be as high as 4·9% in indigenous communities in Australia (Bookallil *et al*., 2005). Despite the relatively low prevalence it is estimated that 20% to 40% of pregnant women with asymptomatic bacteriuria develop pyelonephritis later in gestation (Jolley *et al*., 2010). A prospective hospital based study carried out in Ghana found a prevalence of 9.5% and those in their second trimester had the highest prevalence of significant bacteriuria with women aged between 30-34 years having the highest prevalence (Obirikorang *et al.,* 2009). Studies that have been done globally and regionally have not found any statistical significance between UTIs and trimester although women in their second and third trimesters have been found to have the highest prevalence of UTI, (Obirikorang *et al.,* 2009; Masinde *et al*., 2009; Wamalwa *et al.,* 2013). This could be attributed to the fact that many pregnant women report at the antenatal clinic for booking during these periods.

Another study conducted in Ibadan city, Nigeria reported a prevalence rate of as high as 47.5% (Onkokoet *et al.,* 2009). This high prevalence could be attributed to factors such as poor housing, poor drainage systems, lack of proper personal and environmental hygiene, and population susceptibility factors such as low socio- economic status, sexual intercourse, and pregnancy among others that are common among Nigerian women ((Kolawole *et al.,* 2009)

Variations in prevalence rates from one country to another and among regions of the same country might be due to difference in risk factors within geographical areas and population characteristics such as age, parity, socio-economic status, sexual activity (multiple sexual partners) and health care during pregnancy. The prevalence is also significantly increased in women presenting with certain pre-existing medical conditions, such as sickle cell anaemia, immunosuppressed states, spinal cord injuries and psychiatric illnesses. Other risk factors for the development of cystitis and pyelonephritis during pregnancy include history of *Chlamydia trachomatis* infection and illicit drug use (Goins *et al*., 2010).

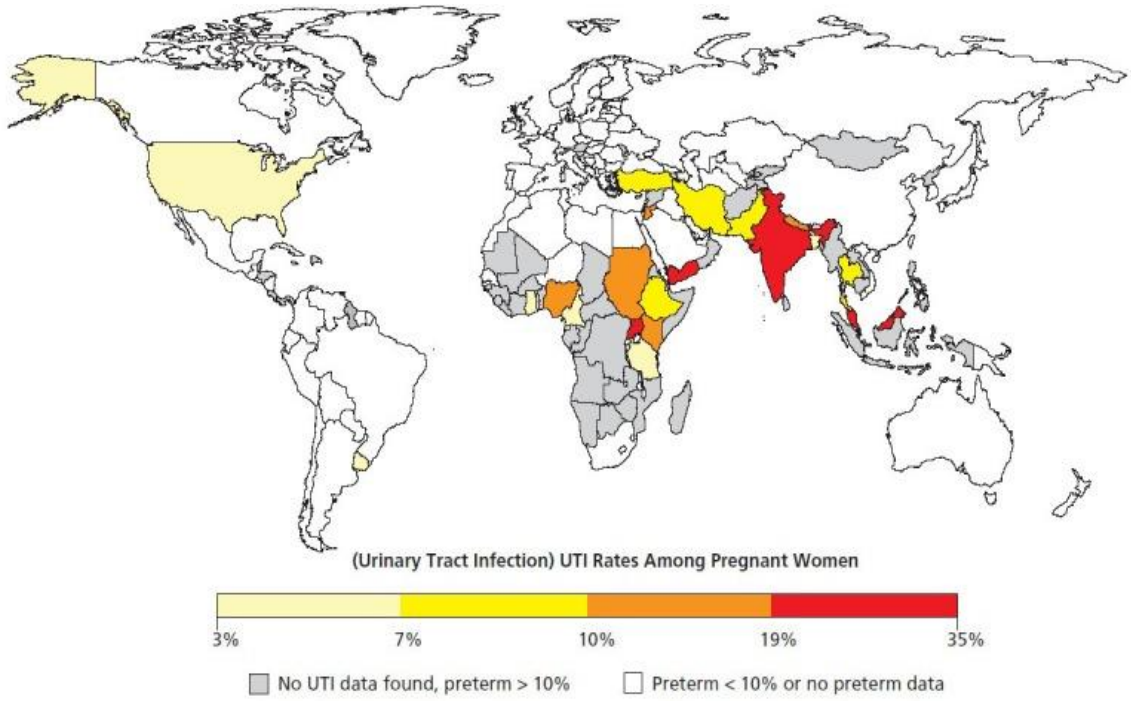

Figure 2. 1: Global prevalence of UTI among pregnant women (Gilbert *et al*., 2013).

Worldwide rates of urinary tract infection (UTI) in pregnant women ranging from moderate (yellow) to high (red). Countries with >10% preterm birth rates but without available data on bacteriuria are colored gray. Countries with <10% preterm birth rates are shown in white.

2.4 Risk factors Associated with Urinary Tract Infections

2.4.1 Gender

Females have fourteen times more chances to develop UTI than male (Hooton *et al.*, 2010). Almost half of all women will experience one UTI during their lifetime partly

due to their relatively short, straight anatomy of the urethra, closer proximity of the anus to the vagina and sexual activity facilitating pathogen entry (Chen *et al.,* 2009). Approximately 1-2% of women who are asymptomatic at initial screening early in pregnancy will develop bacteriuria later in the pregnancy (Ebie *et al.,* 2001).

2.4.2 Age

UTIs are first experienced early in neonatal life and are also frequently observed in the adult life with another peak seen in old age. UTI is one of the most commonly diagnosed infections in older adults. It is second only to respiratory infections in hospitalized patients and community- dwelling adults over the age of 65 years, (Griebling, 2011). As the population ages, the burden of UTI in older adults is expected to grow making the need for improvement in diagnostic, management and prevention strategies critical to improving the health of older adults (Hamdan *et al* 2011). During pregnancy, the prevalence of UTIs increases with maternal age (Obirikorang *et al.*, 2012).

2.4.3 Sexual activity

Sexual activity in women has been established as a major risk factor for UTIs (Dimetry *et al*., 2007) Intercourse traumatizes the urothelium of the distal urethra, leading to an in increased bacterial invasion. The vagina can also act as a reservoir for gastrointestinal bacteria, facilitating inoculation. (Ronald, 1996).

2.4.4 Menopause

Post-menopausal women are at higher risk for UTIs than younger women, partly because they lack estrogen. Estrogen loss thins the walls of urinary tract reducing its ability to resist invading bacteria. It also reduces certain immune factors in the vagina that blocks *E.coli* from adhering to vaginal cells (Hazhir, 2007). The acidity produced by lactobacillus which is a normal vaginal flora acts as a natural host defense mechanism against symptomatic UTIs (Griebling, 2007).

2.4.5 Past history of UTIs

Pastore *et al.* (1999**)** identified two strongest predictors of bacteriuria at prenatal care to be antepartum UTI prior to prenatal care and a pregnancy history of UTI. Similar observations were reported in Pakistan (Haider *et al.,* 2010), Qatar (Aseel *et al.,*2011) and Philippines (Nandy *et al.*, 2007) among asymptomatic pregnant women. Masinde *et al*. (2009) also identified history of UTIs as a risk factor for UTI during pregnancy. However absence of association was reported by Hamdan *et al*. (2011) in Sudan.

2.4.6 Contraceptives

Certain types of contraceptives can also increase the risk of UTIs. In particular, women who use certain types of spermicides and diaphragms tend to develop UTIs (Fallahian *et al.,* 2009). The spring-rim of the diaphragm bruises the area near the bladder, making it susceptible to bacteria. Spermicidal foam or gel used with diaphragms, and spermicidal-coated condoms, also increase susceptibility to UTIs. Majority of spermicides contain nonoxynol-9, a chemical that is associated with increased UTI risk (Fallahian *et al.,* 2009).

2.4.7 Diabetes

People with diabetes are at increased risk of having UTIs since their urine may have a high glucose content, providing an ideal breeding ground for bacteria, (Chen *et al*., 2009). Diabetes may also change the body's defense system reducing its ability to fight a UTI. Susceptibility to UTIs increases as the duration and severity of diabetes increases, (Chen *et al*., 2009).

2.4.8 Genital Mutilation

It is estimated that more than 140 million women today have undergone some type of genital mutilation, which despite bans, continues to be common in at least 28 African countries. (Abdulcadir *et al*., 2011). Several types of female genital mutilation (FGM) exist, often performed on girls aged 4 to 9 years, ranging from clitoridectomy to infibulation. Infibulation is associated with a variety of adverse urologic, gynecologic, and obstetric outcomes such as obstructed labor, stillbirth, hemorrhage, and fistula (GSN *et al*., 2006). FGM not only alters permanently a girl's urogenital anatomy but studies suggests it may fundamentally alter the way her genitourinary mucosa interacts with her genitourinary micro biota (Iavazzo *et al.*, 2013).

2.4.9 Socio-economic status

Socio-economic status is one of the most powerful risk factor for poor health outcomes. Persons of lower socioeconomic status suffer disproportionately from nearly all diseases and have higher rates of mortality than people of higher socio-economic status (Amler *et al*., 1987). Like many other health conditions, low birth weight is strongly associated with socio-economic status. Studies have shown that rates of low birth weight increase with decreasing socio-economic status (Hughes *et al*., 1995). This association persists across various measures of socio-economic status, including occupation of the mother and/or father (Murrells *et al.*, 1985), income and education (Collins *et al*., 1990). At the individual level, a study conducted in Great Britain found the risk of preterm delivery to be 50% higher and the risk of low birth weight delivery to be 95% higher (Fedrick *et al*., 1978) among women in lower social classes. It is not clear just how low socioeconomic status causes an increased risk for low birth weight. It is thought that poverty which is associated with reduced access to health care, poor nutrition, lower education, and inadequate housing may be responsible for some of the increased risk (Klerman *et al*., 1991).

2.5 Microbial aetiology of UTI

Although virtually every organism can be associated with UTIs, certain organisms dominate due to specific virulence and host susceptibility factors (DiPiro *et al*., 2008). The most common agent implicated in both symptomatic and asymptomatic bacteriuria is *E. coli* representing 70–80% of isolates. (Sharma *et al*., 2007; Masinde *et al*., 2009; Obirikorang *et al*., 2012). Other microorganisms include *S. saprophyticus* accounting for 5- 15% of UTI cases (Mittal *et al*., 2005), *Gardnerella vaginalis*, *Chlamydia trachomatis*, *K. pneumoniae*, *Proteus* spp, *P. aeruginosa*, *Enterococcus* spp accounting for 5-10% of UTI cases globally, (Delzell *et al*., 2000).

Specific virulence factors in uropathogenic strains are associated with invasive infection and pyelonephritis during pregnancy. These factors include toxins, adhesins and pilli or fimbriae that permits adherence to uroepithelial cells facilitating multiplication and tissue invasion (Eisenstein *et al*., 1987). These adherence proteins are expressed on the surface of bacterial wall promoting binding to the epithelium of the vagina and urethra, thus increasing the ability of uropathogens to cause UTIs. Incidences of virulence associated determinants are lower in uropathogens linked to asymptomatic bacteriuria as compared with pyelonephritis (Stenqvist *et al.*, 1987).

Additional factors that contribute to virulence of uropathogens include haemolysin production, serum resistance and release of aerobactin. Haemolysin provides uropathogens with a selective advantage by releasing iron from lysed erythrocytes enhancing pathogenicity by destroying phagocytic and epithelial cells (Naveen & Mathai, 2005).

Group B streptococcus (*Streptococcus agalactiae*) infection during pregnancy has been reported to be associated with preterm rupture of the membranes, premature delivery and early onset neonatal sepsis. In a small randomized trial comparing treatment of group B streptococcal bacteriuria with penicillin versus placebo found a reduction in preterm

rupture of membranes and preterm delivery with treatment. As a result of the assumed heavy vaginal colonization, women with group B streptococcal bacteriuria in pregnancy should receive appropriate treatment following prompt diagnosis as well as intrapartum prophylaxis to prevent neonatal infection (Verani *et al*., 2010).

2.6 Diagnosis of UTI

Screening for UTIs during pregnancy is usually requested during the first antenatal visits. This measure allows early start of treatment reducing the rate of progression from asymptomatic to symptomatic infection and it's potentially harmful consequences (Gratacós *et al*., 1994; Fiona *et al*., 2007). A key aspect in the diagnosis of both symptomatic and asymptomatic urinary tract infections is differentiating contamination from significant bacteriuria, (Nicolle *et al*., 2005).

Currently laboratory diagnosis of UTI is based on the color changes of chemical reactants according to urine composition known as dipstick analysis, (Nicolle *et al*., 2005). In primary care settings, leukocyte esterase and nitrite tests are often used to evaluate urinary symptoms, however, they are not useful for diagnosing UTIs in an asymptomatic patient (Colgan *et al*., 2006). The nitrite test is based on the ability of certain bacteria to reduce the urinary nitrates to nitrites. This test has a sensitivity of 50% and specificity of 97%, and can result in false positives when used on urine contaminated with normal vaginal bacteria or highly concentrated urine, given that the test follows colorimetric principles, (Nicolle *et al*., 2005). Infection with non-nitrite–producing microbes, delays between obtaining and testing the sample as well as insufficient time since the last void for nitrites to appear at detectable levels are other limitations of nitrite test, (Colgan *et al.*, 2006). A leukocyte esterase test showing trace or more white blood cells has a sensitivity of 75 - 96 % and specificity of 94 - 98 % for detecting pyuria, however, pyuria is not specific for UTI and may occur with other inflammatory disorders of the genitourinary tract such as vaginitis (Colgan *et al.*, 2006). Both tests have low sensitivity and therefore not suitable as screening tests for diagnosis,

unless used in combination with other tests (McNair *et al*., 2000). Microscopic urine examination has a lower sensitivity 40% - 70% but a high specificity 85% to 95% for the diagnosis of UTI, (Nicolle *et al*., 2005). Pyuria is present in a majority of cases involving pyelonephritis estimated to be about 90%. Presence of pyuria increases the sensitivity to 95% and specificity to 71% for the diagnosis of acute pyelonephritis. White cell casts always point to an upper tract infection (Fihn, 2003).

The microbiologic culture of clean catch midstream urine is considered the gold standard for laboratory diagnosis of UTIs, (Nicolle *et al*., 2005). It is the most accurate method for identification and quantification of bacteria in the urine with high sensitivity. Although urine cultures are expensive, require laboratory expertise and take 24–48 h for results to become available, quantitative culture remains the gold standard for diagnosis of UTIs during pregnancy as the performance of rapid urine screening tests in pregnancy is poor (McNair *et al*., 2000). The most commonly used criterion for defining significant bacteriuria is the presence of a colony count of 10^5 CFU/ml (Rosen *et al.*, 2007). This criterion was established only for women with acute pyelonephritis or women who were asymptomatic but had multiple urine cultures that yielded this number of bacteria; however, the criterion is often applied to other patient populations, (Rosen *et al.*, 2007). Most patients with UTIs, however, do not fall into either category, and 30%–50% of patients with acute urethral syndrome will have colony counts of $<10^5$ cfu/ml, (Rosen *et al.*, 2007). Lower counts or mixed cultures are usually regarded as contaminants. It is in view of this literature that 15% of plates yielding lower counts in this study were analysed alongside the cases. The aim of analyzing these isolates was to asses whether the phenotypes and genotypes of such strains are related to those from confirmed UTI cases. If this is the case, our data would be useful in revising existing diagnosis and treatment guidelines for UTI.

2.7 Treatment

There is no clear consensus in literature on either the duration of therapy or the choice of antibiotic for treatment of UTI during gestation and as a result, practice is more likely guided by national patterns of practice and local resistance patterns than by evidence from clinical trials (Schnarr *et al*., 2008). Beta lactams antibiotics such as penicillins and cephalosporins, are considered safe during pregnancy and are usually prescribed for the treatment of UTIs during the gestational period (Lee *et al*., 2008; Schnarr *et al*., 2008; Guinto *et al*., 2010). Beta lactams are not teratogenic but are sometimes associated with allergic and anaphylactic reactions, (Guinto *et al*., 2010). Additionally, high bacterial resistance rates limit the use of some agents, such as Amoxicillin or Ampicillin (Guinto *et al*., 2010).

Nitrofurantoin has already been demonstrated safe for use in pregnancy, however this only achieves therapeutic levels in the urine therefore cannot be used to treat pyelonephritis, in addition, there is also a theoretical risk of nitrofurantoin-induced hemolytic anemia in the fetus or infants especially those with glucose-6-phosphate dehydrogenase (G6PD) deficiency (Guinto *et al*., 2010). The most common therapeutic regimens currently proposed for the treatment of UTIs during pregnancy according to type is summarized in Table 2.1 (Bruel *et al*., 2000; Mittal *et al*., 2005; Rosen *et al*., 2007; Guinto *et al*., 2010).

Table 2.1: Summery of UTI treatment regimens.

Urinary tract infection	Treatment regimen	Treatment options
Asymptomatic bacteriura	Current standard practice is to treat pregnant women with ASB for at least 3-7days	Cephalexin, nitofurantoin, amoxicillin, norfloxacin and cefuroxime
Urethritis and cystitis	Treatment same as in ASB though longer courses of therapy is usually recommended (7-10 days)	Cephalexin, nitofurantoin, amoxicillin/clavulanic acid, norfloxacin and cefuroxime
Pyelonephritis	Initial treatment is parenteral continued until patient is stable for 48 hours then patient is switched to oral antimicrobial therapy for 2 weeks	Ampicillin, ceftriaxone, cefuroxime, cefazolin, mezlocillin, piperacillin, and ticarcillin/ clavulanate.

Adapted from Guinto *et al*. (2010)

2.7.1 Microbial resistance of urinary tract isolates

Resistance to antimicrobial agents is a problem of global concern and there exists a correlation between antibiotic use and subsequent resistance (Ringertz *et al*., 1990). Empirical use of antimicrobial agents is on the increase around the world and has been recognized as the main reason for the emergence of resistance (Jensen *et al*., 2009). Several studies have also revealed that limited access to medical care and effective treatments, the common practice of self-medication and the availability of counterfeit drugs have contributed to the emergence and spread of drug resistance in the developing world (Kose *et al*., 2007) Resistance to this antimicrobials can be intrinsic, conferred by naturally occurring characteristics of the bacteria, or acquired. (Maltha *et al*., 2014).

The basic mechanisms by which a microorganism can resist an antimicrobial agent include alteration of the receptor for the drug, decreasing the amount of drug that reaches the receptor by altering entry or increasing removal of the drug, destroying or inactivating the drug and developing resistant metabolic pathways. (Shaikh *et al.*, 2015). A bacterium can possess one or all of these mechanisms.

Bacteria can also acquire resistance through mutations of preexisting genes or transfer of resistance determinants from other bacteria (horizontal gene transfer). The horizontal transfer occurs much more commonly than *de-novo* development of resistance through mutation (White *et al*., 2008). It is through horizontal gene transfer that resistance genes, alone or in groups, can spread within bacterial populations and even to other bacterial species leading to emergence of Extended-spectrum β-lactamases (ESBLs) which confer resistance to β-lactams. Thus, very broad antibiotic resistance extending to multiple antibiotic classes is now a frequent characteristic of ESBL-producing enterobacterial isolates. As a result, ESBL-producing organisms pose a major problem for clinical therapeutics (Kiiru *et al*., 2012).

Urinary tract pathogens causing UTIs are almost always predictable with *E. coli* being the primary etiologic agent accounting for 75 to 90% of UTI isolates (Dromigny *et al*., 2005). The mechanism of *E. coli* resistance is the production of beta-lactamases (Dbaibo, 1999). These enzymes are numerous, and they continuously mutate in response to the heavy pressure of antibiotic use, leading to the development of extended spectrum β- lactamases, ESBLs (Park *et al.*, 2009). The ESBL producing bacteria are typically associated with multidrug resistance since genes with other mechanisms of resistance usually reside on the same plasmid as the ESBL gene. As a result, some ESBL producing strains also show resistance to Quinolones, Aminoglycosides, Trimethoprim and Sulfamethoxazole (Kim *et al*., 2010). The antibacterial drugs most commonly prescribed in treatment of UTIs throughout the world are Cephalosporins, semisynthetic Penicillins with or without beta-lactamase inhibitors, Trimethoprim-Sulfamethoxazole, and Quinolones (Chung *et al*., 2010).

Studies by Eryılmaz *et al*. (2010) reported resistance of *E. coli* isolates from a sample size of 110 as 56% to Ampicillin, 24% to Ampicillin / Sulbactam, 9% to Gentamicin, 15% to Ciprofloxacin, 36% to Trimethoprim / Sulfamethoxazole, 12% to Cefazolin and7% to Cefuroxime, with all isolates being susceptible to fosfomycin and nitrofurantoin. However, several other studies have reported a higher percentage of resistance to nitrofurantoin (Jafri *et al*., 2014). This can be attributed to the empirical use of nitrofurantoin in the treatment of UTIs leading to treatment failure in most cases. In Kenya, Kebira *et al.* (2009) reported resistance of *E.coli* to Trimethoprim-Sulfamethoxazole, Norfloxacin, Niproxin, Perfloxacin, Ceftriaxone. All *E. coli* isolates were found to be susceptible to Ticarcilin, Amikacin, and Ofloxacin. In addition, 96% of the isolates were sensitive to Ceftazidime/Fortum and 95% susceptible to Norfloxacin, Ciproxin and Ceftriaxone.

2.8 β -Lactamase phenotypes

β-lactamases are commonly classified according to two general schemes: the Ambler molecular classification and the Bush–Jacoby–Medeiros functional classification (Bush *et al*., 1995); Ambler, 1980). The Ambler scheme classifies β-lactamases into four classes according to the protein homology of enzymes. B-lactamases of class A, C, and D are serine β-lactamase and class B enzymes are metallo-β-lactamases. The Bush–Jacoby–Medeiros functional classification system relies on β-lactamases phenotype to classify the enzymes, (Bush *et al*., 2010). Since the Bush–Jacoby–Medeiros classification scheme groups β-lactamases according to functional similarities based on substrate and inhibitor profiles, this scheme finds more relevance in the hospitals and may help care-givers decide on appropriate therapy (Kiiru *et al.,* 2012). One of the mechanisms of resistance towards β–lactams is production of β-lactamases that hydrolyze the β-lactam ring and render the antibiotic inactive before it reaches the penicillin-binding protein (PBP) target mainly mediated by (Temoniera-1) $_{TEM-1}$ and SulfHydryl Variable-1 ($_{SHV-1}$) enzymes (Dermott *et al*., 2003).

2.8.1 Narrow spectrum β-lactamases (NSBLs)

The NSBLs enzymes have limited hydrolytic activity towards β-lactam antibiotics and are generally susceptible to β-lactamase inhibitors (Jacoby, 2009). The NSBLs include enzymes such as TEM-1, TEM-2, SHV-1 and OXA-1. Based on hydrolytic activity, the primary β-lactamases TEM-1 and TEM-2 are active against penicillins but when produced in large quantities these enzymes can hydrolyze first generation cephalosporins (Kiiru *et al*., 2012). These TEM enzymes are the most widely spread β-lactam degrading enzymes in enterobactereacea and are mainly borne on chromosomes, (Livermore, 1995).

2.8.2 Extended Spectrum β-lactamases (ESBLs)

Extended-spectrum β-lactamases (ESBLs) are a group of β-lactamases conferring resistance to penicillins, first, second and third generation Cephalosporins and Aztreonam but not the cephamycins or carbapenems. They are also sensitive to β-lactamases inhibitors, (Kiiru *et al.*, 2012). These enzymes act on β-Lactam which is a group of antibiotics acting on the cell wall of a bacterial cell (Shaikh *et al*., 2015; Ghafourian *et al*., 2014). ESBL-producing microorganisms exhibit co-resistance to many other classes of antimicrobials, resulting in limited therapeutic options in both hospital and community settings (Livermore, 2008). Mutations arising from the amino acid substitutions or the re-arrangements of the omega loop of parent TEM-1 and SHV-1 enzymes give rise to a variety of β- lactamases. They are encoded by many genes among them *bla*$_{\text{SHV}}$, *bla*$_{\text{TEM}}$, and *bla*$_{\text{CTX-M.}}$

2.8.3 Inhibitor Resistant TEM (IRTs)

Point mutations on the TEM-1 may give rise to TEM-type ESBLs but may also give rise to IRTs. IRT enzyms are derived from both TEM-1 and TEM-2, (Knox, 1995). IRT producing isolates are susceptible to Cephalosporins, Cephamycins, Carbapenems and to Pipperacillin/ Tazobactam combinations. They are however resistant to

Ampicillin/Sulbactam and Amoxicillin/Clavulanic acid inhibitor combinations (Chaibi *et al.,* 1996). Although these enzymes are less prevalent than ESBLs, they are of great clinical significance because they result in therapeutic failure when inhibitor based antimicrobials are prescribed without determining susceptibility profiles of the pathogen (Henquell *et al.,* 1994). The IRTs were initially found in *E. coli* but have recently been detected in *Klebsiella* spp, *Enterobacter cloacae*, *Proteus mirabilis*, *Citrobacter freundi* and *Shigella shonnei* (Chaibi *et al.,* 1996).

2.8.4 Complex Mutant TEM (CMTs)

While point mutaions on $_{\text{TEM-1}}$ or $_{\text{TEM-2}}$ normally generate ESBL and IRT enzymes, combinations of mutations on amino acids that generate ESBLs and those that generate IRTs give rise to enzymes with combined IRT/ESBL characteristics (Kiiru *et al*., 2012). Such enzymes are known as CMTs. Unlike IRTs, CMTs hydrolyse Penicillins and Cephalosporins and are not impeded by inhibitors. They are however susceptible to Carbapenems (Kiiru *et al*., 2012). CMTs have been reported in enterobacteriaceae strains such as *E. coli*, *K. pneumoniae*, *Proteus mirabilis* and *Enterobacter aerogenes* (Henquell *et al.,* 1995). Complex mutant $_{\text{SHVs}}$ have been reported but occur rather rarely, (Randegger *et al*., 2001). Such include $_{\text{SHV-10}}$ identified from urine *E. coli* strains and $_{\text{SHV-49}}$ identified from a *K. pneumoniae* strain (Randegger *et al.,* 2001).

2.8.5 Plasmid-mediated ampicillin β-lactamases (pAmpC)

AmpC enzymes may be found in chromosomes of many Gram-negative bacteria, including *E. coli*, *Citrobacter*, *Serratia* and *Enterobacter* spp where the expression is usually inducible (Jacoby, 2009). Chromosomal overproduction of AmpCs may result from the duplication of the ampC gene leading to higher expression from from multiple transcripts (Caroff *et al*., 2000). Plasmid encoding AmpC have also been reported in *Klebsiella* spp and *E. coli* (Jacoby, 2009). Globally, AmpC are found less frequently than ESBLs among enterobacteriaceae. AmpC are capable of hydrolyzing all major

Cephalosporins and are particularly effective against Cephamycins. They are not inhibited by β-lactamase inbitors but are susceptible to Carbapenems. Due to their ability to spread horizontally and their increase hydrolysis to Cephalosporins, the pAmpC have gained substantial attention in research and hospitals. The AmpC mediated resistance is increasing worldwide (Sheikh *et al.*, 2015).

2.9 β-lactamase genes

2.9.1 TEM bla gene

The TEM-type ESBLs are derivatives of TEM-1 and TEM-2, and are the most commonly encountered β-lactamases among Gram-negative bacteria (Sheikh *et al*., 2015). The first TEM type ESBL was TEM-3 identified in *K. pneumonia* isolates in France and was borne on a plasmid, (Sirot *et al*., 1987). TEM-1 is capable of hydrolyzing Penicicillins and first generation Cephalosporins but is unable to attack the oxyimino cephalosporins (Shaikh *et al*., 2015). TEM encodes for β- lactamases with extended spectrum. TEM-1 is responsible for up to 90% Ampicillin and Penicillin resistance in *E. coli* as well as resistance in *H. influenza, N. gonorrhoea* and *K. pneumoniae* (Kiiru *et al*., 2012). Currently, over 100 TEM-type β-lactamases have been described (Rawat *et al*., 2010). The most common TEM type ESBL is found in *E. coli* and *K. pneumoniae*, however, they could appear in the other Gram-negative bacteria (Livermore, 1995) and also in different genera of Enterobacteriaceae (*Enterobacter aerogenes, Enterobacter cloacae, Morganella morganii, Proteus Mirabilis* and *Salmonella* spp (Morosini *et al.*, 1995). In non Enterobacteriaceae, they are found in *P. aeruginosa* (Nordmann *et al*., 1998).

2.9.2 SHV bla gene

The SHV (SulfHydryl variable) family of β-lactamases appears to be derived from *Klebsiella* spp and is more prevalent than the other types of ESBLs in clinical isolates of bacteria (Jacoby, 1997). The progenitor of the SHV class of enzymes SHV-1, is universally

found in *K. pneumoniae* and appears to be responsible for up to 20% of the plasmid-mediated Ampicillin resistance in this species (Shaikh *et al*., 2015). In many strains of *K. pneumoniae,* the gene encoding $_{SHV-1}$ or its apparent precursor, $_{LEN-1}$ resides within the bacterial chromosomes too. It may be that the gene for $_{SHV-1}$ β-lactamases evolved as a chromosomal gene in *Klebsiella* and was later incorporated into a plasmid which has spread to other bacteria (Shaikh *et al.,* 2015). $_{SHV}$ types of ESBLs have been detected in a wide range of Enterobacteriaceae and outbreaks of $_{SHV}$–producing *Pseudomonas* spp and *Acinetobacter* spp have been reported (Rawat *et al*., 2010)

2.9.3 OXA bla gene

The $_{OXA}$–type β-lactamases are so named because of their oxacillin-hydrolyzing abilities. They are characterized by hydrolysis rates for cloxacillin and oxacillin greater than 50% as that for benzyl penicillin (Bush *et al*., 1995). These beta lactamases predominantly occur in *P. aeruginosa* (Weldhagen *et al.,* 2003) but have also been detected in many other Gram-negative bacteria. The most commom $_{OXA}$-type β-lactamases, $_{OXA-1}$ has been found in 1-10% of *E. coli* isolates (Livermore *et al*., 1995). Recently, the carbapenem-resistant $_{OXA}$ β-lactamases ($_{OXA-48}$) have migrated into the Enterobacteriaceae and are now becoming a major cause of Carbapenem resistance. The emergence of $_{OXA}$ enzymes that can confer resistance to third generation Cephalosporins and Carbapenems, particularly in Enterobacteriaceae, has transformed these β-lactamases from a minor hindrance into a major problem set to demote the clinical efficacy of the carbapenems and cephalosporins (Gutkind *et al*., 2013). The evolution of ESBL $_{OXA}$-type β-lactamases from parent enzymes with narrow spectrum has many parallels with the evolution of $_{SHV}$ and $_{TEM}$ –type of ESBLs (Shaikh *et al*., 2015). Unfortunately, there is scanty epidemiologic data on the geographical spread of $_{OXA}$-type ESBLs (Philippon *et al.,* 1997).

2.9.4 CTX-M bla gene

$_{CTX\text{-}M}$ is derived from "CefoTaXimase Munich" family and constitutes a complex, novel non-homogeneous group of class A beta lactamases with extended spectrum properties (Gutkind *et al*., 2013). $_{CTX\text{-}M}$ β-lactamases are found exclusively in the functional group 2 (Bush *et al*., 2010) and are thought to originate from chromosomal ESBL genes found in *Kluvyera* spp. The term $_{CTX\text{-}M}$ β-lactamase denotes its ability to hydrolyse Cefotaxime. $_{CTX\text{-}M}$ has the ability to hydrolyse Cephalothin better than Benzyl–penicillin and Cefotaxime better than Ceftazidime (Bonnet, 2004). $_{CTX\text{-}M}$ β-lactamase are also able to hydrolyze Cefepime (Tzouvelekis *et al.*, 2000). So far, 128 types of $_{CTX\text{-}M}$ have been reported and are classified as $_{CTX\text{-}M\text{-}1}$, $_{CTX\text{-}M\text{-}2}$, $_{CTX\text{-}M\text{-}8}$, $_{CTX\text{-}M\text{-}9}$ and $_{CTX\text{-}M\text{-}25}$. They are found in different Enterobacteriaceae including *Salmonella* spp (Bradford *et al*., 1998). To date, $_{CTX\text{-}M\text{-}15}$ and $_{CTX\text{-}M\text{-}14}$ enzymes are the most predominant types of ESBLs with $_{CTX\text{-}M\text{-}15}$ showing global distribution (Lahlaoui *et al*., 2014). In Kenya, the predominant ESBL genotype is *bla*$_{CTX\text{-}M}$, most of which are isolated from isolates obtained from the urinary tract (Maina *et al*., 2011). They are also the most important ESBL gene among *E. coli* and in *Salmonella enteric* serovar *typhimurium* (Wang *et al*., 2012).

2.10 Genetic fingerprinting of bacterial isolates

Genetic fingerprinting is a technique used to determine genetic relatedness of bacteria of the same species from the same or different source to study diversity and dynamics of microbial communities (Holden *et al*., 2013). There are several gel-based methods of determining relatedness of bacterial isolates namely; Random amplified polymorphic DNA (RAPD), Pulsed-Field Gel Electrophoresis (PFGE), Restriction fragment length polymorphism (RFLP), Repetitive extragenic palindromic (REP) and Enterobacterial repetitive intergenic consensus (ERIC-PCR) (Mohapatra *et al.*, 2007). Genetic typing techniques based on conserved repetitive regions have been shown to be more accurate and discriminatory than morphological and phenotypic methods for typing bacteria (Rameshkumar *et al*., 2012). These techniques fingerprint bacterial genomes by

examining strain specific patterns obtained from PCR amplification of repetitive DNA elements present within bacterial genomes. Two of such are ERIC-PCR and REP-PCR more common to gram negative enteric bacteria.

ERIC-PCR uses any combination of conserved entrobacterial repetitive intergenic consensus region in order to generate an electrophoretic banding pattern based on the frequency and orientation of ERIC sequences in a bacterial genome. The fingerprinting generated by using ERIC-PCR show characteristic pattern and could be used to differentiate bacterial genomes (De Bruijn, 1992).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The study was carried out at Pumwani Maternity Hospital. Pumwani is an obstetric and referral hospital for delivery of expectant mothers in Nairobi County which constitutes a cosmopolitan affluent, middle class and low economic group. It has 354 obstetric beds, 144 baby cots and 2 theatres. Daily normal deliveries range from 50 – 100, and Caesarean Sections are 10 – 15. It offers antenatal care services to approximately 800 women per month. The hospital is located on the East of Nairobi, Kamukunji constituency in Pumwani ward, **figure 3.1**.

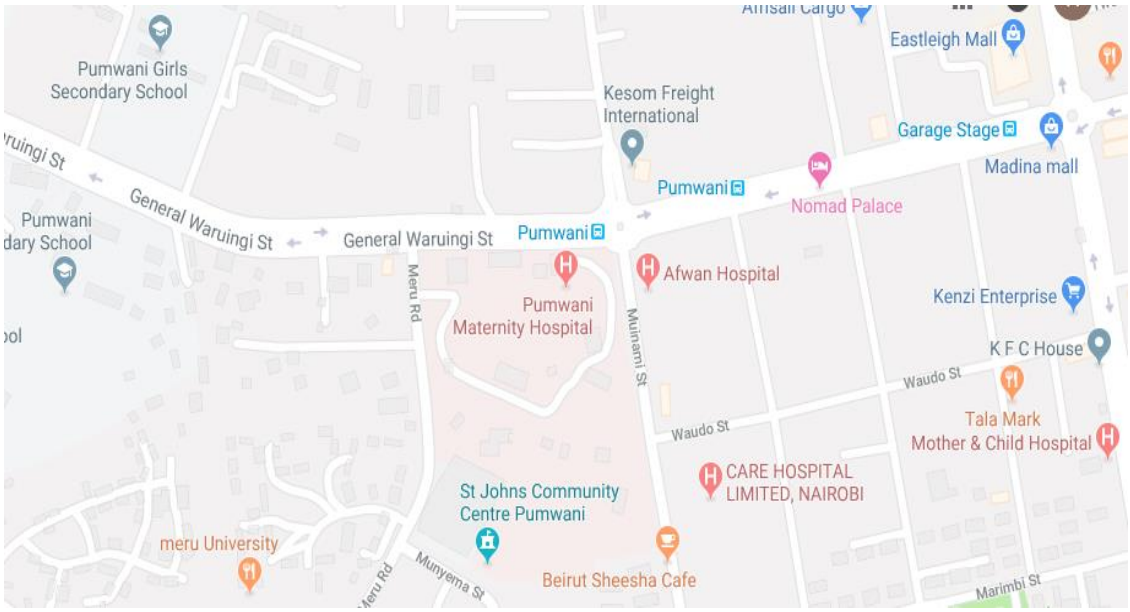

Figure 3.3: Map to Pumwani Maternity Hospital (Adapted from Google, 2018).

3.2 Study Population

The target population was all pregnant women seeking antenatal care services at Pumwani Maternity Hospital at the time of the study.

3.3 Study design

This was a Laboratory based cross-sectional study.

3.4 Inclusion criteria

All pregnant women attending antenatal clinic at Pumwani maternity Hospital at the time of the study and who consented were included in the study.

3.5 Exclusion criteria

Any persons seeking any other medical care other than antenatal care.

3.6 Sample size

The sample size was determined using the Fisher *et al.*, 1998 formulae.

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

Where;

n = Estimated sample size

Z = 1.96 at 95% confidence level

P = Estimated prevalence of UTIs in pregnant women is 14.2% in Kenya (Wamalwa *et al.*, 2013).

Q = 1-p

d = degree of accuracy which is 0.05 at 95% confidence level.

$$\mathbf{n = \frac{1.96^2 \times (0.142)\,(0.858)}{0.05^2}}$$

$$\mathbf{n = 187}$$

3.7 Ethics Statement

Ethical approval was sought from the KEMRI Scientific and Ethical Review Unit (S.E.R.U) - P00043/3329 and the Ethical Research Committee, Pumwani Maternity Hospital. Only urine samples were collected from the expectant mothers. There was no use of invasive procedures that could harm the fetus and no fetal specimen was collected.

3.8 Sampling method

The principal investigator identified eligible participants at the antenatal care clinic after the nurse's assessment irrespective of apparent UTI symptoms. The purpose of this study was adequately explained to them after which informed consent was obtained in a safe, quite room that also guaranteed privacy within the hospital facility, **appendix 1**. Systematic random sampling technique was employed in recruiting participants until the expected study sample was attained. Sampling was done thrice a week, Monday, Tuesday and Wednesday with 10 samples collected each day during the morning hours. Since the facility attends to 100 antenatal mothers weekly, this translated to approximately 400 antenatal mothers monthly. The study was conducted in a period of 3 months and the total number of pregnant women that were expected to attend antenatal clinic was 1200. With a calculated sample size of 187, then the sampling interval was;

1200 = 6.417. This was rounded up to 6**.**

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From the computed sampling interval of 6, and from a random starting point, pregnant mothers were then selected according to sampling interval from the antenatal register until a sample size of 187 was obtained.

3.9 Data collection

A structured questionnaire was administered by the Principal investigator (PI) to gather the socio-demographic data, clinical and lifestyle factors of the study participants such as age, parity, history of pre-term births, gestation, education level, occupation, marital status, history of UTI and recent history of treatment and hospitalization among others, **appendix 3**. No name was indicated on the form but a unique bar code was used. A separate file with names and contacts of the participants for follow up after the results was maintained by the principle investigator.

3.10 Sample collection and processing

Participants were instructed on how to collect clean-catch midstream urine within the hospital facility after signing the consent form. Each sample bottle had a date of collection and bar-code linked to the participant's questionnaire which was in turn linked to confidential patient information file maintained by the PI. No urine sample was accepted from outside the hospital facility. The samples were stored in a cool box ($4^{o}C$) and transported to the Kenya Medical Research Institute (KEMRI) laboratory for processing within 8 hours.

3.10.1 Urine culture

Cysteine, Lactose and Electrolyte Deficient Agar (CLED), MacConkey and blood agar were prepared according to manufacturer’s instructions (Oxoid, Ltd, England) and used

for culture. Known urine aliquot (1µl) was plated directly on the three media and incubated aerobically at 35-37ºC for 24 hours. Determination of colony forming unit (CFUs) was done by counting bacterial colonies on each plate and multiplying the value by the dilution factor plated. Colony counts yielding bacterial growth of ≥100,000 (10^5) CFU/mL were interpreted as UTI infections, while those yielding lower values or mixed cultures were deemed as contaminants. To cater for any design bias and possible errors in identifying UTI cases based on set colony count threshold, a single colony from at least 15% of contaminants were randomly selected and further analyzed alongside those from positive UTI plates. At least 3 colonies of similar morphology per patient were randomly selected from UTI plates and purified on MacConkey's agar before biochemical identification. Special emphasis was given to *E. coli, Klebsiella, Acinetobactor* and *Pseudomonas* due to their unique resistances and frequent implications in UTI.

Gram stain was done for all colonies analyzed and biochemical tests carried out to identify the isolates to the species level (Cheesbrough, 2000). Catalase and coagulase were also added to the panel of tests used for identifying Gram-positive organisms. For Gram-negative organisms, citrate utilization, urease test, Triple sugar iron (TSI), Sulfur indole motility (SIM) were used in identification as detailed in **appendix 5.**

3.10.2 Antimicrobial susceptibility testing

Antimicrobial susceptibilities of the pure isolates was performed according to the Kirby-Bauer disc diffusion method ((Bauer *et al.*, 1966). A loop full of bacteria from a pure culture was transferred to a tube containing 5ml of phosphate buffer saline and mixed gently until it formed a homogenous suspension. The turbidity of the suspension was adjusted to that of the McFarland 0.5 standard and swabbed on Muller Hinton medium to create a lawn of growth. The following antimicrobials was used with their respective concentrations for Gram-negative organisms: Ampicillin (AMP, 10µg), Ciprofloxacin (CIP, 5µg), Nitrofurantoin (NIT, 300µg), Trimethoprim-sulfamethoxazole (SXT,

1.25/23.75µg), Amoxicillin-Clavulanic acid (AMC, 20/10µg), Nalidixic acid (NAL, 30µg), Ceftazidime (CAZ, 30µg), Cefotaxime (CTX, 30µg), Cefoxitin (FOX, 30µg), Cefepime (FEP, 30µg), Ceftriaxone (CRO, 30µg), Chloramphenicol (CHL, 30µg), Imipinem (IPM, 10µg), **Table 3.1.**

Table 3.1: Antimicrobials used for Gram negative organisms

PLATE A		**PLATE B**	
Antimicrobial	**Class**	**Antimicrobial**	**Class**
Ampicillin (**AMP**)	Penicillin	Nitrofurantoin (**NIT**)	Nitrofurans
Cefepime (**FEP**)	4th Generation Cephalosporin	Ciprofloxacin (**CIP**)	Fluoroquinolone
Cefotaxime (**CTX**)	3rd Generation Cephalosporin	Nalidixic Acid (**NAL**)	Quinolone
Ceftriaxone(**CRO**)	3rd Generation Cephalosporin	Chloramphenicol (**CHL**)	Chloramphenicol
Ceftazidime (**CAZ**)	3rd Generation Cephalosporin	Gentamycin (**GEN**)	Aminoglycosides
Amoxicillin/Clavulanic acid (**AMC**)	β-lactam/β-lactamase inhibitor combination	Sulfamethoxazole/Trimethoprim (**SXT**)	Folate and dihydofolate-biosynthesis inhibitor
Cefoxitin (**FOX**)	Cephamycin	Imipinem (**IPM**)	Carbapenem

For gram positive organisms, the following antimicrobials were used in their respective concentrations, Ampicillin (AMP, 10µg), Chloramphenicol (CHL, 30µg), Ciprofloxacin (CIP, 5µg), Erythromycin (ERY 15µg), Nitrofurantoin (NIT, 300µg), Trimethoprim-sulfamethoxazole (SXT, 1.25/23.75µg), Ofloxacin (OFX, 5µg), Amoxicillin-clauvlanic

acid (AMC, 20/10µg), Cefoxitin (FOX, 30µg), Ceftazidime (CAZ, 30µg), Gentamicin (GEN, 10µg), Linezolid (LNZ, 30µg), Doxycycline (DOX, 30µg), Nalidixic acid (NAL, 30µg), Imipinem (IPM, 10µg), Quinupristin (QDA, 15µg), **Table 3.2.**

Table 3.2: Antimicrobials used for Gram Positive organisms

PLATE A		**PLATE B**	
Antimicrobial	**Class**	**Antimicrobial**	**Class**
Ampicillin (**AMP**)	Penicillin	Nitrofurantoin (**NIT**)	Nitrofurans
Gentamycin (**GEN**)	Aminoglycosides	Erythromycin (**ERY**)	Macrolides
Ciprofloxacin (**CIP**)	Fluoroquinolone	Nalidixic Acid (**NAL**)	Quinolone
Imipinem (**IPM**)	Carbapenem	Chloramphenicol (**CHL**)	Chloramphenicol
Ceftazidime (**CAZ**)	3rd Generation Cephalosporin	Doxycycline (**DOX**)	Tetracyclines
Amoxicillin/Clavulanic acid (**AMC**)	β-lactam/β-lactamase inhibitor combination	Sulfamethoxazole/Trimethoprim (**SXT**)	Folate and dihydofolate-biosynthesis inhibitor
Cefoxitin (**FOX**)	Cephamycin	Quinupristin (**QDA**)	Streptogramin
Ofloxacin (**OFX**)	Fluoroquinolone	Linezolid (**LNZ**)	Oxazolidinones

All the antimicrobials used for the study were obtained from Oxoid Ltd. Using a pair of sterile forceps, the antibiotic discs were placed on the agar and incubated at 37^0C for about 18 to 24 hours. The culture was examined for zones of inhibition which were measured using metal calipers. The interpretated results were recorded as either sensitive (S), intermediate (I) or resistant (R) based on the Clinical and Laboratory Standards Institute criteria (CLSI, 2016) guidelines. The standard reference strains, *Staphylococcus aureus* (ATCC-25923), *Escherichia* coli (ATCC-25922 and *P. aeruginosa* (ATCC-27853) were used as quality control organisms to ascertain antibiotic discs potency and

quality of the test media. Susceptibility to Cefoxitin was used for the detection of Methicillin-Resistant *Staphylococcus aureus*- MRSA, (CLSI 2013).

3.10.3 Identification of ESBLs using disc diffusion test

The double disc synergy test was used to test for ESBL production following the CLSI (2015) guidelines. Isolates showing synergy zones between AMC and one or more third generation Cephalosporins were identified as ESBL-producers (Rawat *et al*., 2010). Enlargement or distortion of the inhibition zones to form a keyhole appearance/ghost inhibition zone between the Cephalosporins discs and the AMC disc were interpreted as an ESBL enzyme production phenotype. All other strains that were resistant to at least one third generation cephalosporin was also included in the panel of strains for further analysis for beta-lactamases.

3.10.4 DNA Extraction

DNA used as template in PCR reactions was extracted from pure isolates using the boiling method at 95^{o}C for 15 min (Holden *et al*., 2013). Using a sterile swab, a pea sized amount of inoculum was scrapped from the culture plate and transferred to the corresponding tube. The tubes were placed on the heating block and left to heat for a maximum of 15 min. After cooling, the tubes were placed in a table top centrifuge and contents centrifuged at a 1400 rpm for 5-6 minutes. The supernatant was then transferred to a sterile tube and contents stored at -20^{o}C until use.

3.10.5 Detection of ESBL genes

PCR amplification of selected antimicrobial resistance ESBL genes (*bla*$_{\text{TEM}}$, *bla*$_{\text{OXA}}$, *bla*$_{\text{CTX-M}}$ and *bla*$_{\text{SHV}}$) was carried out in a final volume of 27µl with 1µl DNA template, 1µl of both forward and reverse primers and 12µl each of firepol master mix and PCR water. Appropriate positive control strains were used depending on the test gene while sterile distilled water was used as negative control. Amplification was conducted in 0.2

ml micro centrifuge tube using a programmable Bio-system thermal cycler with an initial denaturation step at 95°C for 7 minutes, followed by 30 cycles of denaturation at 94°C for 1 min, annealing (variable) for 1 min extension at 65°C for 8 min, followed by final extension at 65°C for 16 min.PCR products were analyzed by electrophoresis in 1.5% agarose gels stained with ethidium bromide and visualized under UV light and the image recorded with the aid of a gel documentation system. Primer pairs indicated in **Table 3.3** were used:

Table 3.3: Nucleotide sequences of PCR primers used to amplify ESBL genes

Gene	**Primer sequence**	Expected size (bp)	Annealing Temp(°C)	**References**
TEM	F-5'ATGAGTATTCAACATTTCCGTG 3'	865	55	Zhang *et al* 2017
	R-5'TTACCAATGCTTAATCAGTGAAG 3'			
SHV	F-5'TTATCTCCCTGTTAGCCACC 3'	795	50	Zhang *et al* 2017
	R-5'GATTTGCTGATTTCGCTCGG 3'			
CTX-M	F-5'ATGTGCAGYACCAGTAARGTKATGGC 3'	593	60	Moubareck *et al* 2005.
	R-5'TGGGTRAARTARGTSACCAGAAYCAGCGG 3'			
OXA	F-5'ATGAAAAACACAATACATATCAACTTCGC 3'	820	62	Juma *et al* 2017.
	R-5'GTGTGTTTAGAATGGTGATCGCATT 3'			

3.10.6 Genetic relatedness of the bacterial isolates

Out of the 3 colonies of similar morphology per patient, one was randomly selected for inter-patient species genetic similarity analysis. The inter-species genetic relatedness of

the isolates was done using REP and ERIC PCR. Universal primers based on ERIC sequences were used to generate fingerprinting, ERIC1 (5'-ATGTAAGCTCCTGGGGATTCAC-3') and ERIC2 (5'-AAGTAAGTGACTGGGGTGAGCG-3'); while the single primer for Staph-rep was (5'-TCGCTCAAAACAACGACACC-3') (Versalovic *et al*., 1991). These primers target noncoding repetitive sequences interspersed throughout the bacterial genome and are established approaches for sub-species classification and strain delineation.

The reaction mixture for ERIC-PCR consisted of 1 µl of DNA template, 4µl of 5x Firepol ready to use master mix (SOLIS BIODYNE), 0.7µl of each primer, 15µl of PCR water and 1µl of Butane. Negative control reaction without template DNA was used for each amplified set. Amplification was conducted in 0.2 ml micro centrifuge tube using a programmable Bio-system thermal cycler with an initial denaturation step at 95°C for 7 minutes, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 51°C for 1 min, extension at 65°C for 8 min, followed by final extension at 65°C for 16 min. The reaction mixture for REP PCR was similar with ERIC-PCR except the annealing temperature for REP PCR was 40^{o} C for 1 minute.

The amplified PCR products were subjected to electrophoresis in 1.5 % agarose gel in 1X TBE buffer and stained with ethidium bromide and visualized by UV transillumination against a 1kb DNA ladder. The banding patterns generated using ERIC and REP-PCR were digitized and analyzed using the Bionumerics software using manufacturer's instructions (Maths, Belgium). This enabled the generation of a dendrogram showing the level of genetic similarity among the bacterial isolates.

3.11 Biosafety issues

The biological specimen and the isolates were regarded as infectious and handled using appropriate personal protective equipment at the time of collection, transportation and

processing. Disposal of biological waste was done following KEMRI biosafety guidelines. The bacterial isolates were stored at -40^{0}C indefinitely.

3.12 Study limitations

It was assumed that samples obtained from women population at Pumwani would provide a true picture of microbial etiologies implicated in UTI within Nairobi County and that the resistance data generated could be used to advice on the diagnosis and treatment of UTI during pregnancy. Since this study was conducted in a short duration (within 4 months), it was assumed that the samples taken during this period were a representative of general UTI samples and that the disease does not have seasonal variation. In order to ameliorate these assumptions, samples were collected at the hospital clinic facility and a robust sample size was used with an enough statistical power to cater for any shortcoming regarding these limitations.

3.13 Data analysis

Coding and verification of all collected data was done before analysis. Analysis was done using SPSS version 15. Chi-square test or Fishers Exact Test where applicable was applied for P- value derivation for socio-demographic and risk factors to identify variables associated with UTIs. Binary logistic regression analysis was carried out to generate the adjusted odds ratio with 95% confidence interval for the associations between variables and UTIs. An alpha of less than 0.05 ($P<0.05$) was considered statistically significant. The antibacterial activity was reported as either sensitive, intermediate or resistant and presented in form of tables. ESBL prevalence was calculated from ESBL positive samples. The resulting bands from ERIC and REP PCR were subjected to statistical analysis to enable the generation of a dendrogram showing the level of genetic similarity among the bacterial isolates.

3.14 Risks and Potential benefits for participants

There were no risks associated with this study. However, some of the questions asked may have been uncomfortable or embarrassing, but all care was taken to ensure that the interview was conducted in a safe, quiet place that also guaranteed privacy. The data collected was confidential and only accessible by relevant persons; and was also encrypted to ensure privacy of the participants. Individuals found positive for UTIs were contacted on their mobile numbers and referred to the same facility (Pumwani) for treatment guided by the sensitivity reports.

3.15 Data management

The data was securely stored in hard copies and computers. No name was indicated on the form but a bar code was used. A separate file with names and contacts of the participants for follow up after the results was maintained by the PI. The hard copies were stored in safety cabinets with secured locks accessible only to the PI. The data collected was confidential and would only be accessible to relevant persons. The data would be stored for a maximum of 10 years after which disposition would be done according to KEMRI procedures.

3.16 Dissemination of findings

The study findings were published in Advances in Microbilogy journal, a peer-reviewed journal, presented in conferences and health information briefs.

CHAPTER FOUR

RESULTS

4.1 Prevalence of UTI and major etiologic agents

Among the 210 pregnant women, thirty three (33) were found to have bacteriuria (by sample analysis) translating to a prevalence of 15.7%. The prevalence of asymptomatic (those found to have UTI by sample analysis but lacked any symptoms) and symptomatic bacteriuria was 9 (4.3%) and 24 (11.4%) respectively.

Bacterial isolates were identified and deemed significant for UTI when colonies yielded counts of $\geq 10^5$ CFU/ml with quantitative counts ranging from low to moderate. Colony counts that fell below the set WHO UTI threshold were deemed as contaminants and 15% of these isolates were analysed alongside the UTI cases **(Table 4.1).**

Table 4. 1: Quantitative counts of various bacterial isolates

	Quantitative Counts	***E.coli***	***K. pneumoniae***	***S. aureus***	***P. areoginosa***	***Acinetobacter***	***Enterococcus***
Non UTI cases	Below UTI threshold	3	1	5	1	0	0
	Low:1.00-1.55×10^5	8	4	1	0	2	1
UTI cases	Moderate:1.56-2.55×10^5	5	3	2	0	0	1
	High :2.56-4.00×10^5	2	0	2	2	0	0

Analysis of the quantitative counts exhibited by various bacterial isolates recovered from UTI cases, ranging from low to moderate and 15% of isolates whose counts fell below the UTI threshold, usually regarded as contaminants.

A total of 99 bacterial isolates were identified (3 from each of the 33 UTI cases). From the 99 isolates, 78 (78.8%) were Gram-negative while 21(21.2%) were Gram-positive bacteria, **figure 4.1.** *E. coli* 45 (44.5%) was the most predominant UTI organism followed by *Klebsiella pneumoniae* 21 (21.2%) and *S. aureus* 15 (15.1%). Other organisms included *P.aeruginosa*, *Acinetobacter* spp and *Enterococcus* spp each at 3 (6.1%). Analysis of 15% of the urine samples that would have been classified as contaminations based on CFU cut-off points revealed organisms similar to the pathogens isolated from UTI cases. The organisms were *S.aureus* (50%), *E.coli* (30%), *P.aeruginosa* and *Klebsiella pneumoniae* were at 10% each.

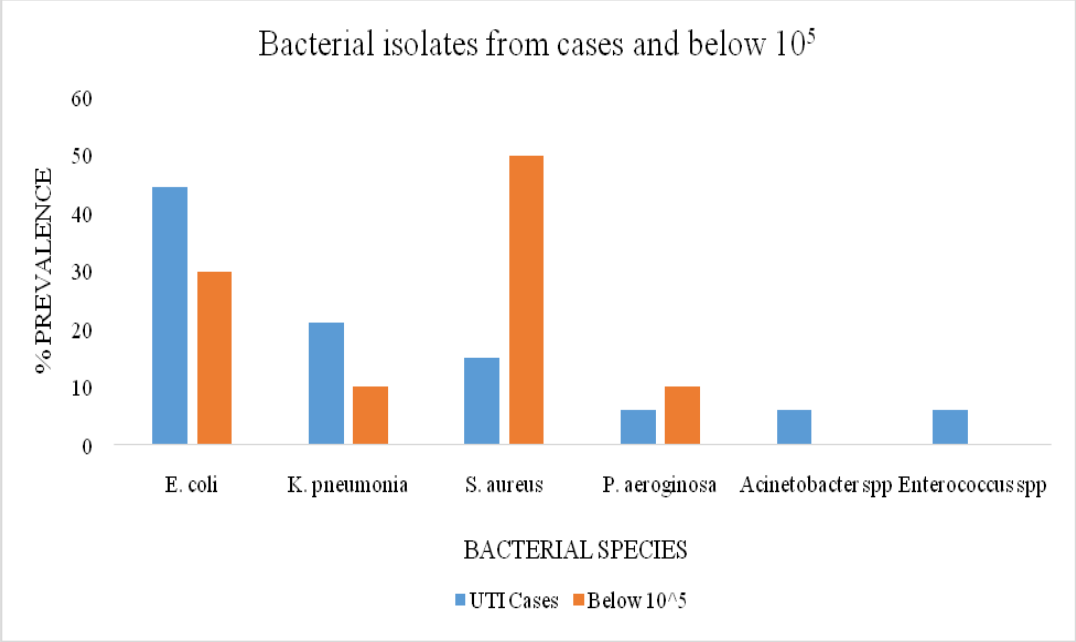

Figure 4. 1: Bacterial isolates UTI cases and below UTI threshold

Bacterial isolates from UTI cases and those recovered from plates that fell below the set UTI threshold of 10^5 CFU/ml.

4.2 Social demographic, clinical and lifestyle factors associated with UTI occurrence

4.2.1 Social-demographic characteristics associated with UTI occurence

Pregnant mothers in the age group of 21-30 years had the highest prevalence of UTI at 25 (75.8%) followed by those aged 31-40 years at 4 (12.1%), below 20 years of age at 3 (9.1%) while those above the age of 40 years had the least prevalence of 1 (3%). UTI prevalence was also high among mothers in the second trimester 20 (60.6%) compared to those in third trimester 8 (24.2%) and first trimester 5 (15.2%). High prevalence of bacteriuria was observed among multiparous (those who have delivered more than one baby before) mothers 24 (72.7%) as compared to nulliparous (those who have never delivered a baby before) 9 (27.3%). Despite these variations, there was no significant association between UTI and maternal age, parity, occupation, gestation, marital status or level of education P>0.05, **Table 4.2**.

Table 4. 2: Selected social-demographic characteristics

Variables	Response	UTI (+)*		UTI (-)*		OR	95% CI	P-value
		N	%	N	%			
Average		**33**	**15.7**	**177**	**84.3**			
Blood Pressure	Normal	28	84.8	163	92.1	0.481	0.161 - 1.441	0.183
	Abnormal	5	15.2	14	7.9	2.079	0.694 - 6.227	0.190
Maternal Age	≤20 years	3	9.1	18	10.2	1.132	0.314 - 4.084	0.850
	21-30 years	25	75.8	134	75.7	0.997	0.419 - 2.373	0.995
	31-40 years	4	12.1	22	12.4	1.029	0.330 - 3.207	0.961
	41-50 years	1	3	3	1.7	0.552	0.056 - 5.472	0.606
Parity	Nulliparous	9	27.3	64	36.2	1.510	0.662 - 3.447	0.325
	Multiparous	24	72.7	113	63.8	0.662	0.290 - 1.511	0.317
Marital Status	Single	4	12.1	14	7.9	0.623	0.191 - 2.025	0.428
	Married	28	84.8	160	90.4	1.681	0.574 - 4.924	0.339
	Divorced	1	3	2	1.1	1.011	0.996 - 1.027	0.540
	Widowed	0	0	1	0.6	0.182	0.011 - 2.982	0.181
Gestation	1st Trimester	5	15.2	43	24.3	1.797	0.653 - 4.942	0.251
	2nd Trimester	20	60.6	84	47.5	0.587	0.275 - 1.253	0.165
	3rd Trimester	8	24.2	50	28.2	1.230	0.520 - 2.910	0.637
Occupation	Employed	15	45.5	107	60.5	1.834	0.868 - 3.877	0.109
	Unemployed	18	54.5	70	39.5	0.545	0.258 - 1.152	0.111
Level of Education	Primary	10	30.3	55	31.1	1.037	0.462 - 2.326	0.930
	Secondary	15	45.5	81	45.8	1.013	0.480 - 2.136	0.974
	Tertiary	8	24.2	41	23.1	0.942	0.395 - 2.247	0.893

Analysis of the social-demographic factors among the 210 pregnant mothers, 33 with UTI and 177 without: None of the factors was found to be associated with UTI, P>0.05.

*Nulliparous- those who have never delivered a baby before; Multiparous- those who have delivered more than one baby before; 1st trimester- 1-3 months; 2nd trimester- 4-6 months; 3rd trimester 7-9 months; Primary class 1-8; Secondary form 1-4; Tertiary-post secondary education.

4.2.2 Clinical factors associated with UTI occurence

At least 70% of the respondents reported to have experienced one or a combination of the UTI symptoms before or during pregnancy with vaginal discharge, urgency to urinate, lower abdominal pain and burning sensation being the most commonly reported symptoms. Out of the 210 pregnant mothers recruited, 8 (3.8%) had been on treatment in the last two weeks for UTI yet they still had significant bacteriuria, 3 (1.4%) had successfully been treated for UTI, while 16 (7.6%) had been on antibiotic treatment for infections other than UTI. In the last 3-4 months, 2 (1%) of the pregnant mothers had been hospitalized while 12 (5.7%) reported to have delivered one month before their due dates. Of these 14, 2 (14%) had significant bacteriuria. Of the 210 participants, 181(86.2%) used contraceptives. The contraceptives used among the 181 mothers were barrier methods, implants (18.2%), injectables (20.4%), intrauterine contraceptive device (IUCD) - (6.6%) and oral contraceptives (11%). The barrier contraceptives used included male condoms (7.2%), female condoms (5.5%), foaming spermicide tablets (17.1%) and the diaphragm (13.8%). The prevalence of UTI amongst barrier contraceptive users was 19 (63.3%) and they were found to be more predisposed to UTI compared to those who used hormonal contraceptives 11(36.7%), (OR=2.62). The association between barrier contraceptive use and UTI occurrence was statistically significant (P<0.05), **Table 4.3.**

Table 4. 3: Contraceptive use and UTI occurrence

	UTI (+)*	UTI (-)*	OR	CI	P-value
Barrier Contraceptives	19	60	2.620	1.164-5.894	0.017
Hormonal contraceptives	11	91	0.382	0.180-0.859	0.229

Analysis of contraceptive users among the 181 pregnant mothers, 30 with UTI and 151 without with barrier contraceptive users being more predisposed to UTI than those who use hormonal contraceptives; **(+)*-** Positive for UTI infection, **(-)*-** Negative for UTI infection.

4.2.3 Lifestyle factors associated with UTI occurence

Among the lifestyle factors surveyed, mothers with multiple partners were two times more likely to develop a UTI (OR 2.45; P>0.05) compared to those with single partners**.** Those who used non-cotton undergarments were three times more likely to develop a UTI compared to those who used cotton undergarments (P<0.05). This study also found that the frequency of changing undergarment was significantly associated with UTI, (P<0.05). Those who changed once were 2 times more likely to develop UTI compared to those who changed twice, (OR 2.282; P<0.05), **Table 4.4.**

Table 4. 4: Lifestyle risk factors associated with UTI occurrence

Variables	Response	UTI (+)*		UTI (-)*		OR	95% CI	P-value
		N	%	N	%			
Average		**33**	**15.7**	**177**	**84.3**			
Weekly Sexual Intercourse	Once only	5	15.2	34	19.2	0.681	0.291 - 1.591	0.373
	Twice	10	30.3	69	39	2.282	1.073 - 4.854	0.129
	Thrice	14	42.4	56	31.6	0.628	0.294 - 1.342	0.228
	>3 times	4	12.1	18	10.2	0.821	0.259 - 2.601	0.737
Number of Sexual partners	One	25	75.8	133	75.1	0.967	0.407 - 2.300	0.139
	Multiple	8	24.2	44	24.9	2.450	1.027 - 5.848	0.326
Material of undergarment	Cotton	10	30.3	76	42.9	1.731	0.778 - 3.851	0.001
	Other than cotton	23	69.7	101	57.1	3.051	1.556 - 5.981	0.004
Frequency of changing undergarment	Once	9	27.3	36	20.3	2.282	0.291 - 1.591	0.047
	Twice	14	42.4	111	62.7	0.681	1.073 - 4.854	0.029
	Thrice	10	30.3	30	16.9	0.469	0.203 - 1.087	0.043

Analysis of the lifestyle factors among the 210 pregnant mothers. Pregnant women with multiple (more than one) sexual partners and those who use undergarments made of materials other than cotton have higher risks of developing UTI than their counterparts; **(+)*-** Positive for UTI infection, **(-)*-** Negative for UTI infection.

4.2.4 UTI awareness among study particippants

When the 210 respondents were asked to state what causes UTI, almost a third 79 (37.6%) had no idea. The remaining proportion of the total respondents indicated that women got UTIs from sexual intercourse 37 (17.6%), poor urogenital hygiene 19 (9%), contaminated toilets 54 (25.7%) and damp panties 21 (10%), **Figure 4.2.** Out of the 33 (15.7%) pregnant mothers who had UTIs, 13 (39.4%) had no idea of what causes UTIs, others indicated other sources of UTIs as sexual intercourse 5 (15.5%), poor urogenital hygiene 2 (6%), contaminated toilets 10 (30%) and damp panties 3 (9.1%). Only 2 (6%) of the mothers with UTI compared to 17 (9.6%) amongst those without could associate UTIs to poor urogenital hygiene, which has been found to be significantly ($P<0.05$) associated to UTI acquisition in this study. Almost a third 66 (37.2%) of women without UTIs had no idea of what causes UTIs compared to 13 (39.4%) who had UTIs.

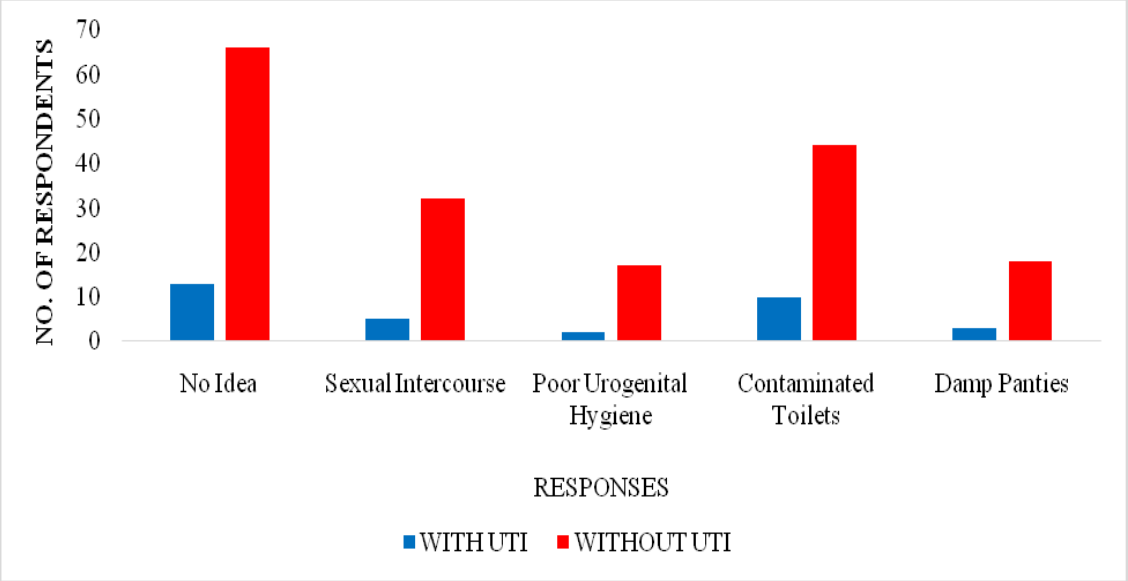

Figure 4. 2: Awareness of UTI sources among pregnant women.

Study participants responses to the various sources of UTI with a majority not having an idea what UTIs are.

4.3 Antimicrobial susceptibility Profiles of the isolated UTI strains

All the *Acinetobacter* isolates recovered in this study showed resistance to AMP, AMC, FOX, NIT and C. None of the isolates was resistant to CAZ, FEP, CIP and SXT, this is in contrast to *E. coli* isolates that showed resistances in the range of (28.9% -62.2%) against same antimicrobials. Resistance to CRO and CTX was 83.3% and 66.7% respectively, **Table 4.5.** All the *Acinetobacter* isolates showed total resistance to Amoxicillin-clavulanic and Nitrofurantoin, the drugs to which *E. coli* isolates showed lower levels of resistance. None of the *E. coli* and *Acinetobacter* isolates showed resistance to IPM. All *K. pneumoniae* and *P. aeruginosa* isolates showed resistance to AMP, CRO, CTX, NAL and SXT. None of *P. aeruginosa* isolates was resistant to CAZ,

FEP, IPM and CIP. *K. pneumoniae* had relatively lower resistance against C (4.8%) and FEP (38.1%), with total resistance against CIP and CAZ, **Table 4.5.**

4.3.2 Antimicrobial susceptibility patterns of Gram-positive isolates

Gram positive isolates exhibited relatively lower resistances to a majority of antimicrobials tested in this study compared to Gram-negative isolates. None of the *S. aureus* isolates recovered in this study was resistant to AMC, NIT, C, SXT, LNZ, QDA, and OFX. All the *Enterococcus* isolates showed no resistance to AMC, OFX, NIT, LNZ and DOX with 16.7% showing resistance to C, SXT and QDA. *S. aureus* showed relatively higher resistances to AMP (100%), NA (86.7%) and E (53.4%), antimicrobials to which *Enterococus* spp showed resistance at 33%, 100% and 50% respectively. Resistance of *S. aureus* isolates to CAZ, FOX, and IPM ranged between (20% -40%). Resistance of *Enterococcus* isolates to FOX, E, AMP and CIP was in the range of 30-50%. All *Enterococcus* isolates were resistant to IPM, **Table 4.5**.

4.3.3 Resistance pattern of presumed contaminants

Isolates recovered from 15% of samples that had colony forming units below the recommended values for UTI (1×10^5 CFU/ml) were also analysed in this study. Comparative analysis revealed that the resistances exhibited by isolates whose counts fell below UTI cut-off were similar to those observed among isolates recovered from UTI cases. *S. aureus*, the predominant contaminant showed no resistance to AMC, NIT, C, SXT, and LNZ. Resistance to FOX, IPM and CIP was in the range of (20%-40%). *E. coli* recorded higher resistances to AMP (100%), NAL (86.7%) and SXT (100%). Resistance to CAZ, CTX and CRO was 44%, 66% and 66% respectively. *K. pneumonia* showed no resistance to IPM and C, **Table 4.5.**

4.3.4 Prevalence of MDR strains

Multi drug resistance (MDR), (resistance to ≥ 3 classes of antimicrobials) was seen in 96% of the isolates, **Table 4.5**. Of the *S. aureus* isolates, 20% were MRSA. Based on the resistance profiles, 22% of *E. coli* isolates and 16.7% of *K. pneumonia* isolates were ESBL-producers. This study found that majority of the ESBL producers exhibited co-resistance to multiple antimicrobials such as fluoroquinolones, aminoglycosides, SXT and third generation Cephalosporins. The most unique resistance phenotypes observed was combined resistance to β-lactams/Fluoroquinolones/ aminoglycosides like gentamicin and this was noted in 38.4% of Gram-negative bacteria.

Table 4. 5: Resistance profiles of UTI isolates and contaminants

Species	Category based on CFUs	Antimicrobials Resistance (%)																		
		n	AMP	AMC	CAZ	CR0	CTX	FEP	FOX	IPM	NAL	CIP	SXT	NIT	CHL	GEN	OFX	ERY	LNZ	QDA	DOX
E. coli	UTI	45	89	13	49	49	62	28	15	0	86	62	86	2.2	31	63	NT	NT	NT	NT	NT
	Contaminant	9	100	0	44	66	66	44	22	0	100	100	100	0	44	56	NT	NT	NT	NT	NT
K. pneumoniae	UTI	21	100	76	100	100	100	38	85	14	100	100	100	66	5	98	NT	NT	NT	NT	NT
	Contaminant	3	100	100	100	100	100	100	100	0	100	100	100	100	0	98	NT	NT	NT	NT	NT
P. aeruginosa	UTI	6	100	100	0	100	100	0	100	0	100	0	100	100	100	100	NT	NT	NT	NT	NT
	Contaminant	3	100	100	0	100	100	0	100	0	100	0	100	100	100	100	NT	NT	NT	NT	NT
Acinetobacter	UTI	6	100	100	0	83	67	0	100	0	33	0	0	100	100	100	NT	NT	NT	NT	NT
S. aureus	UTI	15	100	0	40	NT	NT	NT	20	20	86	20	0	0	0	13	0	53	0	0	26
	Contaminant	15	100	0	86	NT	NT	NT	40	40	93	20	0	0	0	13	6.7	53	0	6.7	33
Enterococcus	UTI	6	33	0	100	NT	NT	NT	50	100	100	33	17	0	17	17	0	50	0	17	0

Antimicrobial susceptibility profiles of the UTI isolates and isolates whose counts fell below the 10^5CFU/ml UTI threshold.

* AMP- Ampicillin; AMC-Amoxicillin-clavulanic; CAZ-Ceftazidime; CRO-Ceftriaxone; CTX-Cefotaxime; FEP-Cefepime; FOX- Cefoxitin; IPM-Imipinem; NAL-Nalidixic Acid; CIP-Ciprofloxacin; SXT-Sulfamethoxazole/Trimethoprim; NIT-Nitrofurantoin; CHL- Chloramphenicol; GEN-Gentamin; OFX-Ofloxacin; ERY-Erythromycin; LNZ-Linezolid; QDA-Quinupristin; DOX- Doxycycline; NT- Not tested

4.3.5 Resistance based on age

This study found that *E. coli* resistance to CAZ, CTX, and CRO was more common among mothers above 30 years of age. However, this pattern was not observed in *P. aeroginosa* and *K. Pneumoniae* isolates. We also noted that regarding species, combined resistance to Cephalosporins, Cephamycins and Floroquinolones was evident among those above 30 years. In contrast, for gram positive isolates like *S. aureus* and *Enterococcus* spp, this pattern was not observed. All *P. aeruginosa* and *K. Pneumoniae* isolates recovered from both age groups showed resistance against AMP, CRO, CTX, NAL and SXT. This pattern was not observed in the Gram-positive isolates. Low resistance against IPM was observed in both Gram-positive and negative isolates, **Table 4.6.**

4.3.6 Resistance based on level of Education

This study found that *E. coli* resistance to Penicillins, Fluoroquinolones and Cephalosporines was higher (75%-100%) among mothers with tertiary level of education, this pattern was however not observed in *K. pneumonia*, *S. aureus* and *Enterococcus*. Similar high (50%-100%) resistances for third generation Cephalosporins, Fluoroquinolones, and SXT was observed in *K. pneumoniae* isolates recovered from participants of all the levels of education. This pattern was however, not observed in all the other isolates. IPM and NIT were highly effective against *E. coli* isolates recovered from participants of all the levels of education, this pattern was not

replicated in *K. pneumoniae*, *P. aeruginosa* and *Acinetobacter*. It was also observed that all Gram-positive isolates showed no resistance against NIT and AMC, as it was for all other Gram-negative isolates, **Table 4.7.**

Table 4. 6: Resistance based on Age

Species	Age		Antimicrobials Resistance (%)																	
		n	AMP	AMC	CAZ	CRO	CTX	FEP	FOX	IPM	NAL	CIP	SXT	NIT	CHL	GEN	OFX	ERY	LNZ	QDA	DOX
E. coli	≤30yrs	36	86	16	41	36	52	19	16	0	83	52	83	3	27	93	NT	NT	NT	NT	NT
	>30yrs	9	100	0	77	100	100	66	11	0	100	100	100	0	44	100	NT	NT	NT	NT	NT
K. pneumonia	≤30yrs	15	100	77	100	100	100	44	83	5	100	100	100	61	5	100	NT	NT	NT	NT	NT
	>30yrs	6	100	50	100	100	100	0	50	33	100	100	100	100	16	100	NT	NT	NT	NT	NT
P. aeroginosa	≤30yrs	3	100	100	0	100	100	0	100	0	100	0	100	100	100	100	NT	NT	NT	NT	NT
	>30yrs	3	100	100	0	100	100	0	100	0	100	0	100	100	100	100	NT	NT	NT	NT	NT
Acinetobacter	≤30yrs	6	100	100	0	83	66	0	100	0	33	0	0	100	0	33	NT	NT	NT	NT	NT
	>30yrs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NT	NT	NT	NT	NT
S. aureus	≤30yrs	12	100	0	41	NT	NT	NT	25	25	16	91	8	0	0	50	0	0	0	0	25
	>30yrs	3	100	0	33	NT	NT	NT	0	0	0	66	66	0	0	66	0	0	0	0	33
Enterococcus	≤30yrs	0	0	0	0	NT	NT	NT	0	0	0	0	0	0	0	0	0	0	0	0	0
	>30yrs	6	33	0	100	NT	NT	NT	50	100	16	100	33	0	16	50	0	0	16	16	0

Antimicrobial susceptibility profiles of the UTI isolates based on age. * AMP- Ampicillin; AMC-Amoxicillin-clavulanic; CAZ-Ceftazidime; CRO-Ceftriaxone; CTX-Cefotaxime; FEP-Cefepime; FOX- Cefoxitin; IPM-Imipinem; NAL-Nalidixic Acid; CIP-Ciprofloxacin; SXT-Sulfamethoxazole/Trimethoprim; NIT-Nitrofurantoin; CHL-Chloramphenicol; GEN-Gentamycin; OFX-Ofloxacin; ERY-Erythromycin; LNZ-Linezolid; QDA-Quinupristin; DOX- Doxycycline; NT-Not tested.

Table 4. 7: Resistance based on the Level of Education

Species	Level	N	Antimicrobials Resistance (%)																	
			AMP	AMC	CAZ	CRO	CTX	FEP	FOX	IPM	NAL	CIP	SXT	NIT	CHL	GEN	OFX	ERY	LNZ	QDA	DOX
E. coli	Pri	12	75	8	33	50	33	16	16	0	75	58	75	8	41	100	NT	NT	NT	NT	NT
	Sec	21	90	11	42	33	66	23	5	0	85	47	85	0	23	85	NT	NT	NT	NT	NT
	Ter	12	100	8	75	75	83	50	33	0	100	91	100	0	33	91	NT	NT	NT	NT	NT
K. pneumoniae	Pri	9	100	90	100	100	100	88	100	11	100	100	100	90	0	100	NT	NT	NT	NT	NT
	Sec	6	100	83	100	100	100	0	100	0	100	100	100	0	0	100	NT	NT	NT	NT	NT
	Ter	6	100	50	100	100	100	0	50	33	100	100	100	100	16	100	NT	NT	NT	NT	NT
P. aeruginosa	Pri	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NT	NT	NT	NT	NT
	Sec	6	100	100	0	100	100	0	100	0	100	0	100	100	100	100	NT	NT	NT	NT	NT
	Ter	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NT	NT	NT	NT	NT
Acinetobacter spp	Pri	3	100	100	0	66	66	0	100	0	33	0	0	100	100	66	NT	NT	NT	NT	NT
	Sec	3	100	100	0	100	66	0	100	0	33	0	0	100	100	66	NT	NT	NT	NT	NT
	Ter	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NT	NT	NT	NT	NT
S. aureus	Pri	3	100	0	0	NT	NT	NT	0	0	66	100	0	0	0	66	0	0	0	0	0
	Sec	9	100	0	55	NT	NT	NT	33	33	0	90	11	0	0	44	0	0	0	0	33
	Ter	3	100	0	33	NT	NT	NT	0	0	0	66	66	0	0	66	0	0	0	0	33
Enterococcus spp	Pri	3	66	0	100	NT	NT	NT	100	100	33	100	66	0	0	100	0	0	33	33	0
	Sec	3	0	0	100	NT	NT	NT	0	100	0	100	0	0	33	0	0	0	0	0	0
	Ter	0	0	0	0	NT	NT	NT	0	0	0	0	0	0	0	0	0	0	0	0	0

Antimicrobial susceptibility profiles of the UTI isolates based on education. * AMP- Ampicillin; AMC-Amoxicillin-clavulanic; CAZ-Ceftazidime; CRO-Ceftriaxone; CTX-Cefotaxime; FEP-Cefepime; FOX- Cefoxitin; IPM-Imipinem; NAL-Nalidixic Acid; CIP-Ciprofloxacin; SXT-Sulfamethoxazole/Trimethoprim; NIT-Nitrofurantoin; CHL- Chloramphenicol; GEN-Gentamycin; OFX-Ofloxacin; ERY-Erythromycin; LNZ-Linezolid; QDA-Quinupristin; DOX- Doxycycline; NT-Not tested; Pri-primary; Sec-Secondary; Ter-Tertiary

4.3.7 Resistance based on Parity

This study found that resistance third generation Cephalosporins (CRO, CAZ, CTX), and Floroquinolones (NAL, CIP) was more common among multiparous mothers. However, for the gram positive strains like *S. aureus*, this pattern was not observed. It was also noted that regarding individual species, combined resistance to Cephalosporins, Floroquinolones and Cephamycin was higher among multiparous mothers. This pattern was not evident in Gram- positive isolates. Regardless of participant's parity, there was no difference in resistance exhibited by *K. pneumoniae* isolates. All *Enterococcus* spp isolates showed total resistance against CAZ, IPM and NAL. This pattern was not observed in *S. aureus* isolates **Table 4.8.**

Table 4. 8: Resistance based on Parity

Species	Parity	Antimicrobials Resistance (%)																		
		N	AMP	AMC	CAZ	CRO	CTX	FEP	FOX	IPM	NAL	CIP	SXT	NIT	CHL	GEN	OFX	ERY	LNZ	QDA	DOX
E. coli	Nulliparous	6	66	0	33	50	50	0	16	0	50	33	30	0	0	100	NT	NT	NT	NT	NT
	Multiparous	39	92	15	51	48	64	33	15	0	92	66	92	2.6	33	89	NT	NT	NT	NT	NT
K. pneumoniae	Nulliparous	9	100	77	100	100	100	55	100	0	100	100	100	55	0	100	NT	NT	NT	NT	NT
	Multiparous	12	100	75	100	100	100	25	75	25	100	100	100	75	8	100	NT	NT	NT	NT	NT
P. aeruginosa	Nulliparous	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NT	NT	NT	NT	NT
	Multiparous	6	100	100	0	100	100	0	100	0	100	0	100	100	100	100	NT	NT	NT	NT	NT
Acinetobacter	Nulliparous	6	100	100	0	88	66	0	100	0	33	0	0	100	100	66	NT	NT	NT	NT	NT
	Multiparous	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NT	NT	NT	NT	NT
S. aureus	Nulliparous	6	100	0	50	NT	NT	NT	16	16	0	83	16	0	0	50	0	0	0	0	50
	Multiparous	9	100	0	33	NT	NT	NT	22	22	22	88	22	0	0	0	0	0	0	0	11
Enterococcus spp	Nulliparous	0	0	0	0	NT	NT	NT	0	0	0	0	0	0	0	0	0	0	0	0	0
	Multiparous	6	33	0	100	NT	NT	NT	50	100	16	100	33	0	16	50	0	0	16	16	0

Antimicrobial susceptibility profiles of the UTI isolates based on Parity. * AMP- Ampicillin; AMC-Amoxicillin-clauvlanic; CAZ-Ceftazidime; CRO-Ceftriaxone; CTX-Cefotaxime; FEP-cefepime; FOX- Cefoxitin; IPM-Imipinem; NAL-Nalidixic Acid; CIP-Ciprofloxacin; SXT-Sulfamethoxazole/Trimethoprim; NIT-Nitrofurantoin; CHL- Chloramphenicol; GEN-Gentamycin; OFX-Ofloxacin; ERY-Erythromycin; LNZ-Linezolid; QDA-Quinupristin; DOX- Doxycycline.

4.4 Prevalence of β-lactamase Phenotypes

Bacterial strains that exhibit Narrow Spectrum β-lactamase Phenotypes (NSBLs) accounted for 22 (23.6%) in this study. The classical ESBLs which exhibits a wide hydrolytic activity was noted in 19 (20.4%) isolates. Advanced β-lactamase phenotypes plasmid-mediated ampicillin β-lactamases and Complex mutant TEMs (pAmpC and CMT) were found in 16 (17%) and 28 (30%) isolates respectively. The inhibitor-resistant TEM phenotype (IRTs) was present in 8 (8.6%) isolates, **Table 4.9**.

Table 4. 9: β- lactamase phenotypes encountered among the 93 isolates analysed

Antibiotics to which isolates were resistant							
Penicillin	Third generation cephalosporins	Fourth generation cephalosporin cefepime	β-lactamase inhibitor (AMC)	Cephamycin (Cefoxitin)	Carbapenem e.g.imipenen	Most probable phenotype	Total n (%)
AMP	None	None	-none	-none	- none	NSBL	22 (23.6)
AMP	None	-none	AMC	-none	- none	IRT	8 (8.6)
AMP	CTX /CAZ/CRO	-none	-none	-none	- none	ESBL	19 (20.4)
AMP	CTX,CAZ/CRO	FEP	AMC	-none	- none	CMT	28 (30)
AMP	CTX,CAZ/CRO	FEP	AMC	FOX	- none	PAmpC	16 (17)

β-lactamase phenotypes observed in the isolates were defined as: **NSBL**-resistance to penicillins but susceptible to other classes of β-lactam antibiotics; **IRT**- resistance to β-lactamase inhibitors (Amoxicillin-Clavulanic acid) and ampicillin but with concomitant susceptibility to all other classes of β-lactams; **ESBL**- resistance to Penicillins and cephalosporins with concomitant susceptibility to a β-lactamase inhibitor, cephamycins and carbapenems; **CMT**-resistance to most β-lactams and β lactamase inhibitor (AMC)

but Susceptible to cephamycins and carbapenems; **pAmpC**- resistance to all generations of β lactam antibiotics, were susceptible to carbapenems.

4.4.1 Distribution of β-lactamase phenotypes across species

Analysis of the diversity of the phenotypes across species revealed that 22 (41%) and 6 (11%) of the NSBL and IRT phenotypes respectively originated from *E.coli* species. A majority of the ESBL phenotype also originated from *E. coli* 12 (22%), *K. pneumonia* 4 (17%), *P. aeroginosa* 2 (22%) and *Acinetobacter* 1 (17%). The CMT and pAmpC phenotypes were prevalent in *K. pneumonia* species at 13 (54%) and 7(29%) respectively, **Table 4.10.**

Table 4. 10: Distribution of β-lactamase phenotypes across species

	n	**NSBLs**	**IRTs**	**ESBLs**	**CMTs**	**pAmpC**
E.coli	**54**	22 (41%)	6 (11%)	12 (22%)	9 (17%)	5 (9%)
K. pneumonia	**24**	0	0	4 (17%)	13 (54%)	7 (29%)
P. aeroginosa	**9**	0	1 (11)	2 (22%)	3 (33%)	3 (33%)
Acinetobacter spp	**6**	0	1 (17%)	1 (17%)	3 (50%)	1 (17%)

Distribution of the various phenotypes across the 93 isolates analysed. **NSBLs**-Narrow spectrum β-lactamases; **IRT**-Inhibitor resistant TEM phenotype; **ESBL**-Extended Spectrum β-lactamases; **CMTs**- Complex mutant TEMs phenotypes; **pAmpC**-plasmid-mediated ampicillin β-lactamases phenotypes.

4.4.2 Prevalence of β-lactamase genes

The most prevalent β-lactamase gene in all isolates was *bla*$_{TEM}$ (58%) followed by *bla*$_{CTX-M}$ (45.2%). Other classes of β-lactamase genes detected were *bla*$_{SHV}$ and *bla*$_{OXA}$ accounting for 29% and 22.6% respectively. In this study, co-existence of multiple *bla* genes in the same isolate was observed in *E. coli* and *Klebsiella pneumonia*, in the following combinations; *bla*$_{CTX-M/TEM}$, *bla*$_{CTX-M/SHV}$ and *bla*$_{SHV/TEM,}$ **Plates 4.1 and 4.2.**

Among *E.coli* isolates, *bla*$_{TEM}$ were 72.2%, *bla*$_{CTX-M}$ 38.8%, with *bla*$_{OXA}$ and *bla*$_{SHV}$ being 5.6% each. All the *K. pneumoniae* isolates had the *bla*$_{SHV}$ gene with 50% of the isolates expressing *bla*$_{TEM}$ and *bla*$_{CTX-M}$. A majority (66.6%) of *P. aeroginosa* isolates had the *bla*$_{OXA}$ gene, **Table 4.11**. Acinetobacter spp had *bla*$_{TEM}$ and *bla*$_{OXA}$ genes at 50% each.

Table 4. 11: Distribution of selected genes across species

	n	***bla*$_{TEM}$**	***bla*$_{SHV}$**	***bla*$_{CTX-M}$**	***bla*$_{OXA}$**
E.coli	54	39 (72)	3 (5)	21 (39)	3 (5)
K. pneumoniae	24	12 (50)	24 (100)	15 (63)	12 (50)
P. aeroginosa	9	0 (0)	0 (0)	3 (33)	6 (67)
Acinetobacter	6	3 (50)	0 (0)	5 (83)	0 (0)

Table shows number (%) of selected β-lactamase producing genes in isolates obtained from urine among pregnant women attending antenatal clinic at Pumwani Maternity Hospital.

The PCR gene results of a few representative isolates are shown in **Plates 4.1 and 4.2**.

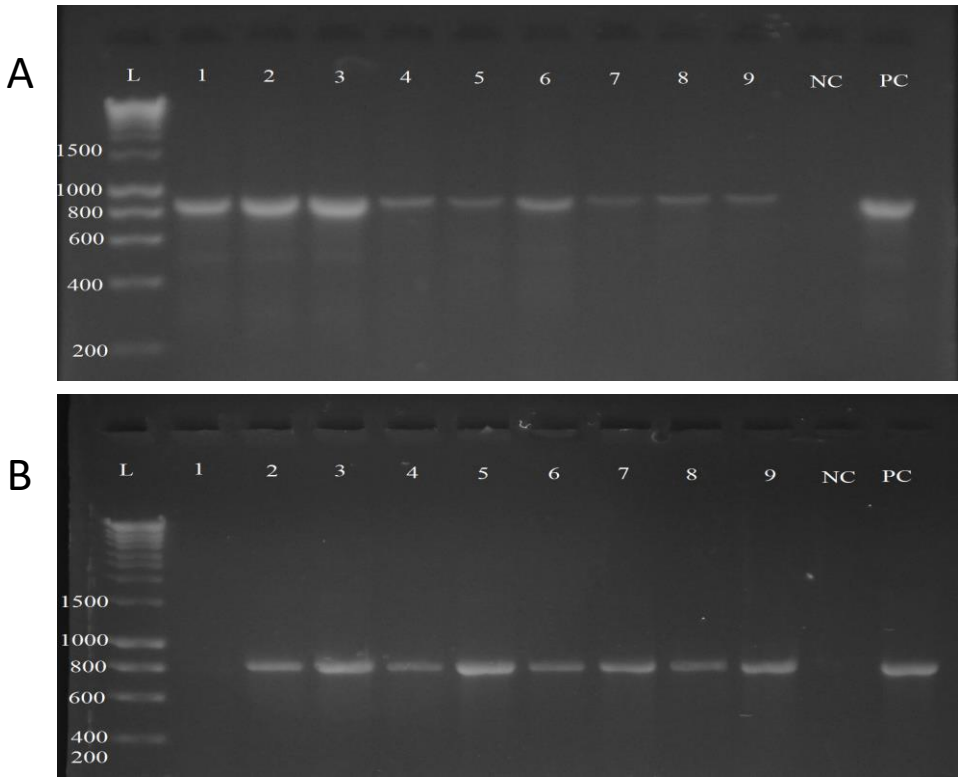

Plate 4. 1: Electrophoresis gel results for blaTEM and blaSHV gene

Plate A: *bla*$_{TEM}$ gene (865 bp); **Plate B**: *bla*$_{SHV}$ (795bp); **L**-Molecular weight Ladder; **NC**-Negative Control (Sterile distilled water); **PC**-Positive Control (known positive control strains).

*****Numbers at the top represent random DNA numbers of the UTI isolates.

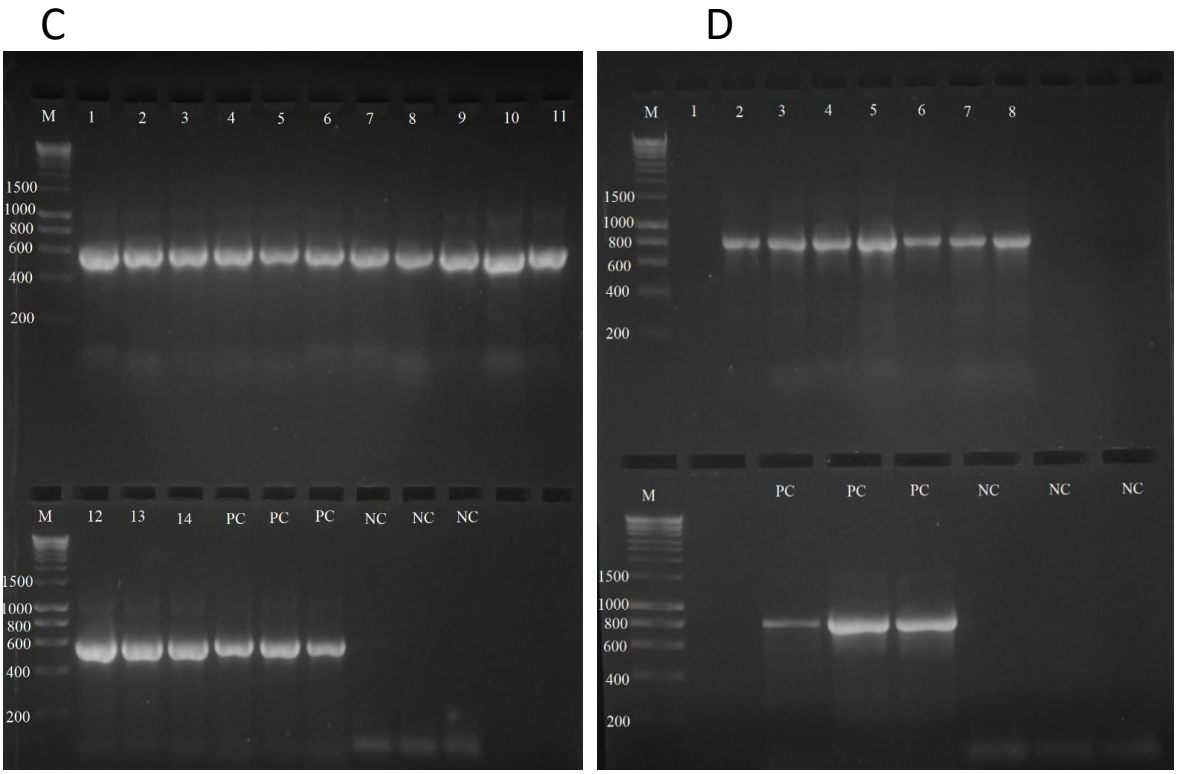

Plate 4. 2: Electrophoresis gel results for blaCTX-M and blaOXA genes

Plate C: *bla*$_{CTX-M}$ gene (593 bp); **Plate D**: *bla*$_{OXA}$ (820bp); **M**-Molecular weight Ladder; **NC**-Negative Control, (Sterile distilled water); **PC**-Positive Control, (known positive control strains).

*****Numbers at the top represent random DNA numbers of the UTI isolates

4.5 Genetic diversity of the UTI isolates

4.5.1 Genetic diversity of E. coli isolates

The results of genetic similarity testing revealed that out of the 18 *E. coli* isolates, 14 exhibited 90% similarity with four (4) demonstrating approximately 82% similarity. Clusters 1-3 had heterogenous resistance phenotypic characteristics, parity and the quantitative counts. Cluster 1 had two isolates, a contaminant and a UTI isolate having 99% genetic similarity. The UTI isolate had a combined resistance to Cephalosporins and SXT while the contaminant showed resistance against Fluoroquinolones and SXT. Sub –clusters 4-6 had 5 isolates (four from multiparous women). Two of these isolates had similar resistant phenotypes with 3 showing resistance against Cephalosporins, floroquinolones and SXT. The quantitative counts of these isolates ranged from below UTI threshold to moderate counts. Clusters 7-10 had 5 isolates, four from multiparous women with CFUs/ml ranging from low to moderate. A majority (80%) of the isolates showed resistance against NAL and SXT. The isolates in this clusters were isolated from asymptomatic UTI cases. Clusters 11 and 12 had 3 isolates with 96% genetic similarity, different resistance phenotypes and the quantitative counts ranged from moderate to high, **Figure 4.3.**

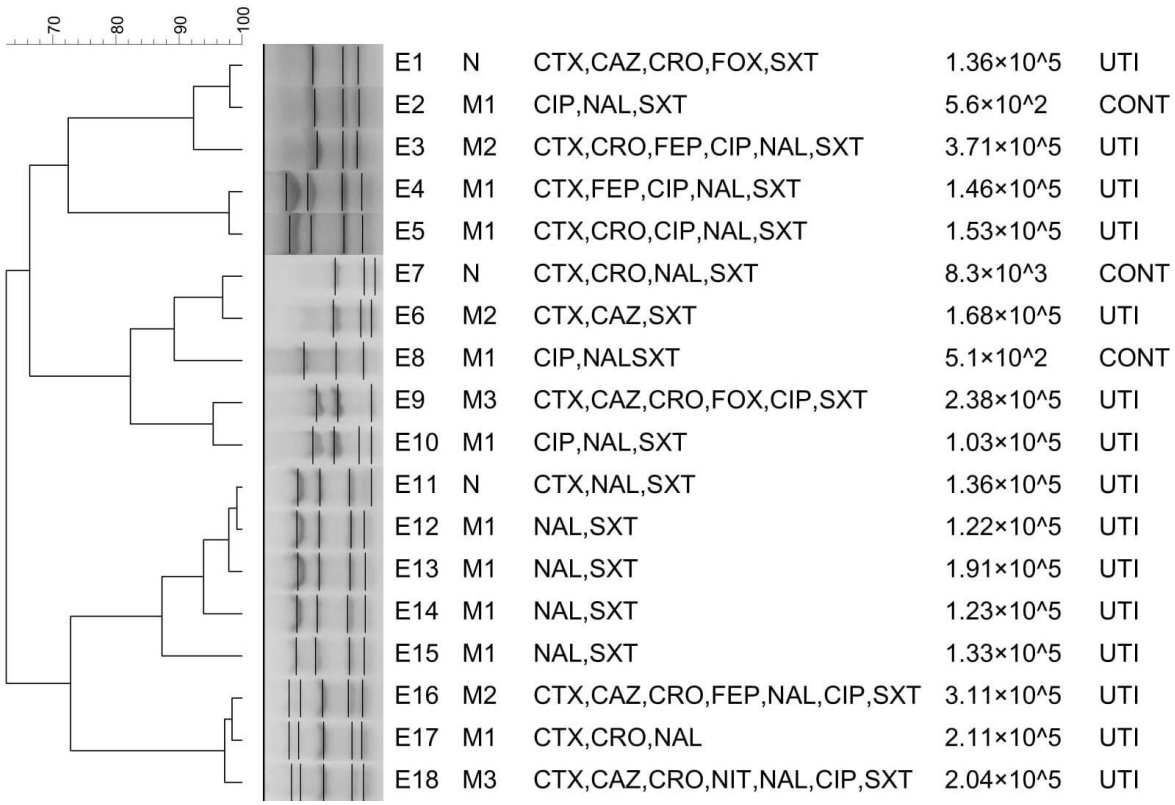

Figure 4. 3: Dendrogram showing the genetic diversity of *E. coli* isolates.

*E-Prefix denotes *E. coli*, N-Nulliparous, M1-Multiparous, one child; M2- Multiparous, two children; M3- Multiparous, three children, CAZ-Ceftazidime; CRO-Ceftriaxone; CTX-Cefotaxime; FEP-cefepime; FOX- Cefoxitin; NAL-Nalidixic Acid; CIP-Ciprofloxacin; SXT-Sulfamethoxazole/Trimethoprim; NIT-Nitrofurantoin; UTI-Urinary Tract Infection; CONT- Contaminant- isolates from samples with less than 1×10^5 CFU/mL.

4.5.2 Genetic diversity of K. pneumoniae and S. aureus isolates

The *K.pneumoniae* isolates in this study shared 98% genetic similarity. Cluster 1 had two isolates originating from both nulliparous and multiparous mothers. These isolates showed combined resistance against cephalosporins, cephamycin and fluoroquinolones.

Isolates in cluster 2 and 3 originated from multiparous women, had heterogenous resistant phenotypes with counts ranging from low to moderate. Cluster 4 had 2 isolates which originated from nulliparous mothers with CFU/mL ranging from low to moderate counts. Among the *K. pneumoniae* isolates combined resistance against Cephalosporins, Floroquinolones and Cephamycin was observed in 75% of the isolates, **Figure 4.4 (A).**

Out of the 10 isolates, 6 (60%) had 55% genetic similarity and 4(40%) being 30% similar. Cluster 1-2 comprised of 3 isolates whose counts fell below the UTI threshold (contaminants), had different resistant profiles and 60% genetic similarity. Clusters 3-6 comprised of 5 isolates from UTI cases. Three of the isolates were from multiparous mothers while 2 were from nulliparous. The resistant phenotypes of the isolates were not similar. Isolates in cluster 7 were contaminants which originated from nulliparous mothers and had homogenous phenotypic characteristics. From this dendrogram, isolates whose counts fell below the UTI shreshold (contaminants) didn't cluster together with any of the *S, aureus* isolates that caused UTI in this study, **Figure 4.4 (B)**. This is contrary to the Gram-negative isolates in which the contaminants clustered together with the UTI strains.

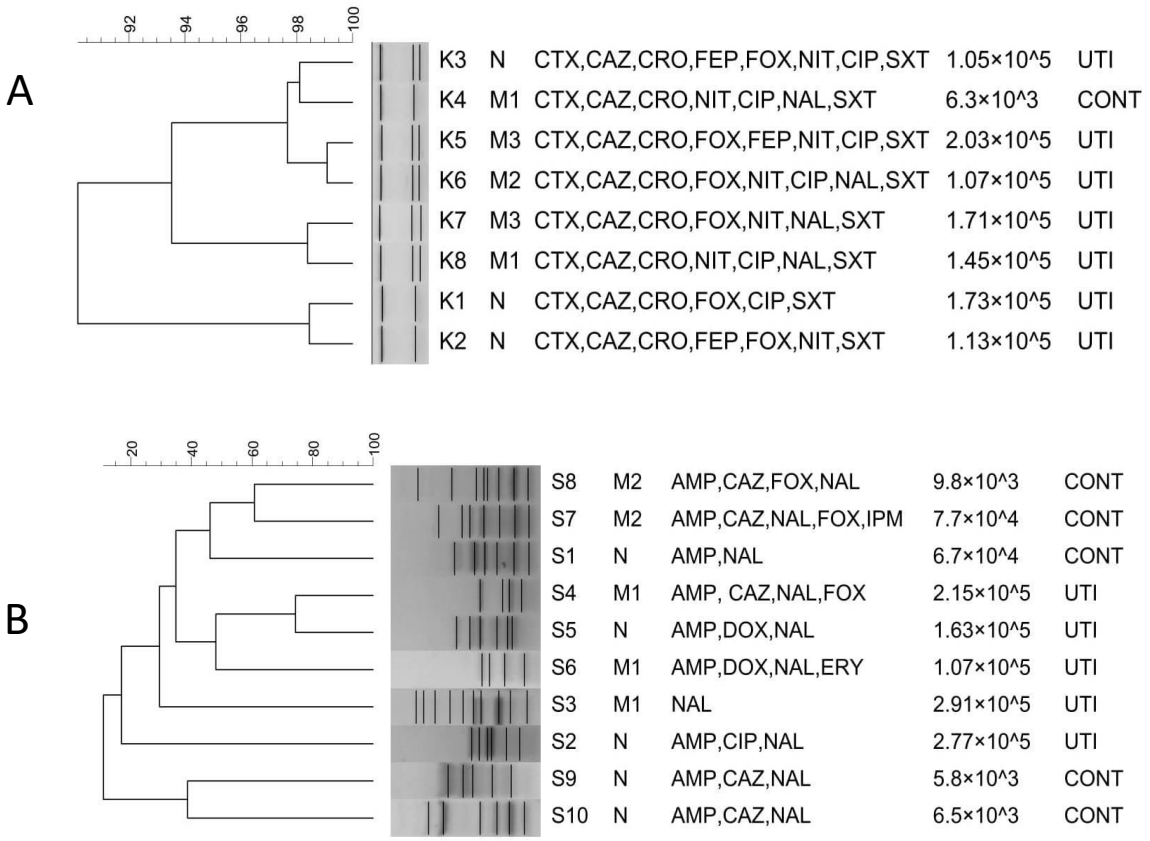

Figure 4. 4: Dendrogram showing the genetic diversity of (A) *K. pneumonia* and (B) *S. aureus* isolates.

*K-Prefix denotes *K. pneumoniae*, S denotes *S. aureus*; N-Nulliparous, M1-Multiparous, one child; M2- Multiparous, two children; M3- Multiparous, three children, CAZ-Ceftazidime; CRO-Ceftriaxone; CTX-Cefotaxime; FEP-cefepime; FOX-Cefoxitin; NAL-Nalidixic Acid; CIP-Ciprofloxacin; SXT-Sulfamethoxazole/ Trimethoprim; NIT-Nitrofurantoin; DOX- Doxycycline; IPM-Imipinem; ERY-Erythromycin UTI-Urinary Tract Infection; CONT- Contaminant- isolates from samples with less than 1×10^5 CFU/ml.

4.5.3 Genetic diversity of P. aeroginosa, Acinetobacter spp and Enterococcus spp

The *P. aeroginosa* isolates had 99% genetic similarity. Isolates in the two clusters originated from multiparous mothers. The two UTI isolates had relatively high quantitative counts ranging from 2.73-2.78 $\times 10^5$. The isolates had different phenotypic characteristics **Figure 4.5 (A).**

The two Acinetobacter isolates which originated from multiparous mothers had 100% genetic similarity and identical phenotypic characteristics. The two isolates showed resistance against ceftriaxone, cefoxitin, nitrofurantoin and chloramphenicol **Figure 4.5 (B)**.

The two *Enterococcus* spp had 30% genetic similarity, formed 2 sub-clusters with dissimilar resistant phenotypes. The isolates were from multiparous mothers who had UTI **Figure 4.5 (C).**

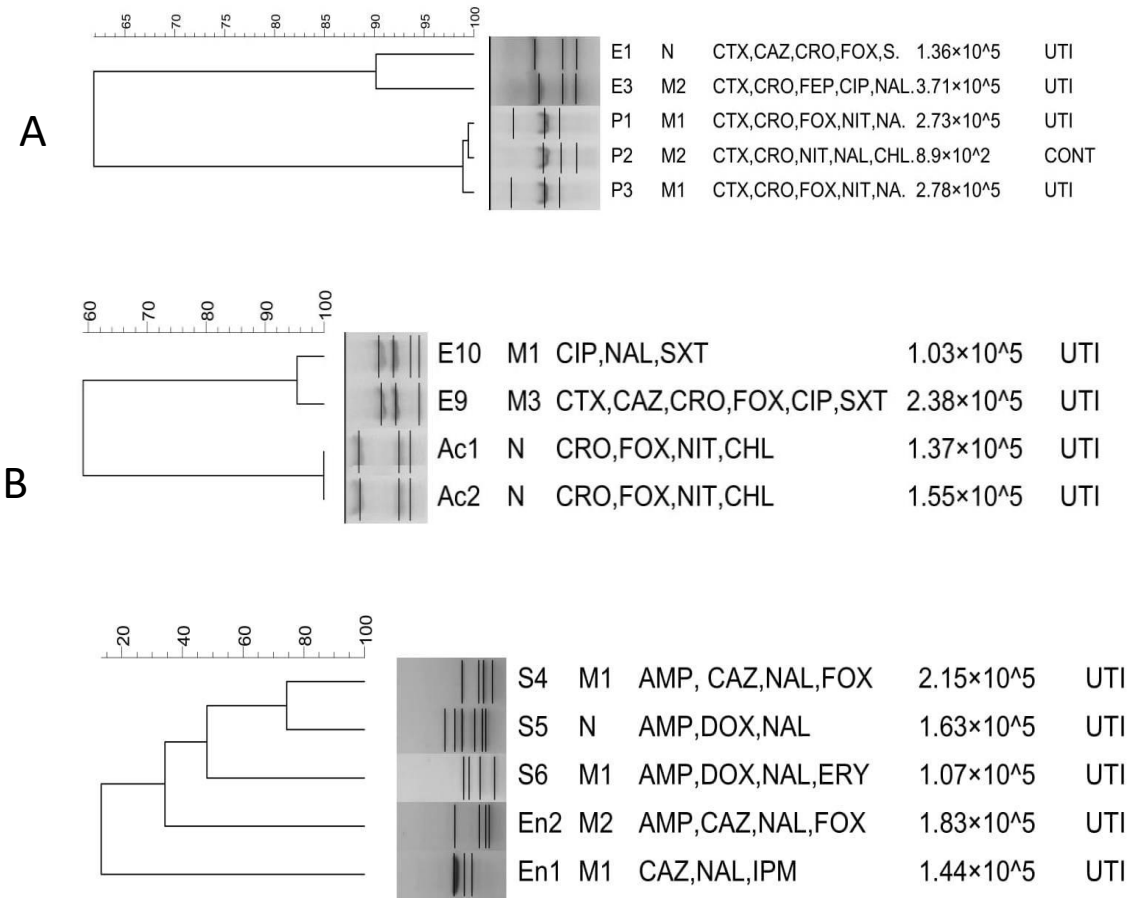

Figure 4. 5: Dendrogram showing the genetic diversity of (A) *P. aeroginosa*, (B) *Acinetobacter* spp and (C) *Enterococcus* spp.

*P-Prefix denotes *P. aeruginosa*; Ac- *Acinetobacter* spp, En- *Enterococcus* spp. N-Nulliparous, M1-Multiparous, one child; M2- Multiparous, two children; AMP-Ampicillin; CRO-Ceftriaxone; CTX-Cefotaxime; CAZ- Ceftazidime; FOX- Cefoxitin; NAL-Nalidixic Acid; CIP-Ciprofloxacin; SXT-Sulfamethoxazole/Trimethoprim; NIT-Nitrofurantoin; CHL-Chloramphenicol; IPM-Imipinem UTI-Urinary Tract Infection; CONT- Contaminant- isolates from samples with less than 1×10^5 CFU/mL. N/B- The E and S-prefix organisms were used as references in these dendrograms.

CHAPTER FIVE

DISCUSSION

5.1 Discussion

The overall prevalence of urinary tract infection among pregnant women attending antenatal clinic at Pumwani Maternity Hospital in this study was 15.7% regardless of the women's age, parity and gestation. This falls within the global prevalence of 13%-33% (Agersew *et al*., 2012) and is comparable to the prevalence reported in Tanzania 15.5% (Masinde *et al.,* 2009), but higher than studies in Uganda 13.3% (Andabati *et al*., 2010) and Ethiopia 10.4% (Agersew *et al*., 2012). This was however lower than the prevalence of 31.3% reported in Egypt (Dimetry *et al*., 2007). Variations in prevalence rates from one country to another and among different regions of the continent can be attributed to environmental, economic and social habits of a community. In this study, knowledge of UTIs among the pregnant mothers was very low (37.6%) and only 9% could link UTI acquisition to poor urogenital hygiene which was found to be significantly associated with these infections. This may partially fuel the high prevalence because fewer women seek treatment in time leading to poor treatment outcomes especially in well-established infections.

Gram-negative bacteria isolates were more prevalent (78.8%) than Gram-positive bacteria (21.2%). Similar rates of isolation has been reported in Tanzania and Kenya (Moyo *et al.,* 2010;Nabbugodi *et al*., 2015). Since most UTI pathogens are of feacal origin, their prevalence can be attributed to the anatomy of the female genitalia-close proximity to the anal opening, the up-massaging of the Gram-negative feacal bacteria from the perenium up the vagina during sexual intercourse, this coupled with short urethra in females and poor urine flow during pregnancy favours their aetiology in UTIs. *E. coli* was the most predominant pathogen with overall isolation rate of (44.5%) similar to other findings in Tanzania, Sudan and Ethiopia (Masinde *et al.,* 2009; Hamdan *et al*.,

2011; Agersew *et al*., 2012). Difficulty in maintaining personal hygiene during pregnancy as well as the anatomical and functional changes that occur during this period may increase the risk of acquiring UTIs from *E. coli* being a commensal of the bowel. *K. pneumonia* was the second most prevalent uropathogen similar to related studies in the neighboring Tanzania (Masinde *et al.,* 2009), and Korea (Chin *et al.,* 2011). Among Gram-positive organisms, *S. aureus* was dominant at 15.1% followed by *Enterococcus* spp at 6.1%. The bacterial isolates (*E. coli*, *K. pneumoniae*, *S. aureus*, *P. aeruginosa*, *Acinetobacter* and *Enterococcus* spp) identified in this study are comparable to those of many other studies either regionally or internationally, however, different results have also been reported. The differences and similarities in the distribution of the pathogens causing UTIs may be due to different environmental conditions, host factors and practices such as health care, education programs, socio-economic status and hygiene practices in each country.

Different socio-demographic factors have been documented to contribute to occurrence of UTIs among pregnant women. This includes gestation, age, parity, level of education and occupation (Dimetry *et al.,* 2007;Smaill *et al.,* 2007; Wamalwa *et al.,* 2013). In this study, there was no significant association between UTIs and maternal age, parity, occupation, gestation, marital status or level of education making it comparable to other studies (Masinde *et al.,* 2009; Sheikh *et al.,* 2000). A large proportion of the mothers (75.7%) who had UTIs belonged to the age group 21-30 years, this age group is the peak reproductive period in most societies, Kenya included. This compares well with studies done by Wamalwa *et al.* (2013), Masinde *et al.* (2009) and Gulfareen, (2010). Multiparous women had a higher prevalence of UTIs (72.7%) compared to nulliparous women (27.3%). These findings are however contrary to reports by El Sheikh *et al,* (1999) who in their study in Sudan reported a decreased incidence of bacteriuria with age of patients and significantly high bacteriuria in nulliparous than multiparous women. In their study, this finding was attributed to the trauma caused by the movement of the

penis in the vagina hence the increased prevalence of UTIs among young and nulliparous women.

Herein, we further observed that pregnant women in their second trimester of pregnancy had the highest prevalence of UTI (60.6%) followed by the third and first trimesters which is in agreement with the findings of Masinde *et al.* (2009) and Obirikorang *et al.*(2012). It is however contrary to the findings of Turpin *et al*. (2007) who reported a high percentage of UTIs during first and early second trimesters. Results from this study were in concordance with those from other studies that have reported that majority of the mothers who suffered UTIs are in the second trimester. The risk of UTI is set to begin in the 6^{th} week and peaks during weeks 22 to 24, due to decreased tone, urethral dilatation, decreased urine concentration and increased stasis as well as hormonal changes, (Delzell *et al*., 2000), all these factors contribute to the risk with increased pregnancy duration. The high prevalence of UTIs in the second trimester can partially be attributed to the rapid changes in the physiology and immunology of pregnant women and due to the frequency of UTIs tests during this phase.

Increasing rates of resistance among bacterial uropathogens has caused growing concern in both developed and developing countries. A rise in bacterial resistance to antibiotics complicates treatment options for UTIs. Currently, most patients are treated empirically without laboratory based bacteriological investigation and this could be fuelling resistances due to use of sub- optimal agents (Kariuki *et al*., 2011). Out of the 210 pregnant mothers recruited in this study, 8 (3.8%) had been on UTI treatment in the last two weeks yet they still had bacteriuria. This implies that some of the UTIs persist despite treatment further indicating that the current treatment guidelines may not be effective.

The overall prevalence of UTI among barrier contraceptive users was high (63.3%)**.** There was about a two-fold increase in risk of the development of UTI among barrier contraceptive users (OR=2.62) compared to hormonal contraceptive users. This finding

is consistent with other studies (Hooton *et al.,* 1996;Fallahian *et al.,* 2009). Condom use is still a major factor to UTI due to poor lubrication on the condom resulting into friction during intercourse making it possible for pathogens to enter and colonise the urethra (Dimetry *et al.,* 2007). Couples should therefore be advised to ensure sufficient lubrication. On the basis of these findings, contraceptive use is a significant risk factor for acquiring UTIs with the barrier methods being more predisposing. This calls for immediate intervention in terms of health education, promotion and encouragement of the use of contraceptive methods that carry lesser risks of urinary tract infection.

Among the lifestyle factors surveyed, high rate of bacteriuria was found among women who used undergarments made of non-cotton material and those who had sex more than twice a week. Previous studies indicates that simple hygiene habits such as voiding before and after sexual intercourse, use of absorbent cotton undergarments and wiping from anterior to posterior are advocated to decrease the risk of UTI (Griebling *et al.,* 2007; Dimetry *et al*., 2007). Material of the undergarment and frequency of changing the undergarments were also found to be significantly contributing to the occurrence of UTI ($P<0.05$), similar to a previuos study conducted in Egypt (Dimetry *et al.,* 2007). There is a possibility that the moisture trapped by non-cotton undergarments favor the proliferation of UTI agents including yeast hence increasing the chances of UTIs. Majority of pregnant women in this study used undergarments made of materials other than cotton that is absorbent. This finding could be explained by the fact that cotton undergarments are expensive hence most mothers opt to buy affordable and attractive ones made of non-cotton materials.

From the foregoing, it is clear that limited knowledge on UTIs may play a critical role in acquisition of UTIs. Being with multiple sexual partners had a two- fold increase to UTI occurrence. This can be attributed to cross contamination from partners in the sex network. Frequency of sexual intercourse (≥2 times/ week) had moderate association with UTI occurrence similar to findings from a study conducted in Egypt (Dimetry *et al.,* 2007). In general, UTIs are not considered as sexually transmitted infections (STIs)

but this data suggest that males may be an important factor in the spread of UTIs between partners. There is a need to include both sexual partners for UTI diagnosis and treatment especially during pregnancy.

Beta-lactam antibiotics, such as Penicillins and Cephalosporins, are considered safe during pregnancy and are usually prescribed for the treatment of UTIs during the gestational period (Guinto *et al*., 2010). All the Gram-negative isolates except *Acinetobacter* showed resistance in the range of 89%-100% to AMP and SXT. These results are similar to those reported in two related studies in Kenya indicating that *E. coli* recovered in Kenya from urine are more resistant to these antimicrobials than among similar isolates recovered in developed countries such as Japan (Bii *et al.*, 2017). This is particularly worrying because SXT is heavily relied upon as a prophylaxis against opportunistic infections among individuals infected with HIV in Kenya.

Resistance of Gram-negative isolates against Fluoroqunolones was in the range of 20-100% depending with the species. This trend needs to be monitored closely since Fluoroquinolones are thought to be the most effective antibiotic agents against *E. coli* infections (Oladeinde *et al.,* 2015).

In recent years, Kenya has reported development of resistance to Fluoroquinolones and extended-spectrum beta-lactams in uropathogenic *E.coli* (Kariuki *et al.,* 2007). This high rate of resistance against flouroquinolones has also been reported by other studies (Gobernado *et al.,* 2007; Sabharwal, 2012). In several studies, it has been shown that the prescribing habits of the physicians are the driving factor for the antibiotic resistance for this group of antibiotics (Goossens *et al.,* 2005). The apparent increase in fluoroquinolone-resistant strains in Kenya and the world could be attributed to the wide use of these antimicrobial against ESBL-producers and in the treatment of UTIs and respiratory infections. Other reports have indicated that the overuse of fluoroquinolones in human and veterinary medicine could also breed resistance (Schwarz *et al*., 2001). This continued increase in fluoroquinolone resistance rates affects patient management

and necessitates a change in some current treatment guidelines. This study shall strengthen the epidemiological database and would help foster prudent decisions in the countrys forthcoming antibiotic policies.

An alarming finding in this study was the high prevalence of resistance to third generation Cephalosporins among the Gram-negative uropathogens. An earlier study conducted in Kenya reported similar resistance patterns in uropathogens (Bii *et al*., 2017). Cephalosporins have been deemed safe during pregnancy and are commonly prescribed for the treatment of UTIs (Guinto *et al.,* 2010). Third generation Cephalosporins have been in use for a long period of time and must have been abused and overtime organisms have developed a wide range of resistance mechanisms. Resistance to third generation Cephalosporins would increase the cost of treatment because more expensive antimicrobials are required for their replacement. Pregnant women with such highly resistant strains are more likely to have recurrent UTIs, thus the spread of such resistant strains may lead to poor perinatal and maternal outcomes in developing countries where patients may not afford more effective but fairly expensive antimicrobials. Resistance to NIT (another important antimicrobial for UTIs) was noted in 50% of the isolates. Nitrofurantoin has already been demonstrated safe for use during pregnancy, however it only achieves therapeutic levels in the urine therefore cannot be used to treat pyelonephritis.

In this study, 14.3% of *K. pneumoniae*, 20% of *S. aureus* and all *Enterococcus* showed resistance to Imipinem. Although our sample size was rather small to determine national trends in resistance to Carbapenems, these findings are worrying considering that carbapenemase-producers in this study were recovered from otherwise asymptomatic participants. While this class of drugs remain useful for the treatment of serious infections, isolates resistant to carbapenems pose a serious challenge because none or few chemotherapeutic options remain for the seriously ill patient. The use of carbapenems as an alternative in developing countries is also a challenge because few

patients can afford this antimicrobials. Increased resistance to this class of antimicrobial will therefore lead to significant increase in mortality and morbidity of patients.

This study revealed high resistance prevalence in pregnant mothers with tertiary level of education compared to other levels of education. There is a possibility that this category of mothers relies on self-medication hence the high resistances exhibited. Healthcare facilities have inadequate diagnostic capacity and the population has limited access to formal healthcare services as demonstrated by the prevalence of self-medication. Retail pharmacies are frequently operating without a license and appear to be more accessible to most patients; they are located within the community, do not charge consultation fees, have shorter waiting times and are usually willing to negotiate treatment protocols to meet the financial needs of clients (Goel *et al*., 1996). A number of pilot studies in Kenya estimated that 65% of pharmacies dispense without a doctor's prescription (Kwena *et al.*, 2008). The fact that these drug retailers also appear to be an important source of information about illnesses in general could be a contributing factor to this group resorting to self-medication.

Multi drug resistance (MDR) was observed in 96% of the isolated uropathogens, this is comparable to the findings in Ethiopia (Agersew *et al.*, 2012) and is indicative of very high resistance to commonly used antibiotics. Antibiotic resistance has been recognized as the consequence of antibiotic use and abuse (Albrich *et al*., 2004). This alarming phenomenon can be attributed to use of sub-optimal agents and inappropriate administration of antimicrobials in empiric therapies that can result in a shift to increased prevalence of resistant isolates in the community.

Antimicrobial resistance patterns of isolates whose counts fell below the UTI threshold were similar to those of strains recovered from UTI-cut point specimen (cases). This confirms that indeed these isolates may be implicated in UTIs and the low counts may partially be due to a receding or an establishing infection. If these "contaminants" represent a resistant population of uropathogens, they may become established and lead

to serious and untreatable UTIs. Based on these results, it is therefore important to revise the existing diagnostic guidelines for UTIs. Further investigation based on genome analysis may in future reveal the genetic relatedness between these contaminants and the UTI isolates.

Based on the resistance profiles of the isolates investigated, 20.4% of all isolates were ESBL-producers. This study found out that majority of the ESBL- producers exhibited co-resistance to multiple antimicrobials such as Fluoroquinolones, Aminoglycosides, SXT and third generation Cephalosporins. Treatment of ESBL-producing bacteria is limited to carbapenems such as imipinem or alternatively fluoroquinolones and aminoglycosides which may be used if these antibiotics exhibit *in vitro* activity (Paterson *et al*., 2005). However, co-resistance of third generation Cephalosporins with Aminoglycocosides and/or Fluroquinolones among ESBL-producers from this study is worrisome. The use of any of these classes of antimicrobials can lead to treatment failure if empirically prescribed for Gram-negative infection causing strains in the absence of culture and susceptibility testing. The use of Carbapenems would not be a tenable option in developing countries since they are expensive to the majority and their increased use may further drive antimicrobial resistance resulting to even more limited therapeutic options.

Clinically, ESBLs limit the efficacy of β-lactams, including cephalosporins, and are associated with high morbidity and mortality (Rawat & Nair, 2010). MRSA can easily spread within the community and cause multiple infections especially to persons who are immune-compromised. There is no doubt that the ESBLs and MRSA strains are playing a critical role as causes of UTIs in Kenya and this will pose a serious treatment challenge because of the ever-reducing treatment options. In the face of increasing resistance, there is a need to devise infection control methods that can reduce the incidences of UTIs. Such measures will also include educating people, especially the most vulnerable groups (women) on predisposing and risk factors for UTIs.

In Kenya, there are limited reports on the prevalence of various β-lactamase phenotypes and genotypes in urinary isolates from pregnant women and this is a critical area of public health concern. Based on the resistance profiles exhibited in this study, IRTs, ESBLs, CMTs and pAmpC producers pose the greatest clinical and chemotherapeutic challenges and should be the most importantly monitored bacterial strains to prevent their spread in the community. Although IRTs are less prevalent than ESBLs, they are of great clinical significance because they result in therapeutic failure when inhibitor-based antimicrobials are prescribed without determining susceptibility profiles of the pathogen (Henquell *et al.,* 1994). ESBL-producing microorganisms exhibit co-resistance to many other classes of antimicrobials, resulting in limited therapeutic options in both hospital and community settings (Livermore, 2008). Increase in the prevalence of such highly resistant strains may lead to ineffectiveness of most β-lactam antibiotics thus creating limited therapeutic options in management of hospital and community acquired infections in Kenya. The high use of antimicrobials to treat infections emperically has led to the selection of high resistance profiles encountered in this study. This gross misuse of β-lactam antibiotics has arisen from inadequate surveillance and limited culture and susceptibility data, non-compliance in antibiotic use, self medication, poverty and lack of enforcement of antibiotic policy in Kenya. This partially explains the complex phenotypes such as ESBLs, CMT and pAmpC encountered in this study.

A large proportion (58.1%) of the isolates with NSBL phenotype tested positive for *bla*$_{\text{TEM}}$. This *bla*$_{\text{TEM-1}}$ encodes for β- lactamases with extended spectrum responsible for up to 90% of Ampicillin and Penicillin resistance in *E. coli* as well as resistance in *H. influenza*, *N. gonorrhoeae* and *K. pneumoniae,* it is the most commonly encountered β-lactamase gene among Gram-negative bacteria (Kiiru *et al.,* 2012). The *bla*$_{\text{SHV}}$ accounted for 29% of all the isolates analyzed. The *SHV*-1 β-lactamases are most commonly found in *K. pneumoniae* and is responsible for up to 20% of the plasmid-mediated ampicillin resistance among Enterobacteriaceae species. All the *K. pneumoniae* isolates in this study were positive for *bla*$_{\text{SHV}}$, thus there is need to closely

monitor the spread of NSBLs since mutations arising from these derivatives of NSBLs are responsible for the exponential increase in antimicrobial resistance worldwide.

Further analysis revealed that *bla*$_{\text{CTX-M}}$ accounted for 45.2% of the *bla* genes in these isolates. Majority of these ESBL isolates were resistant to Cefotaxime, a known indicator for carriage of *bla*$_{\text{CTX-M}}$ genes. The most plausible explanation to these findings is that in most instances, $_{\text{CTX-M-15}}$ enzymes are found alongside $_{\text{TEM-1}}$ enzymes in a common plasmid and dissemination of these plasmids can be passed conjugatively among enterobacteriacea. This therefore makes $_{\text{CTX-M-15}}$ the most common prevalent ESBL enzyme globally.

Among the $_{\text{CTX-M}}$ genes, the *bla*$_{\text{CTX-M-15}}$ gene is the most important because is associated with the globally disseminated multi-drug resistant clone *of E. coli* ST131 strains frequently associated with urinary tract and bloodstream infections in both community and clinical settings (D'Andrea *et al.,* 2013). Many ST131 strains exhibit resistance to multiple antimicrobials with key resistance to fluoroquinolones (Price *et al*., 2013). Based on this previous reports, it is highly probable that some of our isolates carrying the *bla*$_{\text{CTX-M}}$ and exhibiting concomitant resistance to fluoroquinolones are members of this clonal complex because resistance to fluoroquinolones with or without the production of ESBL is the primary indicator of members associated with this sequence type (Johnson *et al*., 2013). Previous studies in Kenya have also reported ST131 strains bearing the *bla*$_{\text{CTX-M-15}}$ and/or ciprofloxacin resistance among clinical isolates from hospitalized and non-hospitalized patients (Kiiru *et al.,* 2012). Thus, these strains are likely to present drastic negative health implications to both the mother and child since infections arising from these strains are associated with high possibilities of treatment failure.

The *bla*$_{\text{OXA}}$ gene was the least encountered in this study, with only 22.6% prevalence. The most common $_{\text{OXA}}$- type β-lactamase, $_{\text{OXA-1}}$ has been found in 1-10% of *E. coli* isolates (Livermore, 1995). Although they are predominantly found in *P. aeruginosa*,

they have been detected in many other Gram-negative bacteria. From this study, a large proportion 66.6%% of *bla*$_{OXA}$ gene was found in *P. aeruginosa*, 50% in *K. pneumonia* and 5.6% in *E. coli* isolates. Resistance to AMC in this study can partially be attributed to the carriage of this gene. This hypothesis is partially based on findings from a previous study conducted in Kenya that described *bla*$_{OXA\text{-}1}$ enzymes in *Salmonella* strains that contain mutations in the promoter and confer resistance to β-lactamase inhibitors (Boyle *et al.*, 2011). AMC is one of the commonly used antimicrobials for the treatment of infections caused by ESBL-producers and any increase in the resistance due to a combination of ESBL and the $_{OXA\text{-}1}$ enzymes would jeopardize the clinical significance of this antimicrobial.

From this study, *bla*$_{CTX\text{-}M}$, *bla*$_{TEM}$ and *bla*$_{SHV}$ were the most prevalent *bla* genes combinations. Co-carriage of these *bla* genes partially explains why a significant number of ESBL isolates from this study were resistant to a wide range of β-lactams. While the presence of the ESBL was generally associated with varying degrees of resistance to different classes of antibiotics, the presence of a particular genotype could not predict the susceptibility pattern to a particular drug with the exception of *bla*$_{CTX\text{-}M}$ which was associated with resistance to CTX. Due to limited time and resources, these genes were not sequenced thus it wasn't possible to do plasmid profiles but from the data, it is assumed that plasmids and other mobile genetic elements are involved in this type of resistances.

Genetic analysis using ERIC-PCR revealed diverse genotypic and phenotypic characteristics of the isolates clustered in small groupings of highly similar strains. The genetic diversity among the isolates in this study can be explained by the fact that the isolates were not epidemiologically related; they had been isolated from pregnant women living in different locations within Nairobi County. Currently, most primary care settings rely on dipstick analysis and urine microscopic examinations for diagnosis of UTIs. Based on the findings of this study, despite the close genetic relatedness among these urine isolates, there were diverse resistance profiles observed on individual isolates

that clustered together. It is imperative that culture and sensitivity be used for diagnosing UTI and treatment should always be guided by sensitivity reports. An important finding in this study is the clustering together of the supposed contaminants with the pathogenic strains that cause UTI suggesting that these isolates are actually pathogenic despite giving lower quantitative counts. There is a likelihood of cross transfer of resistance genes among these isolates hence the similar resistance phenotypes exhibited.

This study also revealed close genetic relatedness among microbes that caused asymptomatic bacteruria as they clustered together and had near homogenous phenotypic characteristics. Since these isolates originated from different patients from different locations, this suggests possible exchange of strains between communities. Advanced techniques are recommended to ascertain whether these exchanges are due to clonal expansion or not. When these strains gain resistance determinants, then the potential for an increase in the proportion of resistant strains is likely to be very high in the near future and their subsequent dissemination to the general public is unavoidable. Staph-rep analysis suggested that the isolates clustered based on the pathogenicity. This study demonstrated that pathogenic *S. aureus* didn't cluster together with the supposed "contamintants" suggesting a possible difference in the genetic make-up of the strains that caused UTIs and those whose counts didn't meet the UTI threshold.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

1. This study concluded that UTIs are prevalent among antenatal mothers in Pumwani maternity hospital. UTIs are still a burden to maternal and child health and can therefore vastly contribute to poor perinatal and maternal outcomes.
2. Unsatisfactory personal hygiene practices such as use of undergarments made of materials other than cotton and frequency of changing undergarments have a significant role in developing UTIs during pregnancy. Moisture trapped by these undergarments creates a conducive environment for proliferation of uropathogens.
3. This study further found moderately high level of resistance against first-line drugs and high level of resistance against 3rd generation cephalosporins and fluoroquinolones. It's possible that recent heavy use of these classes of antimicrobials is driving resistances to these antimicrobials. Unless an intervention to deaccelerate this trend of resistance is effected, another class of antimicrobials (e.g) carbapenems may be required. It is therefore possible that morbidity and mortality due to such highly resistant strains is likely to rise.
4. IRTs, ESBL, CMT and pAmpC phenotypes and *bla*$_{TEM}$, *bla*$_{CTX-M}$ pose the greatest clinical and chemotherapeutic challenges since they are associated with varying degrees of resistance to different classes of antibiotics thus limiting therapeutic options. *bla*$_{CTX-M}$ enzymes are found alongside *bla*$_{TEM}$ enzymes in a common plasmid and dissemination of these plasmids can be passed conjugatively among enterobacteriacea.
5. Genetic clustering of the isolates revealed the presence of strains with identical genotypes but with different phenotypic characteristics and vice versa. The diversity of the genotypes and the complex resistance phenotypes as well as the

potential for wide spread dissemination of those prevalent isolates detected in this study suggests possible exchange of resistant genes between possibly less virulent strains and highly drug-resistant strains. When less virulent strains gain resistance determinants, then the potential for an increase in the proportion of resistant strains will be high in the near future and the subsequent dissemination to the general public is inevitable.

6. This study further revealed that the organisms usually considered as urine "contaminants" have similar resistance patterns as well as a high genetic similarity to the isolates causing UTIs. Despite giving lower quantitative counts, this organisms are genetically and phenotypically similar to the pathogenic strains thus may be capable of causing complications during preganancy.

6.2 Recommendations

1. Health education on personal hygiene practices such as wearing cotton undergarments, frequently changing the undergarments, voiding before and after sexual intercourse as well as wiping from anterior to posterior should be emphasized by health care professionals during antenatal visits. All women of reproductive age should be sensitized to seek antenatal care services as soon as they conceive.
2. Measures such as appropriate prescription practices, adequate patient education, adequate diagnostic facilities, limiting unauthorized sale of antimicrobials and appropriate functioning drug regulatory mechanisms should be put in place to minimize resistance pressure. These measures may help to preserve the potency of these antibiotics and increase/improve successful treatment rates.
3. The study emphasizes the need for microbiology laboratories to adequately screen for ESBL-producing strains that cause UTI since infections caused by these organisms are not efficiently treated with β-lactam antibiotics. This will assist in revising existing empiric treatment regimens to periodically reflect prevailing resistance phenotypes. There is also need for efficient infection-

control practices for containment of outbreaks; and intervention strategies, e.g., antibiotic rotation to reduce further selection and spread of these increasingly resistant pathogens.

4. This study has demonstrated the need to combine phenotypic and molecular methods in order to understand important aspects of antimicrobial resistance in developing countries. Continuous surveillance of phenotypic and genotypic drug resistance data, as well as clinical characteristics and treatment outcome for the prevalent strains should be done so as to understand the spread of those successful strains, inorder to make better infection control measures.
5. This study also recommends a review on the existing diagnostic guidelines based on CFUs to pregnant mothers. Due to a combination of the physiological, hormonal and mechanical changes that occur during gestation, lower counts of 10^3-10^4 CFUs/ml could pose a threat to the mother and child hence a review is of utmost importance in preventing any complications that may arise from these uropathens.
6. As antimicrobial resistance among bacterial pathogens is an evolving process, regular monitoring and surveillance is required to establish reliable information about resistance pattern of uropathogens for optimal empiric therapy of patients with UTI. This approach will help slow the emergence of resistance. This study forms a strong basis of future larger studies that should include whole genome analysis that can unravel transmission routes and molecular diversity of the UTI isolates.

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APPENDICES

Appendix 1: Research Consent Form.

TITLE: Prevalence, Antimicrobial Susceptibility Profiles and Genotypic Characterization of Isolates obtained from Urine Samples of Pregnant Women Attending Antenatal Clinic at Pumwani Maternity Hospital, Kenya.

What is the purpose of this research?

You are being invited to take part in this study. We aim to obtain information about the prevalence of urinary tract infection, most commonly implicated bacterial pathogens and antimicrobial susceptibility profiles of these pathogens among pregnant women attending antenatal clinic at Pumwani maternity hospital. Please read all of the following information and also kindly listen as the investigator carefully explains this information to you. You are advised to seek explanation on any words, terms, or sections that are not clear to you. You are also free to ask any questions that you have about this research study. If you accept to take part in this study, we'll request you to sign this form. You should keep your copy for your records. It has information about important names and telephone numbers which you can use in the future either to make any inquiries about this study.

We are requesting you to consent or to decline consent to do the following:

1. To allow us obtain urine samples from you.
2. To allow us keep specimen obtained from you by freezing or using any other means of long term preservations
3. To allow us ship the specimen (Isolates or DNA) from KEMRI to local and international labs in developing countries in this or future studies related to UTI

investigations in order to perform advanced tests such as whole genome sequencing that are not available or are very expensive locally.

I would also like to disclose to you the following;

a) That some questions I will ask may be uncomfortable or embarrassing but all care will be taken to ensure that the interview is conducted in safe, quite place that also guarantees privacy. The data collected will also be confidential and will only be accessible to relevant authorities. The data will also be encrypted to ensure your privacy.
b) That you may find it embarrassing to collect urine specimen. However, I will explain to you the best way and the most comfortable way to do so.
c) That I intend to do tests on your urine to determine whether you have a urinary infection or not. The isolates will also be subjected to antimicrobial susceptibility test after which the genetic relatedness of the isolates will be determined to enable me make good study conclusions. The tests will only be done for research purposes only.
d) That there are no monetary gains for participating in this study.
e) That there are no penalties for declining participation in this study.
f) That you can withdraw from this study at any time.
g) That you may decline to answer any of my questions.
h) My role is to provide the data to help the doctor treat you but you may still be required to meet treatment bills arising from your illness.

Your part in the research

You will be investigated for urinary tract infection causing bacteria. In order to investigate this disease, I ask you to consent on your behalf. Once you grant us the consent, I will obtain a urine sample. **The urine samples will not be stored but will be disposed according to KEMRI biosafety procedures immediately after culture.**

What additional procedures will there be for patients who help with research?

Urine will be taken and should you be found to have a urinary tract infection, your results will be given to a medical doctor who will then treat you. In order to obtain urine specimen, I shall give you a sterile universal container and give you instructions on how to collect and pack the clean catch midstream urine specimen. I intend to collect the

urine specimen once it is available but I will only take specimen collected within two hours.

What will happen after the study?

The data I obtain from this study will be used to know the prevalence, antimicrobial susceptibility profiles, the social, clinical and lifestyle predictors to Urinary tract infections. The data will provide crucial information for your treatment should you be positive for UTI. This data will also be given to the Pumwani maternity hospital administration to help come up with health policies with regards to management of UTI in pregnancy, antimicrobial usage and incorporation of urine culture as routine antenatal care.

What if I change my mind about helping with this research?

If you agree to participate in this study and later change your mind, you are free to withdraw at any time. You will not be discriminated against in any way in the future due to your decision to withdrawal or to decline to participate.

Who will read or hear about information collected from me?

The information collected from those who help with this research will be stored using codes so that each individual cannot be recognized. Coded information will be held on computers protected by passwords known to the research team only.

Do you have any questions that you would like me to answer now? If you would like to know more details about the research or have any issues about your rights that needs to be discussed in the future you can contact any of the following people with priority being the SERU contact.

1. **The Secretary, KEMRI/SERU. P.O Box 54840-00200, Nairobi Tel. 2722541 Ext. 3307.**
2. **Hellen Onyango – 0721293820.**
3. **Dr. John N Kiiru, CMR- KEMRI - 0721-805285**

Laboratory number…….

Telephone number……

I have read the above information and have had the opportunity to ask questions and all of my questions have been answered satisfactorily. I consent to participate in the study as has been explained and as I have understood it.

Signature …….

Date …….

Right or Left hand Thumb print for those who cannot sign

Name of principle investigator……

Signature……
Date…….

Appendix 2: Questionnaire

County_____

Date of interview _____

Name of interviewer _____

Client‘s number _____

Height _____Meters

Weight _____ Kilograms

(A). SOCIAL DEMOGRAPHIC FACTORS

1. Blood pressure _____mmHg
2. Age: _____years (completed years)
3. Marital status (Tick one)

   Single ☐

   Married ☐

   Divorced ☐

   Widowed ☐

4. Parity (number of pregnancies) of the respondent _____

5. How old is your pregnancy? _____

6. Occupation of the respondent (Tick one)

   Employed ☐

   Unemployed ☐

7. Level of education of the respondent (tick one)
   None ☐

   Primary ☐

   Secondary ☐

   Tertiary ☐

(B) CLINICAL FACTORS

8. Have you ever experienced any of these symptoms?

   Pain while passing urine ☐

   Vaginal discharge ☐

   Burning sensation ☐

   Lower abdominal pain ☐

   Urgency to urinate ☐

9. If you have ever experienced any of these symptoms are you experiencing any today?

   Yes ☐ ☐ No

10. If yes to which one (s)?

Pain while passing urine ☐

Vaginal discharge ☐

Burning sensation ☐

Lower abdominal pain ☐

Urgency to urinate ☐

11. Have you been on any medication in the last two weeks?

Yes ☐ No ☐

If yes, what condition were you being treated for.....

12. Have you had a history of hospitalization in the last 3-4 months?

Yes ☐ No ☐

13. Have you ever had a birth one month before your expected date of delivery?

Yes ☐ No ☐

14. Have you used a contraceptive before?

Yes ☐ No ☐

If yes, which one.....

(C) LIFE STYLE FACTORS

15. On average, can you tell me how many times you engage in sexual intercourse in a week?

Yes ☐ No ☐

If yes, how many times?

Once only ☐

Twice ☐

Three times ☐

More than three times ☐

16. Is it possible for you to tell me the number of sexual partners you have?

Yes ☐ No ☐

17. If yes, how many do you have currently?

One ☐

More than one ☐

18. What material of undergarment do you usually use?

Cotton ☐ Non cotton ☐

19. How often do you change your undergarment in a day?

Once ☐ Twice ☐ Thrice ☐

20. In your own thoughts, what do you think causes infection of the urinary system?

No idea ☐

Sexual intercourse ☐

Poor urogenital hygiene ☐

Contaminated dirty toilets ☐

Damp panties ☐

LABORATORY SPECIMEN

Urine findings

A) Urinalysis_____

B) Microscopy_____

C) Culture and Sensitivity_____

Appendix 3: Colonial Morphology, Gram Stain and Biochemical Tests

Colonial morphology will be carried out based on:

1. Size – pin point, small, moderate, or large
2. Pigmentation- Colour of the colony
3. Form – circular, irregular, or rhizoid
4. Margin – Entire, lobate, undulate, serrate, filamentous
5. Elevation – Flat, raised, convex, umbonate

Gram stain

Gram stain will be used to differentiate the microorganisms into either gram positive or gram negative. A smear will be prepared from the colonies using a clean grease free slide, allowed to air dry then heat fixed. The smear will be flooded with crystal violet for 1 minute then washed in water. Lugols iodine will be added to the smear, allowed to stain for 1 minute then washed in water. The smear will be decolorized rapidly in acetone alcohol. Finally the smear will be counter stained with safranin for 2 minutes, washed in water, allowed to air dry then examined under oil immersion objective the morphology of the bacteria (cocci, bacilli) and the gram reaction, purple colour for gram positive and red/pink for gram negative (CLSI, 2005).

Biochemical Tests

Catalase

Catalase test will be used to differentiate staphylococcus from streptococcus. It tests the ability of an organism to produce enzyme catalase which acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. 2-3 ml hydrogen peroxide solution will poured into a test tube, using a sterile wooden stick, a good growth of the test organism will be immersed in the hydrogen peroxide solution. Active bubbling will be considered positive for catalase.

Coagulase

Coagulase test will be used to differentiate *S. aureaus* which produces enzyme coagulase from *S. epidermidis* and *S. saprophyticus* which do not produce coagulase. Coagulase causes plasma to clot by converting fibrinogen to fibrin. A drop of plasma will be placed onto a clean grease free slide. A colony of the test organism will then be emulsified onto the plasma and gently mixed. Clumping within 10 seconds will be a positive test.

Citrate utilization

Citrate utilization tests the ability of an organism to utilize citrate as the only source of carbon and ammonium salt as the nitrogen source. Growth in the medium is shown by a change in colour of the indicator from light green to blue. Saline suspension of test organism will be inoculated into Simmons medium Incubated at 37^0C for 96 hours and observed for growth indicated by a colour change from light green to blue.

Urease

This test is used to identify bacteria capable of hydrolyzing urea using the enzyme urease. It is commonly used to distinguish the genus *Proteus* from other enteric bacteria. The test organism is inoculated in urea broth medium and incubated at 37^0 for 24 hours. Hydrolysis of urea forms a weak base, ammonia, as one of its products. This weak base raises the pH of the media above 8.4 and the pH indicator, phenol red, turns from yellow to pink.

Sulfur indole motility

This test is used to identify the ability of an organism to reduce sulfur, produce indole and be motile. SIM is commonly used to differentiate members of *Enterobacteriaceae*. The test organism is inoculated on SIM medium using a single stab to the bottom of the tube and incubated at $37^0$for 24 hours. If hydrogen sulfide is produced, a black color forms in the medium. Bacteria that have the enzyme tryptophanase, can convert the amino acid, tryptophane to indole. Indole reacts with added Kovac's reagent to form rosindole dye which is red in color -indole positive. If an organism is motile then the growth will radiate from the stab mark and make the entire tube appear turbid.

Triple sugar iron

This test is used to differentiate microorganisms by their ability to ferment carbohydrates, produce hydrogen sulphide (H_2S) and gas. The test organism is inoculated onto TSI medium by stubbing the butt and streaking the slant, the tube is then incubated at 37^0 for 24 hours. If lactose or sucrose is fermented, a large amount of acid

is produced, which turns the phenol red indicator yellow both in butt and in the slant. Some organisms generate gases, which produces bubbles/cracks on the medium. If lactose is not fermented but the small amount of glucose is, the oxygen deficient butt will be yellow but on the slant the acid will be oxidized to carbon dioxide and water by the organism and the slant will be red. If neither lactose/sucrose nor glucose is fermented, both the butt and the slant will be red. If H_2S is produced, the black color of ferrous sulfide is seen

Appendix 4: KEMRI ethical approval

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel: (254) (020) 2722541, 2713349, 0722-205901, 0733-400003, Fax: (254) (020) 2720030
E-mail: director@kemri.org, info@kemri.org, Website. www.kemri.org

KEMRI/RES/7/3/1 **October 04, 2016**

TO: **HELLEN A. ONYANGO**
PRINCIPAL INVESTIGATOR

THROUGH: **PROF. SAMUEL KARIUKI,**
THE DIRECTOR, CMR,
NAIROBI

forwarded 11/10/2016

Dear Madam,

RE: **KEMRI/SERU/CMR/P00043/3329 (*RESUBMISSION 2 OF INITIAL SUBMISSION*) PREVALENCE, ANTIMICROBIAL SUSCEPTIBILITY PROFILES AND GENETIC DIVERSITY OF ISOLATES OBTAINED FROM URINE SAMPLES OF PREGNANT WOMEN ATTENDING ANTENATAL CLINIC AT PUMWANI MATERNITY HOSPITAL KENYA (VERSION 1.2. 27/09/2016)**

Reference is made to your letter dated 28th September 2016. The KEMRI Scientific and Ethics Review Unit (SERU) acknowledges receipt of the revised study documents on September 30, 2016.
This is to inform you that the Committee noted that the issues raised during the 254th Committee C meeting of the KEMRI/Scientific and Ethics Review Unit (SERU) held on **August 25, 2016** have been adequately addressed.

Consequently, the study is granted approval for implementation effective from **4th October, 2016** for a period of one year. Please note that authorization to conduct this study will automatically expire on **October 03, 2017.** If you plan to continue with data collection or analysis beyond this date, please submit an application for continuation approval to the SERU by **August 22, 2017**.

You are required to submit any proposed changes to this study to SERU for review and the changes should not be initiated until written approval from SERU is received. Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of the SERU and you should advise the SERU when the study is completed or discontinued.

You may embark on the study.

Yours faithfully,

T-OK **DR. EVANS AMUKOYE**
ACTING HEAD,
KEMRI SCIENTIFIC AND ETHICS REVIEW UNIT

Appendix 5: Pumwani ethical approval

NAIROBI CITY COUNTY

Telephone: 020 344194
Web.www.nairobi.go.ke

City Hall
P. O. Box 30075 - 00100
Nairobi
Kenya

COUNTY HEALTH SERVICES:
PUMWANI MATERNITY HOSPITAL

PMH/DMOH/75/0612/2016

31ST OCTOBER 2016

TO:
HELLEN A. ONYANGO
JKUAT.
P. O. BOX 62000
NAIROBI

RE: APPROVAL OF RESEARCH PROPOSAL

This is to inform you that the research entitled "**Prevalence, Antimicrobial Susceptibility Profiles and Genetic Diversity of Isolates Obtained from Urine Samples of Pregnant Women Attending Antenatal Clinic at Pumwani Maternity Hospital, Kenya**" has been approved.

You are expected to pay Kshs. 6000/- only.

You are hereby allowed to collect data. We look forward to receiving a summary of the research findings upon completion of the study.

Yours sincerely,

PUMWANI MATERNITY HOSPITAL
P. O Box 42619-00100, NAIROBI
TEL. NRB. 6763291-4/ 5762965

DR. L.O. KUMBA
MEDICAL SUPERINTENDENT

Appendix 6: Publication