Antimicrobial Resistance Among Pregnant Women With Urinary Tract Infections Attending Antenatal Clinic at Levy Mwanawasa University Teaching Hospital Lusaka, Zambia

By

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Declaration

I, Kekelwa Inyambo Yeta, declare that this dissertation is an exhibition of my own work. It is being submitted for the MSc Degree in Epidemiology and Biostatistics at the University of Zambia, Lusaka. It has never been submitted for any degree at this or other Universities.

Signature: _____

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Certification of completion of dissertation

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The University of Zambia approves this dissertation of Kekelwa Inyambo Yeta in partial fulfilment of the requirements for the award of the degree in Master of Science in Epidemiology and Biostatistics.

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Abstract

Introduction: Globally, bacterial infections are a major cause of morbidity and mortality, particularly in low income countries. The global emergence of antimicrobial resistance undermines the management of infectious diseases. Internationally, there is a growing concern over antimicrobial resistance (AMR) which is currently estimated to account for more than 700, 000 deaths per year worldwide.

Objectives: The main objective of this research was to determine the prevalence of antimicrobial resistance (AMR) and factors associated with AMR among pregnant women with Urinary Tract Infections (UTIs) attending antenatal clinic at Levy Mwanawasa University Teaching Hospital (LMUTH), Lusaka Zambia.

Methodology: This was a hospital based, cross sectional study conducted at levy Mwanawasa University Teaching Hospital among pregnant women attending antenatal clinic. Interviewer administered questionnaire was used to assess the socio-demographic characteristics. Basic descriptive statistics (proportions, mean) of study participants were used to describe the characteristics of the variables of respondents. Categorical variables were summarized in the form of numbers and percentages and presented in table format. Chi square was used to assess the association between categorical variables. The logistic regression analysis was carried out to generate the adjusted odds ratio with 95% confidence interval.

Results: The prevalence of UTI was 60% (95% CI,53.3%-66.7%) with the most isolated bacteria being E. coli (59), Staphylococcus aureus (17) and Klebsiella (21). There was significant association between gestational age and HIV with UTI. The prevalence of AMR was found to be 53% (95% CI,46.1%-59.8%). The highest resistant drugs being Nalidixic acid (88.3%), Norfloxacine (58.5%) and ampicillin (77.8%), the least resistant were chloramphenicol (20%), ciprofloxacin (30.5%) and nitrofurantoin (40%). There were no significant predictor variables to AMR.

Conclusion: Generally, a much higher prevalence of UTI than most studies undertaken has been found in this study, indicative of the level of menace urinary tract infections. Therefore, early screening of pregnant women for UTI causing uropathogens and determining their antibiotic susceptibility pattern is an important intervention to prevent complications that may endanger the life of both the pregnant women and the fetus. The prevalence of AMR was as well high. This is attributed to the misuse of drugs hence need to re-enforce prescription-only policies and implementing antimicrobial stewardship programmes least we be in an error in which we have ineffective antibiotics.

Key words: *Antimicrobial resistance (AMR), Susceptibility patterns, Urinary tract infections (UTI), Prevalence, Bacterial uropathogen*

Dedication

This dissertation is dedicated to my father, Snr Chief Inyambo Yeta who first taught me the value of education and critical thought. A special feeling of gratitude to him, his words of encouragement and push for tenacity ring in my ears. He has always been there for me, showing me nothing but unconditional love and believed in me even in moments I did not.

To my mother Muliya Ngenda, thank you so much for your unconditional love and support you have shown me. May God bless you.

I also dedicate this dissertation to my friends Evelyn Kunda Ng'andu and Misozi Blessings Nyimbiri, you are simply amazing.

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Abbreviations

ATCC	American Type Culture Collection
AMR	Antimicrobial Resistance
CLED	Cysteine Lactose Electrolyte-Deficient
CDC	Centres for Disease Control and Prevention
DDD	Defined Daily Dose
E. coli	Escherichia coli
GP	General Practice
LMUTH	Levy Mwanawasa University Teaching Hospital
WHO	World Health Organization
UTIs	Urinary Tract Infections

Definition of Terms

Antimicrobial resistance: The ability of a microbe to resist the effect of an antimicrobial agent (Australian Commission on Safety and Quality in Health Care, 2013a)

Community-acquired urinary tract infection: Positive E. coli urine culture obtained within the first 48 hours of admission (including cultures from non-admissions such as outpatient clinics)

Extensive drug resistance: Non-susceptibility to at least one agent in all but two or fewer antimicrobial categories" (Magiorakos *et al.,* 2012)

Hospital-acquired urinary tract infection: Positive E. coli urine culture obtained more than 48 hours after admission and within 48 hours of discharge.

Multidrug-resistance: Non-susceptibility to at least one agent in three or more antimicrobial categories" (Magiorakos *et al.,* 2012, p. 277)

Pandrug-resistance: Non-susceptibility to all agents in all antimicrobial categories (Magiorakos *et al.,* 2012)

Urinary tract infection: Urinary tract infection (UTI) is a collective term that describes any infection involving any part of the urinary tract, namely the kidneys, ureters, bladder and urethra (Tan, 2016).

1.0 CHAPTER ONE: INTRODUCTION

1.1 Background

It is estimated that about 150 million people worldwide are diagnosed with Urinary Tract Infections (UTIs) each year costing the global economy in excess of 6 billion US dollars (Gupta *et al.,* 2010). UTIs are one of the most common bacterial infections encountered by both the general community and in hospitals.

About 50% of women will suffer from at least one urinary tract infection (UTI) during their adult life. Women are more susceptible to UTI when compared to men, and this is largely due to short urethra, absence of prostatic secretion, pregnancy and easy contamination of the urinary tract with faecal flora (Tazebew, 2012).

UTIs account for around 5% of consultations in General Practice and are the second commonest infection after respiratory infections expectedly leading to 15% of community use of antibiotics for UTIs (Larcombe, 2012).

Globally, bacterial infections are a major cause of morbidity and mortality, particularly in low income countries. In resource-poor and tropical countries, infectious diseases are still the major source of morbidity and death (Demain *et al.,* 2009). The global emergence of antimicrobial resistance undermines the management of infectious diseases (Urassa *et al.,* 2010).

In 1981, World Health Organization (WHO) set out a goal towards "health for all by 2000" which required a combination of effective antimicrobial agents, immunization, and improved public health programs. This goal experienced a major setback because of the global emergence and spread of antimicrobial resistance (AMR) (Wax *et al.,* 2007). AMR results in the therapeutic failure of standard treatment, and longer duration of treatment, leading to an increased risk in the spread of infections (WHO 2014). AMR is a broad term that includes resistance to all antimicrobial agents.

Internationally, there is a growing concern over antimicrobial resistance (AMR) which is currently estimated to account for more than 700, 000 deaths per year worldwide (Tadesse *et al.,* 2017). If no appropriate measures are taken to halt its progress, AMR will cost approximately 10 million lives and about US$100 trillion per year by 2050. Antimicrobial resistance is a problem that concerns development as resistant pathogens do not respect orders (Tadesse *et al.,* 2017).

The 2014 World Health Organization (WHO) report identified Africa and South Asia as the regions without established AMR surveillance systems.

There are many different pathogenic microorganisms (bacteria, fungi, protozoa and viruses) which cause UTIs generally and among pregnant women. Bacteria are usually more prevalent and invasive. For instance, E. coli and other Enterobacteriacae are the most common bacterial pathogens and accounts approximately 75% of the isolates (Getenet *et al.,* 2011). The relative frequencies of the pathogens vary with age, sex, catheterization, and hospitalization (Wondewosen, 2011).

According to a study by (Mwaka *et al.*, 2011), Escherichia coli (*E. coli*) causes 75-90% of acute uncomplicated cystitis while *S. Saprophyticus* accounts for 5-15%, mainly in younger women worldwide. *Enterococcus spp* and aerobic gram-negative rods, *K. pneumoniae* and *P. mirabilis*, were isolated from the cases of UTI (Mwaka *et al.,* 2011). UTIs are commonly encountered diseases in developing countries, with an estimated annual global incidence of at least 250 million (Wondewosen, 2011). For instance, the Ugandan Bureau of Statistics (UBoS) 2009/2010, National Household Survey found the national prevalence of UTIs to be 0.2%.

Antibiotics are among the most commonly used medications globally and are of enormous importance to global health; despite their importance, the sustained effectiveness of antibiotics is endangered by the development of resistance. The excessive and unnecessary use of antibiotics has been the main cause of antibiotic resistance (Wax *et al.,* 2007).

1.2 Statement of the problem

Antimicrobial drugs have helped drastically in curing patients suffering from bacterial infections. However, emerging antimicrobial resistance in bacteria threatens to undermine the management of bacterial infections. Developing countries have greater burden of infectious diseases. A number of factors, which may promote antimicrobial resistance such as availability of antimicrobials without prescription, use of counterfeit or substandard antimicrobial drugs, suboptimal hygiene, immuno-suppression due to malnutrition or HIV, may be more frequent in developing countries. At the same time, consequences of antimicrobial resistance may be felt harder in resource-poor settings, since second-line antimicrobial drugs for resistant bacteria may be unavailable or unaffordable. There are many unresolved questions regarding antimicrobial resistance in general, including regarding its impact on patient outcome.

The abuse of antibiotics by the public is an important risk factor for antibiotic resistance (Melander *et al.,* 2000). There have also been reports that some pregnant women are ignorant of the management of common infections which has resulted in AMR and this gap in knowledge has extended to health practitioners in some cases (Wise *et al.,* 1998). Thus, it is crucial to gain an understanding of AMR among pregnant women.

Prospective and retrospective studies have both shown an increased risk of death from UTI infections if it is caused by AMR pathogens (Hawkings *et al.*, 2015).

In Zambia, like in many other countries, there is emerging evidence of antimicrobial resistance (AMR) in several pathogens (Chisanga *et al.*, 2017). The University Teaching Hospital, the highest-level hospital in Zambia has been detecting multi-drug resistant pathogens, resistant to the first, second and third line antimicrobial agents which has left very limited options for antimicrobial therapy for infectious diseases (Chisanga *et al.*, 2017).

Despite the large number of antimicrobial agents available, these infections have remained a significant problem among pregnant women in Zambia (Chisanga *et al.*, 2017). The indiscriminate use of antimicrobial drugs has led to resistance in most patients. Concurrent resistance to different antimicrobial agents has given rise to multidrug resistance which also complicates the therapeutic management of UTIs (Gupta *et al*., 2001; Akram *et al*., 2007; Tripti and Singh, 2010).

AMR is evident among UTI pregnant women in Zambia and particularly Lusaka but not so many studies have been carried out to determine its prevalence. Although some studies on antimicrobial resistance have been done, by and large, the issue has received far too little attention other than comments from stakeholder without empirical evidence. It is upon this background that the study is carried out to determine essentially AMR in UTI bacteria among pregnant women and remove the topic from the realm of conjecture and speculation.

This comes with a view that patients with infections caused by bacteria resistant to a specific antibacterial drug generally have an increased risk of worse clinical outcomes and death, and consume more healthcare resources, than patients infected with the same bacteria not demonstrating the resistance pattern in question. Available data are insufficient to estimate the wider technological, scientific, societal and economic implications when effective treatment for an infection is completely lost as a result of resistance to all available drugs.

1.3 Significance of the study

The study findings will contribute to local and international knowledge on the types of uropathogens responsible for urinary tract infections (UTIs) in the area of study. The findings will augment information which will help Clinicians, Medical Officers, and Ministry of Health (MoH) in making decisions for empirical treatment of UTIs and management antimicrobial resistance.

The findings of the study will provide scientific backing to the rampant increase in antimicrobial resistance among pregnant women with UTIs. The aim of this study is to

gain more knowledge of bacterial infection and their resistance patterns at Levy Mwanawasa University Teaching Hospital. This will increase to the already existing evidence to make sensible decisions on antimicrobial therapy both at the level of practicing clinicians and at authority level responsible for developing guidelines and policies.

1.4 Research questions

1. What is the prevalence of antimicrobial resistance among pregnant women with UTIs attending antenatal clinic at LMUTH?
2. What are the factors associated with Antimicrobial resistance among pregnant women with UTIs attending antenatal clinic at LMUTH?

1.5 General Objective

To determine the prevalence of antimicrobial resistance (AMR) and factors associated with AMR among pregnant women with UTIs attending antenatal clinic at Levy Mwanawasa University Teaching Hospital (LMUTH).

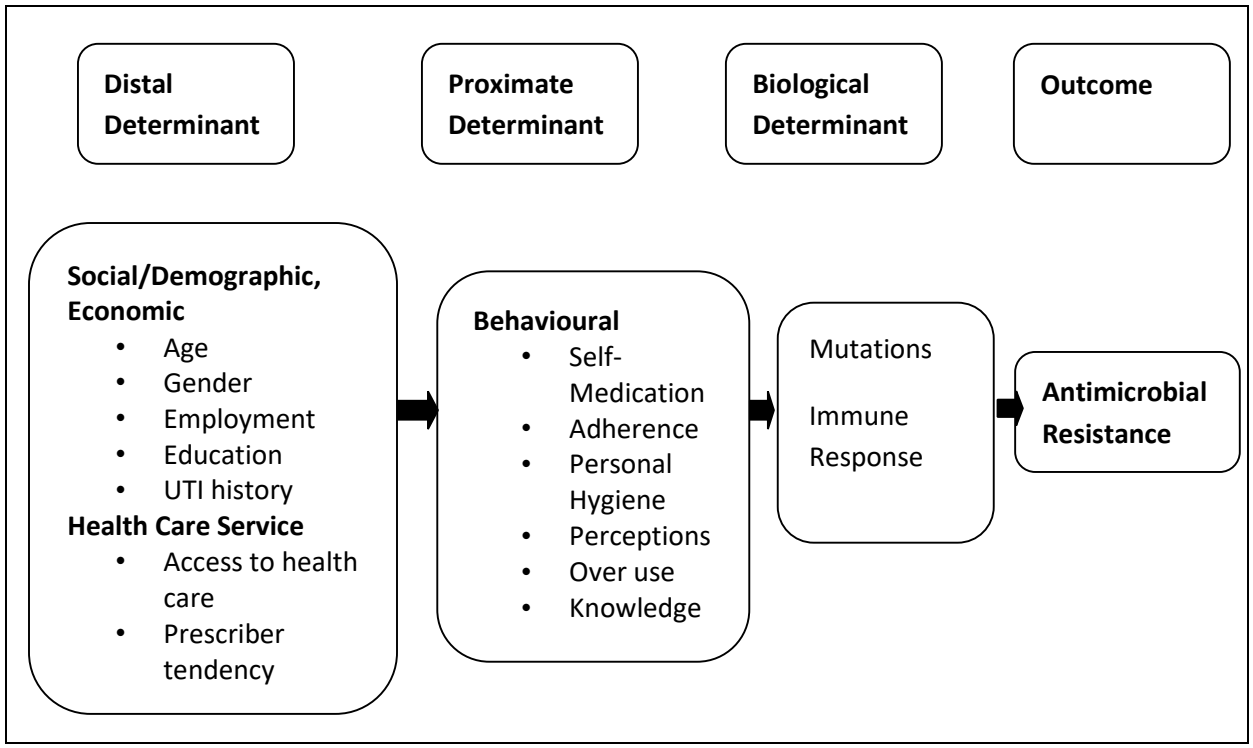
1.6 Specific Objectives

1. To identify the susceptibility patterns of the isolated pathogenic bacteria among pregnant women attending antenatal clinic at LMUTH.
2. To determine the prevalence of Antimicrobial resistance among pregnant women with UTIs attending antenatal clinic at LMUTH.
3. To determine the demographic, social-economic and clinical factors associated with antimicrobial resistance among pregnant women with UTIs attending antenatal clinic at LMUTH.

1.7. Conceptual framework

For this study, a proximate determinants conceptual framework was adopted. This model is an adaptation of Boerma and Barnighausen analytical framework 2003.The model aims at examining the distal and proximate determinants in order to determine their influence on antimicrobial resistance.

Figure1. Conceptual framework for understanding the determinants of antimicrobial resistance among pregnant women with urinary tract infections.



Conceptual framework adapted from Boerma et al., and Barnighausen et al., 2003.

The models for this study include distal determinants whereas the other models have focused only on proximate determinants. These distal determinants comprise the contextual variables (socio-economic and demographic factors such as age and education) as well as health care services. These must operate through proximate determinants (behavioural such as misuse of antibiotics, adherence, knowledge etc.) in order to influence antimicrobial resistance.

The model aimed at examining the underlying and proximate determinants in order to determine their influence on antimicrobial resistance. The model employed regression analysis in order to determine which determinants are predominant in influencing antimicrobial resistance.

The schematic presentation above shows how different conditions interact with antimicrobial resistance as the outcome. It shows that resistance is influenced by more than just the number of mutations. It points out the distals, determinants, proximate determinants as well as biological determinants that influence antimicrobial resistance.

2.0. CHAPTER TWO: LITERATURE REVIEW

2.1. Introduction

This literature review was conducted to understand historical and current research related antimicrobial resistance in pregnant women with urinary tract infections. The risk of urinary tract infection (UTI) in pregnant women and Antimicrobial resistance (AMR) within a wide range of infectious agents is a growing public health threat of broad concern to countries and multiple sectors (Leopold *et al.,* 2014).

Internationally, there is a growing concern over antimicrobial resistance which is currently estimated to account for more than 7000, 000 deaths per year worldwide. If no appropriate measures are taken to halt its progress, AMR will cost approximately 10 million lives and about US$ 100 trillion per year by 2050 (WHO report, 2014).

Furthermore, without effective antimicrobials for prevention and treatment of infections, medical procedures such as organ transplantation, cancer chemotherapy, diabetes management and major surgery (for example, caesarean sections or hip replacements) become very high risk (WHO Fact sheet, 2017).

On the other hand, Antimicrobial resistance increases the cost of health care with lengthier stays in hospitals and more intensive care required.

2.2 Prevalence of Antimicrobial resistance

Antimicrobial resistance is a global threat as it is present in all parts of the world and it means that there is a shortage of effective antibiotics to treat simple infections and diseases, also statistics reveal that because of antimicrobial resistance patients' morbidity and mortality is increased, as well as healthcare related expenditures (Diminskyte, 2016). The latest report from the World Health Organization "Antimicrobial resistance: Global report on surveillance" pointed out that Southeast Asia has among the highest rates of antibiotic resistance worldwide (Mohrs, 2015).

A study done in Maiduguri – Nigeria, reported a high incidence of UTI in both nonpregnant and pregnant women. The predominant organism in this report was *E.coli* and was found to be highly sensitive to quinolones, while resistant to Co-amoxicillin, Cotrimoxazole and Nalidixic acid (Initiative & W.H.O., 2003).

A study was conducted in 2010 on Bacterial isolates and drug susceptibility patterns of urinary tract infection among pregnant women at Muhimbili National Hospital in Tanzania by Sabrina et al. The study findings showed a low rate to moderately high rate antimicrobial drug resistance against first line drugs namely, nitrofurantoin 18.7 % (n=16), co-trimoxazole 38.5 % (n=13) and ampicillin 57.7 % (n=26). Resistance was seen against second line drugs: ciprofloxacin 13.6 % (n=22) and amikacin 5 % (n=20).

High rate of resistance was observed in third generation cephalosporin cefotaxime 31.2 % (n=16). Of the Gram-positive organisms tested against vancomycin and methicillin, resistance was found in 25 % (n=13) and 25 % (n=4), respectively.

In conclusion, *E. coli* was found to be the common cause of UTI among the pregnant women. Low to moderately high level of resistance was found in first line drugs while high level of resistance was found in third generation cephalosporin. It is recommended to monitor the levels of resistance for nitrofurantoin, fluoroquinolone and cefotaxime and to screen for Extended Spectrum Beta Lactamase production among cefotaxime resistant *E. coli* and *Klebsiella spp.*

A study by (Agyepong *et al.,* 2018), found *E. coli* and *K. pneumoniae* to be the most predominant pathogens implicated in UTI and most common infections frequently caused by *E.coli* and *Klebsiella pneumonia* have high resistance to broad spectrum antibiotics in Ghana. Furthermore, a study in Uganda found urinary tract infection prevalent (26.8%). Bacterial isolates responsible for the infections were *Proteus* (39.5%), *Escherichia coli* (32.1%), *Staphylococcus aureus* (14.8%), *Klebsiella spp*. (6.2%), *Staphylococcus haemolyticus* (2.5%), *Staphylococcus intermedius* (2.5%), *Citrobacter* (1.2%) and *Morganella* (1.2%). The pathogens exhibited high-level of resistance to commonly used antibiotics like Cotri-moxazole, Amoxicillin, Nalidixic Acid, Nitrofurantoin, Gentamicin, Erythromycin, Chloramphenicol, Ampicillin, Ciprofloxacin, Tetracycline and Azithromycin while the isolates showed no resistance to pharmaco-enhanced Amoxicillin and oral Cefotaxime. In contrast, other studies, (Afriyie *et al.*, 2014) found that the predominant bacterial isolates were *Coliforms* (44.2%) and *Escherichia coli* (36.2%) and they had high susceptibility was seen with Nitrofurantoin and Gentamicin.

A cross sectional study was conducted at Khartoum north teaching hospital Antenatal Care Clinic between February-June 2010, to investigate epidemiology of UTI and antibiotics resistance among pregnant women (Sahm *et al.,* 2001).The prevalence of bacteriuria among symptomatic and asymptomatic pregnant women were (12.1%), and (14.7%) respectively, with no significant difference between the two groups (P = 0.596), and the overall prevalence of UTI was (14.0%). *Escherichia coli* (42.4%) and *Staphylococcus aureus* (39.3%) were the commonest isolated bacteria. *E. coli* isolates, showed resistance to amoxicillin, Nalidixic acid, nitrofurantoin, ciprofloxacin, cotrimoxazole, amoxicillin or clavulanate and norfloxacine, respectively. The study further concluded that *Escherichia coli* were the most prevalent causative organisms and showing multi drug resistance pattern, asymptomatic bacteriuria is more prevalent than symptomatic among pregnant women. Urine culture for screening and diagnosis purpose for all pregnant women is recommended.

2.3 Risk factors of antimicrobial resistance in pregnant women with UTI

Antimicrobial resistance occurs when bacteria change in some way that reduces or eliminates the effectiveness of the drugs, chemicals or other agents designed to cure or prevent infections. The bacteria survive and continue to multiply causing more harm. There is a growing concern regarding antimicrobial resistance worldwide among causative agents of UTI in pregnant women. (Chakupurakal *et al*., 2010). A rise in multidrug resistance to UTI is mainly attributed to an extensive unrestrained antibiotics usage habit in developing countries mainly due to the habitual trend of empirical antimicrobial treatment which is commonly started without obtaining the laboratory results of urine culture. (Haque *et al*., 2015). A number of studies have been done to assess the risk factors and their findings are in the below literature that has been reviewed.

Reasons for multidrug-resistant organisms in developing countries are numerous, but the inadequate access to effective drugs, the unregulated manufacture and dispensation of antimicrobials, and the lack of money available to pay for appropriate, high-quality medications are some of the major poverty-driven factors contributing to anti-microbial resistance (Planta, 2007). Some other factors that are associated with antimicrobial resistance are listed below:

1. **Production of enzymes**

   According to Monica C, 2006, 'bacteria become resistant to antimicrobial agents by a number of mechanisms, the commonest being production of enzymes which inactivate or modify antibiotics'.

2. **Misuse of antibiotics**

   The European Centre for disease prevention and Control (ECDC), 2014; it underscored that the main origin of resistance to antibiotics is misuse and this misuse was attributed to the following;

   i. Unnecessary prescription of antibiotics for viral infections against which they have no effect.
   ii. Too frequent prescription of broad spectrum antibiotics in place of a better targeted antibiotic, through more specific diagnosis
   iii. Inadequate use by the patient that is not respecting either dosage or duration of treatment.

   Studies by Behailu *et al.,* 2016; Masinde *et al.,* 2009 further highlighted that antimicrobial resistance has been recognized as the consequence of repeated antibiotic use and abuse. The reasons for this alarming phenomenon might be due to inappropriate and incorrect administration of antimicrobial agents in empiric therapies.

3. Social demographics

A study conducted in India, found that there was no association between age, educational level and multiparity with UTI and antimicrobial resistance (Singh *et al.*, 2014). However, a study by (Kovavisarach, Vichaipruck and Kanjarahareutai, 2009), found that lower education was the only significant risk factor related to antimicrobial resistance. There was no association between bacteriuria or resistance and risk factors such as gestational diabetes, past urinary tract infection, multiparity, advanced maternal age, lower education level, advanced gestational age and lower socioeconomic status (Perera *et al.*, 2012).

Different risk factors expose pregnant women to UTI including increasing parity status, increasing age, frequent sexual intercourse, diabetes, sickle cell disease and previous history of UTI. Most importantly UTI in pregnancy is mainly related to poor hygiene and low socio-economic status of developing countries. (Vardi *et al*., 2012). Many studies have been done in line with this and their findings are stipulated below.

A recent study found that factors that affect the frequency of bacteriuria during pregnancy to be multiparity, gestational age, previous medical history of UTI, diabetes mellitus and anatomic urinary tract abnormalities. In addition, anaemia, socio-economic status, educational status, sexual activity and catheterization are also associated with increased risk of UTI (Dar, 2013).

Factors proposed to affect the frequency of bacteriuria during pregnancy include multiparity, gestational age, previous medical history of UTI, diabetes mellitus and anatomic urinary tract abnormalities. In addition, anaemia, socio-economic status, educational status, sexual activity and catheterization are also associated with increased risk of UTI (Tazebew E, *et al.*, 2013).

A recent study found that advanced maternal age (> 35 years) and younger maternal age (< 20 years) are risk factors related to ABU in pregnancy (Kovavisarach, Vichaipruck and Kanjarahareutai, 2009). However, Chongsomchai *et al* and Fatima and Ishrat found that advanced or younger maternal age was not a risk factor related to ABU in pregnancy. A study by (Perera *et al.*, 2012), found that there was no association between bacteriuria resistance and risk factors such as advanced maternal age, lower education level, advanced gestational age and lower socioeconomic status.

Owing to risk factors associated with UTI in pregnancy, treatment is more aggressive and should begin within the soonest possible time. However, the development of resistance has become a common feature among Uropathogens.

3.0. CHAPTER THREE: METHODOLOGY

3.1. Study design

This was a hospital-based cross-sectional study which was based on quantitative approach. The study was conducted between November 2018 and May 2019.

3.2. Study setting

The study was conducted at the Levy Mwanawasa University Teaching Hospital. Levy Mwanawasa University Teaching Hospital is a 250-bed tertiary-care. The hospital has a total number of 528 both medical and administrative staff with a catchment area of 8 districts within Lusaka province, an estimated population of over 3 million. The clinical Laboratory department, one of the diagnostic units at the institution, offers services to clients in six key disciplines which include Clinical Chemistry, Haematology/Immunology, Blood Transfusion services, and the Microbiology/Parasitology. The department runs an estimated 67000 tests a year for both in and out-patients.

This hospital was purposely selected because it serves as a reference Centre hence has a large number of in and outpatients and represents patients from large geographical areas.

3.3. Study population

This study focused on all pregnant women who were attending antenatal clinic at Levy Mwanawasa University Teaching hospital.

3.4. Eligibility criteria

3.4.1. Inclusion criteria

The study included all pregnant women aged 18 years and above, attending the antenatal clinic at Levy Mwanawasa University Teaching Hospital, with a urinalysis test suggestive of Urinary tract infection and consenting to participate in the study.

3.4.2 Exclusion criteria

Pregnant women who were on antimicrobial therapy for UTI two weeks prior to selection were excluded from the study. Pregnant women whose urine samples were; less than 10 ml, collected more than 2 hours before processing, submitted in leaking or dirty unsterile containers and revealing growth of more than two types of bacteria on culture were as well excluded from the study.

3.5. Sample size and sampling procedures

The sample size was calculated using the single size population formula by Fisher (Mugenda and Mugenda 1998) based on 95% confidence interval and 14% prevalence rate of antimicrobial resistance, (Behailu *et al*., 2016).

Formula:

$n = [Z^2 P\ (1 - P)]/d^2$

Where:

n = the required sample size
Z = the critical value associated with the level of significance
P = the estimated prevalence (0. 14) d = degree of precision
chosen for the study Z = 1.96 for 95% level of confidence
d = 0.05 degree of precision

$$n = \frac{z^2 p(1-p)}{\varepsilon^2}$$

$$n = \frac{1.96^2\ 0.14\ (1 - 0.14)}{0.05^2}$$

$$n = 185.0$$

$$\underline{n \approx 185}$$

Therefore, the desired sample size was185. Taking into account a 10% non-response rate, the minimum sample size was 203 participants.

The estimated prevalence of 14% was used as it is of similar population setting.

A purposive sampling technique was used to enroll consecutive pregnant women attending antenatal care in the hospital during the study period. Patients who tested positive for UTIs were recruited until the expected study sample number of participants was attained.

3.6 Data collection

An interviewer-administered standard questionnaire was used to gather sociodemographic and lifestyle data from the participants in order to collect all variables of interest from the patient perspective.

3.7 Laboratory procedures

Upon completion of the interview, participants were sent to the hospital laboratory with a request form. Instructions were given to them by trained medical laboratory personnel on how to collect the specimen. Participants were advised to place 10–20 mL clean catch midstream urine specimen into a sterile screw-capped, wide-mouthed, sterile disposable plastic container after signing the consent form. (Laboratory Standard Operation Procedure 2008). Each sample bottle was labelled with date and time of collection then immediately sent to the microbiology department for Microscopy, culture and antimicrobial susceptibility. A unique sample number was linked to the participant`s questionnaire which was in turn linked to confidential patient information.

Urine specimens were inoculated onto CLED, MacConkey and Blood Agar plates (OXOID, Ltd, Basingstoke, UK) by using the streak method following the standard microbiological procedures. The plates were incubated at 37°c for 24 hours then later examined for significant growth. Diagnosis of UTI was based on the presence of ≥10^5 colony forming units per milliliter of Mid-stream urine of one or two types of bacterial species. Specimens with more than two types of bacteria species were regarded as contamination, sample collection was repeated. The identification of bacteria isolate was done using biochemical tests.

Antimicrobial susceptibility testing was performed for the bacterial isolates identified from urine cultures with significant growth by using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (Oxoid Ltd, bangistoke, UK) according to the criteria set by the Clinical and Laboratory Standards Institute (CLSI) to determine the susceptibility patterns of the commonly used antibiotics.

The procedure for antimicrobial susceptibility testing was as follows: Briefly 4–6 morphologically identical colonies of bacteria from pure cultures were collected with an inoculating loop and transferred into a tube containing 5 mL of nutrient broth, then mixed gently until a homogenous suspension is formed, and incubated at 37°C for 3–5 hours until the turbidity of the suspension becomes adjusted to the density of 0.5 McFarland standards, which yield a uniform suspension containing 105–106 cells/mL.

Using a sterile nontoxic dry cotton swab, sample of the standardized inoculums (turbidity was adjusted to obtain confluent growth) were taken and streaked on the entire surface of the dried Mueller–Hinton agar plate three times, turning the plate at 60° angle between each streaking to ensure even distribution. The inoculum was allowed to dry for 5–15 minutes with the lid in place. Using sterile forceps, the selected antibiotics disks will be applied to the plates at a distance of 15 mm away from the edge and 24 mm apart from each other. After incubating the plates at 37°C for 24 hours, diameters of the zone

of bacterial growth inhibition around the disks were measured to the nearest millimetre. The susceptibility or resistance to the agent in each disk will be determined, and the isolates will be classified as sensitive, intermediate, or resistant according to the standardized table

3.8 Summary of variables:

The following table gives a summary of variables of interest.

Dependent Variable	**Indicator**	**Scale of Measurements**
Antimicrobial resistance	Resistance or susceptibility calculated as percentage	Binary
Independent Variables		
Age	Age in years	Continuous
UTI history	Occurrence of UTI in previous month	Nominal
Gestation age	Trimester presented in weeks	Nominal
History of antibiotic use	Antibiotic usage in the last month	Nominal

3.9 Data analysis and management

The culture results from the samples collected were used to calculate prevalence of UTI, characterize the type of microbial growth (isolates) and to test for antimicrobial susceptibility test.

The data generated from the questionnaires was checked for completeness, consistency and accuracy and then entered an excel spreadsheet. After manual verification and cleaning, the data processing and statistical analysis was performed using STATA software version 14.0 (Stata™ Corporation, Texas, TX, USA).

Basic descriptive statistics (proportions, mean) of study participants were used to describe the characteristics of the variables of respondents.

Categorical variables were summarized in the form of numbers and percentages and presented in table format. Continuous variables such as age were assessed for normality assumptions using qq-plots. Statistics such as means and their respective standard

deviations were reported if normality assumptions are fulfilled, otherwise, medians and their respective interquartile ranges were reported.

The main outcome variable, which is antimicrobial resistance (binary) in pregnant women, was classified and potential factors associated with the outcome were tested individually. Chi-square test was used to assess statistical differences between categorical variables with significance level set at $p<0.05$ and 95% confidence interval. It was used to assess the association between the categorical variables if the assumptions are met otherwise Fisher`s exact test was performed.

Variables with a p-value of less than 0.05 were then entered into the logistic regression model to identify independent predictors for antimicrobial resistance in pregnant women. The logistic regression analysis was carried out to generate the adjusted odds ratio with 95% confidence interval. Variables from a univariate model that were statistically significant were entered into a multivariable model. The final step involved selecting a model which explained the data well between the full model and the nested model, there are several tests available that asses the adequacy of a model. The study used r squared, Akaike information criterion and the Bayesian information criterion. Results were presented using tables and graphical representation for a better interpretation. A p value less than 0.05 ($p <0.05$) was considered statistically significant.

3.10 Data quality control

Data collected was verified for completeness, it was double entered into the excel spread sheet to ensure accuracy and reliability. Culture and biochemical tests were performed by laboratory scientist using the standard operating procedures to ensure quality results. The American Type Culture Collection (ATCC) reference strains such as Escherichia coli (ATCC-25922), Staphylococcus aureus (ATCC-25923), and Pseudomonas aeruginosa (ATCC-27853) were used as quality control parameters of Laboratory tests.

3.11 Ethical considerations

The objectives of the study were explained well to the head of the hospital and each pregnant woman. In this regard, oral and written consents were obtained from participants before starting the study and participation was voluntary for all those who agreed and signed the consent form.

The identity of all participants was protected, and the information given was not traced to their names. The responses and concerns of participants were only used for research; names were not used in analysis and the only link with the results was via identity numbers (ID No).

There were no risks or costs incurred on the part of the participants. Information sheet clearly stated that no benefits will be granted to participants, however, it was noted that information collected through participation may not benefit anyone at the time of study, but it is hoped future generations benefit.

Patient information about their Urinary Tract Infections status was kept in outermost confidentiality and patients with UTIs were encouraged to seek medication, critical results were availed to the participants so that they seek medication attention.

The researcher ensured that the proposed research methodology for conducting the study was followed in order to avoid alteration of the research findings aimed at satisfying the researcher's views.

Ethical approval to undertake the study was sought from University of Zambia Biomedical Research Ethics Committee (UNZABREC), Reference number 002-09-18. Written permission was obtained from the Ministry of Health (MOH) and the Senior Medical Superintendent of Levy Mwanawasa University Teaching Hospital (LMUTH).

4.0. CHAPTER FOUR: RESULTS

4.1. Socio-demographic, obstetrics, and clinical characteristics

A total of 203 pregnant women were included in this study. As shown in Table 1, most of the pregnant women were married with a proportion of 80.9%. The majority of the study participants were in the age range of 25 to 29 years and 30 to 34{(59% and28.8%)} respectively. Most of these women came from low cost areas having a proportion of 68.2% and most of them had gone as far as secondary education having a proportion of 52.0%. Majority of the women having a proportion of 24.4% had an income ranging from 2001 to 3000 kwacha followed by those who had no income having a proportion of 23.4% Most of the urinary infected pregnant women 61% had a history of previous urinary tract infection and most of them 50%. were in their second trimester. The population description is summarized in table 1 below.

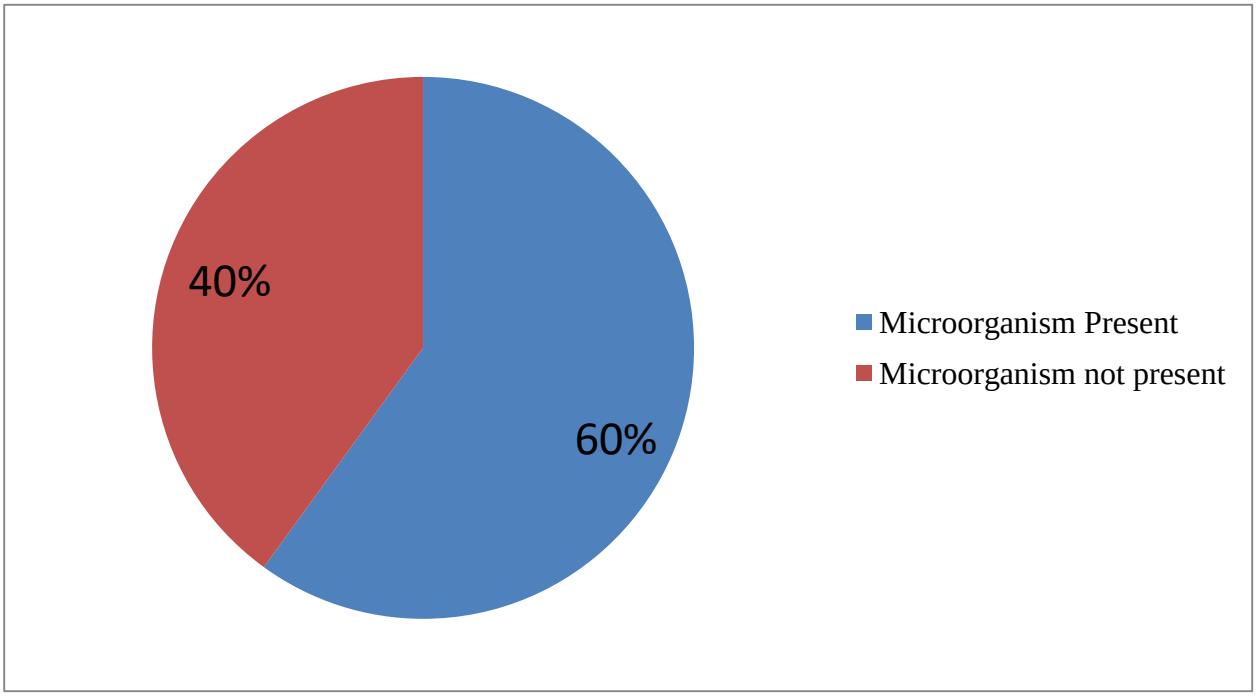
Table 1: Socio-demographic, obstetric and clinical characteristics of pregnant women attending antenatal clinic at Levy Mwanawasa University Teaching Hospital (n=204)

Characteristics	**Total (n=)**	
	Patient (n)	**Percentage (%)**
Marital status		
Single	36	17.6
Married	165	80.9
Separated	1	0.4
Widowed	1	0.4
Divorced	2	1.0
Age in years		
Less than 20	9	4.3
20 to 24	43	20.0
25 to 29	59	28.8
30 to 34	59	28.8
35 to 39	26	12.7
40 and above	9	4.4
Residence		
High cost	15	7.8
Low cost	131	68.2
Medium cost	46	23.0
Education level		
Primary	28	14.3
Secondary	102	52.0
Tertiary	66	33.7

Monthly Income		
No income	48	23.4
1001 to 1500	42	20.5
1501 to 2000	26	12.7
2001 to 3000	50	24.4
Above 3000	39	19.0
HIV status		
Reactive	74	36.8
Non-Reactive	127	63.2
History of urinary tract infection		
Yes	125	61.0
No	80	39.0
Pregnancy Trimester		
First	19	10.8
Second	88	50.0
Third	69	39.2

The prevalence of urinary tract infection among pregnant women who were attending antenatal clinic at Levy Mwanawasa university Teaching hospital was found to be 60% (95% CI, 53%-66.7%) as shown in figure 1.

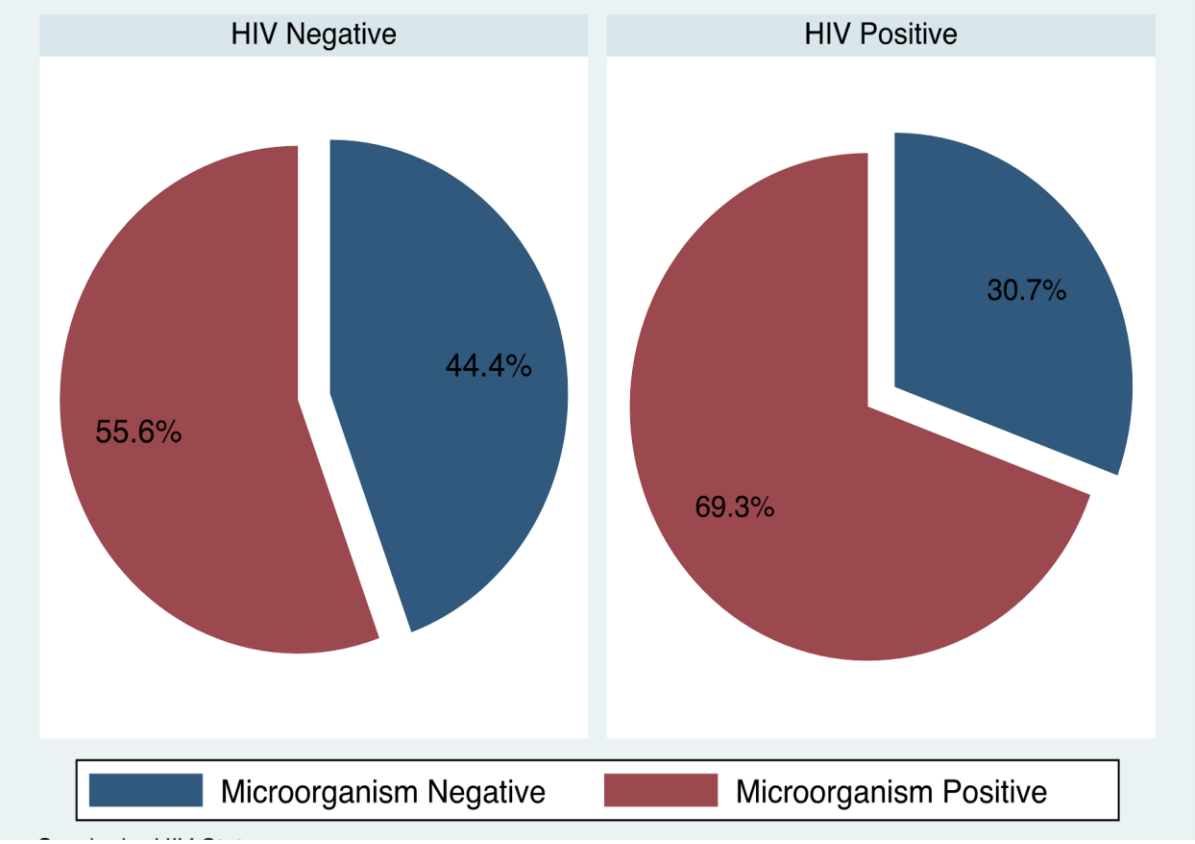
Figure 1: Prevalence of urinary tract infection among pregnant women at LMUTH



4.3: Microorganism positivity rate and related factors among pregnant women with UTI

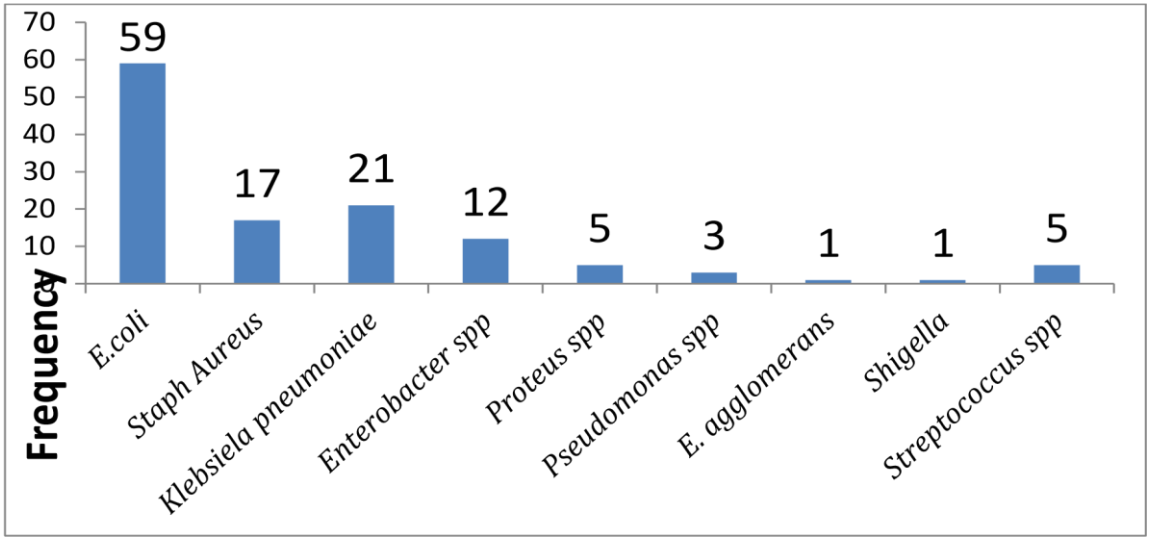
The total number of microorganisms isolated or tested positive from the urine samples of the pregnant women was 124 (60.5%) with 81 (39.5%) testing negative. The total number of women that were HIV positive was 74 (37.3%) and 126 (62.7%) were HIV negative. With reference to HIV status, more bacteria were isolated from HIV positive pregnant women compared to HIV negative pregnant women, having proportions of 69.3% and 55.6% respectively (Figure 2).

Figure 2: Microorganism isolation by HIV status



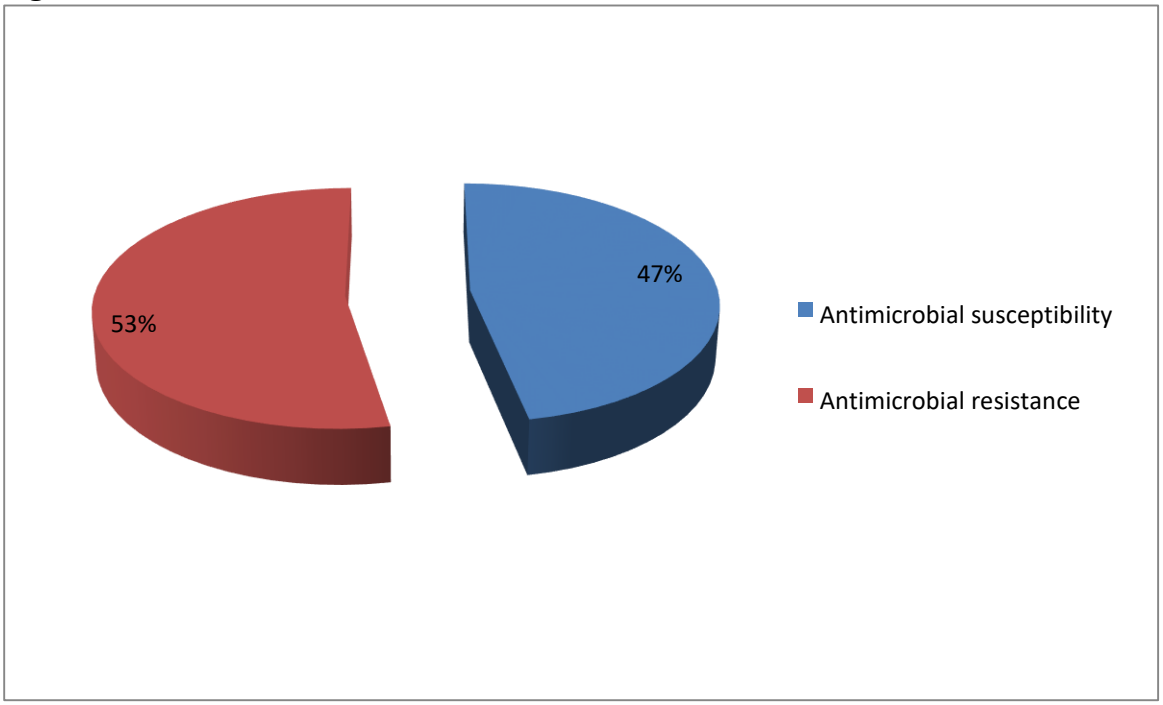
As shown in Figure 3, the following bacterial uropathogens were identified: *E. coli*, 59 isolates (28%); *S. aureus*, 17 isolates (8.29 %); *Klebsiella pneumonia*., 21 isolates (10.24 %); *Enterobacter spp*, 12 isolates (5.85 %); *Proteus* spp., 5 isolates (2.44 %); *Pseudomonas spp*., 3 isolates (1.46 %), *Streptococcus species*, 5 isolates (2.44%) and the least isolates were *E. Agglomeran*s, 1 isolate (0.49%) and *Shigella*, 1(0.49%).

Figure 3: Microorganisms isolates prevalence



Prevalence of Antimicrobial resistance among pregnant attending antenatal at LMUTH. Out of 124 cases with significant bacteria growth, 65 (53%), (95% CI,46.1%-59.8%) cultures plates were resistant to one or more drugs used in this study and 58(47%) were susceptible to all the drugs (Figure 4).

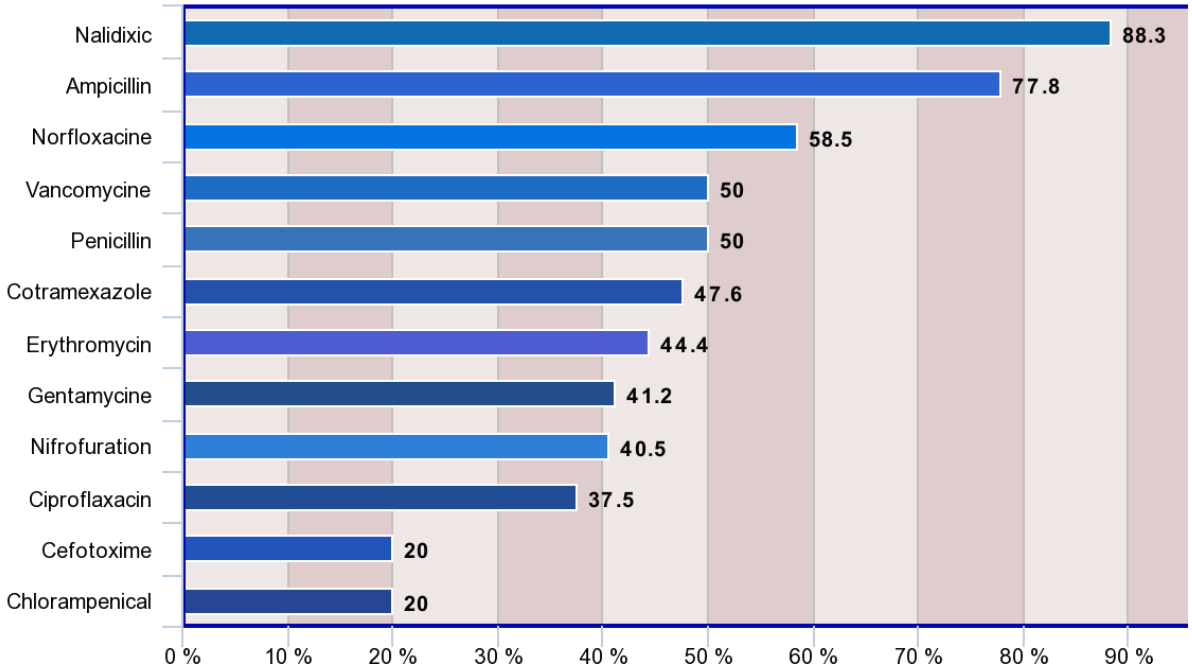
Figure 4: Prevalence of antimicrobial resistance



4.4. Urinary tract infection antibiotic resistance distribution of pregnant women

In figure 5. The prevalence of resistance in descending order was as follows; nalidixic acid 88.3%, ampicillin 77.8%, norfloxacine 58.5%, vancomycin 50%, penicillin 50%, co-trimoxazole 47.6%, erythromycin 44.4%, gentamicin 41.2%, nitrofuratoin 40.5%, ciprofloxacin 37.5, cefotaxime 20% and chloramphenicol 20%.

Figure 5: Resistance of isolated bacteria to antibiotics among pregnant women at Levy Mwanawasa University Teaching Hospital



4.5. Gram positive and Gram-negative antimicrobial resistance profile of urinary tract infections among pregnant women

In Table 5. Among the gram positive bacteria, the total number of 50 *Staphylococcus aureus* were isolated, maximum resistance was shown to norfloxacine and nalidixic acid at 3(60%) respectively, Ciprofloxacin 1(17%), then followed by Chloramphenicol1 (25%), Nitrofurantoin 2(40%).The total number of *Streptococci* isolated was 10. The maximum resistance was shown to norfloxacine 1(100%) followed by Co-tramoxazole and nitrofuratoin at 1 (50%) respectively. The drugs Cefotaxamine, Gentamicin, Penicillin and Ampicillin were not administered for *strep Spp*. Gram-negative bacteria isolates were more prevalent than gram-positive bacteria isolates (25%).

Among the gram-negative isolates, the drugs most resistant to *E.coli* were; ampicillin 2(67%), norfloxacine 14(52%) and nalidixic acid 1(50%) and the least resistant was nitrofuratoin 14(37). The drugs most resistant to *Klebsiella* were; nalidixic acid 14(82%), ciprofloxacin 3(75%) and the least resistant chloramphenicol 0(0%). The drugs

most resistant to *Enterobacter spp* were; nalidixic acid 9 (90%), norfloxacine 2(67%) and the least resistant was ciprofloxacin 0(0%). The drugs most resistant to *proteus spp* were; nalidixic acid 4(100%), norfloxacine 3(75%). For the Gram positives drugs including penicillin, cefotaxime, and chloramphenicol showed 100% sensitivity. Among the gram-negative isolates drugs including; chloramphenicol showed maximum sensitivity of 100%.

Table 2: Drug resistance of isolated gram-positive bacteria

Antibiotics tested	***Staphylococcus aureus* (n= 50)**		***Strep Spp* (n = 10)**	
	Total	Resistance (n %)	Total	Resistance n (%)
Nitrofurantoin	5	2(40%)	2	1(50%)
Nalidixic acid	5	3(60%)	0	0(0%)
Chloramphenicol	4	1(25%)	1	0(0%)
Norfloxacine	5	3(60%)	1	1(100%)
Co-trimoxazole	7	3(43%)	2	1(50%)
Ciprofloxacin	6	1(17%)	3	1(33%)
Cefotaxime	1	0(0%)	-	-
Gentamicin	6	3(50%)	-	-
Penicillin	2	0(0%)	-	-
Ampicillin	2	1(50%)	-	-
Erythromycin	7	4(57%)	1	0(0%)

Table 3: Gram negative and antimicrobial sensitivity profiles of UTI among pregnant women

Antibiotics tested	***E.coli* (n= 126)**		***Klebsiella* (n = 54)**	
	Total	Resistance (n %)	Total	Resistance n (%)
Nitrofurantoin	38	14(37%)	16	8(50%)
Nalidixic	2	1(50%)	17	14(82%)
Chloramphenicol	15	3(20%)	1	0(0%)
Norfloxacine	27	14(52%)	9	6(67%)
Co-trimoxazole	7	4(57%)	3	2(67%)
Ciprofloxacin	15	7(47%)	4	3(75%)
Cefotaxime	9	2(22%)	-	Drug not given
Gentamicin	7	1(14%)	2	2(100%)

Penicillin	2	1(50%)	-	Drug not given
Ampicillin	3	2(67%)	2	2(100%)
Erythromycin	1	0(0%)	-	Drug not given
Antibiotics tested	***Enterobacter SPP* (n= 29)**		***Proteus spp* (n = 17)**	
	Total	Resistance (n %)	Total	Resistance n (%)
Nitrofurantoin	10	3(30%)	4	1(25%)
Nalidixic	10	9(90%)	4	4(100%)
Chloramphenicol	2	0(0%)	1	0(0%)
Norfloxacine	3	2(67%)	4	3(75%)
Co-trimoxazole	1	0(0%)	1	0(0%)
Ciprofloxacin	3	0(0%)	-	Drug not given
Cefotaxime	-	Drug not given	-	Drug not given
Gentamicin	-	Drug not given	1	1(100%)
Penicillin	-	Drug not given	-	Drug not given
Ampicillin	-	Drug not given	1	1(100%)
Erythromycin	-	Drug not given	1	0(0%)
Antibiotics tested	***Pseudomonas* (n= 18)**		***E.Agglomerus* (n = 3)**	
	Total	Resistance (n %)	Total	Resistance n (%)
Nitrofurantoin	3	2(67%)	1	1(100%)
Nalidixic	3	2(67%)	1	1(100%)
Chloramphenicol	-	Drug not given	-	Drug not given
Norfloxacine	4	2(50)	1	1(100%)
Co-trimoxazole	7	3(43%)	-	Drug not given
Ciprofloxacin	1	0(0%)	-	Drug not given
Cefotaxime	-	Drug not given	-	Drug not given
Gentamicin	-	Drug not given	-	Drug not given
Penicillin	-	Drug not given	-	Drug not given
Ampicillin	-	Drug not given	-	Drug not given
Erythromycin	-	Drug not given	-	Drug not given

4.6 Association of demographic factors with antimicrobial resistance

This section presents the results of the relationship between antimicrobial resistance and background characteristics. Table 4 below shows the distribution of antimicrobial resistance by the independent variables (age, current use of antibiotics, HIV status, duration of antibiotic use, symptoms, history of UTI infection, trimester, residence and marital status). Socio-demographic characteristics have been identified as factors that may affect antimicrobial resistance in pregnant women. The results presented in Table 5 below show the Pearson's Chi-square test results. The study found that HIV positive pregnant women (71%) were more likely to have antimicrobial resistance compared to HIV negative pregnant women (67%) at $p<0.64$, indicating an insignificant relationship between antimicrobial resistance and HIV status of the pregnant women. Other analysis, as shown in Table 5, showed that pregnant women with a history of antibiotic use were more likely have antimicrobial resistance, but this was not statistically significant at $p<0.05$. There was no association between antimicrobial resistance and the following; age in years, gestational age, marital status, education level and residence among the pregnant women with urinary tract infections at LMUTH.

Table 4: Association of antimicrobial resistance with socio-demographic characteristics of study participants at Levy Hospital

Characteristics	No Resistance	Resistance	P-values
Age group			
18-24	8 (26.7%)	22 (73.3%)	0.681
25-29	10 (27.8%)	26 (72.2%)	
30-34	9 (25.7%)	26 (74.3%)	
≥35	10 (47.8%)	12 (52.2%)	
Residence			0.7760
High density area	18 (45%)	47 (55%)	
Middle	11 (35.5%)	20 (65.5%)	
Low density	8 (34.8%)	15 (65.2%)	
Marital Status			0.8370
Single	5 (22.7%)	17 (77.3%)	
Married	32 (32.7%)	68 (67.3%)	
Gestational age			0.385
1st trimester	3 (16.7%)	15 (83.3%)	
2nd trimester	21 (34.4%)	40 (65.6%)	
3rd trimester	13 (31.1%)	31 (68.9%)	
History of UTI infection			0.0580
No	5 (33.3%)	10 (66.7%)	
Yes	32 (30.3%)	76 (69.7%)	

Current Use of Antibiotics No Yes	 30 (30.3%) 8 (32%)	 69 (69.7%) 17 (68%)	0.0270
Knowledge of antibiotic Resistance No Yes	 36 (32.7%) 2 (14.3 %)	 74 (67.3%) 12 (85.7%)	 0.223** 0.133*
Duration of antibiotic Use 7 days 14 days	 24 (29.3%) 9 (34.6%)	 58 (70.7%) 17 (65.4%)	0.2660
Income 0-1500 1501-3000 ≥3001	 15 (40.5%) 15 (40.5%) 7 (18.9%)	 36 (41.9%) 37 (43%) 13 (15.1%)	0.2786
Education level attained Primary Secondary Tertiary	 7 (38.9%) 16 (25.8%) 14 (34.2%)	 11 (61.1%) 46 (74.2%) 27 (65.9%)	0.416
HIV status Negative Positive	 23 (32.9%) 15 (28.9 %)	 47 (67.1%) 37 (71.2%)	0.2238
Symptoms dysuria/nocturia/urgency dysuria Haematuria	 16 (45.7%) 14 (40%) 5 (14.3%)	 33 (42.9%) 32 (41.6%) 12 (15.6%)	0.0859
Kidney Infection No Yes	 35(29.4%) 3 (60%)	 84 (70.6%) 2 (40%)	0.167** 0.167*

Note: Pearson- **

Fishers' Exact- *

4.7 Determinants of Antimicrobial Resistance in pregnant women attending antenatal clinic at LMUTH.

Table 5: Logistic Regression Model

Variables	Univariate Analysis		
	Crude Odds Ratio	95% CI	P value
Marital Status	0.41	0.75-2.99	0.246
Age			
25-29	1.12 1.56	0.25-5.04 0.30-	0.876
30-34	0.22	8.03	0.594
>35		0.03-1.38	0.108
Education Level			
Primary	1		
Secondary	2.11	0.34-13.1	0.423
Tertiary	1.58	0.22-11.5	0.652
Previous UTI	0.32	0.17-0.59	0.000*
HIV Status	0.33	0.16-0.68	0.002*
Income			
Low	1		
Medium	0.75	0.17-3.28	0.708
High	0.67	0.08-4.99	0.695
Kidney Infection	0.99	0.66-1.56	0.987
Duration of antibiotic Use	0.61	0.24-1.55	0.310
Residence			
High	1		
Medium	1.53	0.35-6.56	0.568
Low	0.36	0.86-1.88	0.227

Symptoms			
Nocturia/Urgency	1		
Dysuria	1.08	0.31-3.72	0.906
Haematuria	1.36	0.12-9.37	0.754
Gestational Age			
1st Trimester	1		
2nd Trimester	0.59	0.11-3.30	0.556
3rd Trimester	0.85	0.12-6.03	0.871
UTI Medication	1.00	0.57- 1.73	0.000*
Familiarity to Antibiotics	0.41	0.19-0.86	0.019*

Statically significant P-value (P<0.05)

C.I: Confidence Interval

Table 6: Multivariable Analysis

Variables	Univariate Analysis			Multivariable Analysis		
	COR	**95% CI**	**P-value**	**AOR**	**95% CI**	**P-value**
Previous UTI	0.32	0.17-0.59	0.000*	0.69	0.28-1.69	0.427
HIV Status	0.33	0.16-0.68	0.002*	0.36	0.23-0.94	0.034*
UTI Medication	1.00	0.57-.73	0.000*	0.33	0.13-0.83	0.020*
Familiarity to antibiotics	0.41	0.19-0.86	0.019*	1.09	0.44-2.70	0.851

COR: Crude odds ratio, AOR: Adjusted odds ratio, CI: Confidence Interval

**** Statistically significant P-value (P<0.05)***

Interpretation of Univariate Analysis

The logistic regression model was used to test for statistical significance association between the dependent variable (Antimicrobial resistance) and explanatory variables (age, current use of antibiotics, HIV status, duration of antibiotic use, symptoms, history of UTI infection, trimester, residence and marital status). The model allowed estimating the probability of a binary outcome.

Marital status having an odds ratio of 0.41 at 95% CI indicated that marital status had 59% of affecting resistance, it had a p value of 0.246 which was greater than 0.05 hence showing statistical insignificance in association with the outcome variable. Age ranges between 30-34 were two times more likely to affect antimicrobial resistance as compared to the age ranges 25-29 and those greater than 35 years of age with odds ratios of 1.52, 1.12 and 0.22 respectively at 95% CI, all had p values greater than 0.05 hence statistically insignificant in association with the outcome variable. Education level had secondary and tertiary levels two times more likely of affecting antimicrobial resistance as compared to primary level which had odds ratios of 2.11, 1.58 and 1 at 95% C.I. Their p values were all greater than 0.05, these values show that level of education was however statistically insignificant when associated with the outcome variable. Income was statistically insignificant in association with antimicrobial resistance as its p value was greater than 0.05, it nonetheless showed that all the string variables had a lesser likelihood of affecting antimicrobial resistance as odds ratios were less than 1. kidney infection, duration of antibiotic use and gestational age all indicated a lesser odd of affecting the outcome variable as they all had odds ratios of less than 1, their p value were as well statistically insignificant as they were greater than 0.05. The variables residence and symptoms showed likelihood of affecting the outcome variable as they both had odds ratios of greater than 1, they were however statistically insignificant as their $P>0.05$.

The variables; previous UTI, HIV status, history of UTI medication and familiarity with medication all showed statistical significance in association with the outcome variable as they had $P<0.05$, the P values where respectively as follows; (0.000, 0.002, 0.000, 0.019). With reference to odds ratio values, previous UTI and HIV status showed -67% likelihood of affecting the outcome variable, history of UTI medication had an odds ratio of 1.00 indicating it would in no way increase the likelihood of affecting antimicrobial resistance. Familiarity with medication indicated a -59% likelihood of affecting the outcome variable.

Interpretation of Multivariable Analysis

Upon completion of the univariate analysis, the statistically significant variables were assessed in the multivariable analysis for adjustment of confounding factors, screening

the relevant variables and for prediction purposes. In the multivariable analysis only UTI medication (OR-0.36 ,95%CI, P value-0.034) and HIV status (OR-1.09,95%CI, P value-0.020) were statistically significant with P values < 0.05 hence concluding that they are predictors of the outcome variable (antimicrobial resistance).

With all the determinants having an influence on resistance either directly or indirectly as illustrated using the logistic regression model above, we examined which determinants can be deemed predictors of resistance. Investigator led stepwise regression model was used to select the best fitting model for predictors of resistance as illustrated in table 7 below. The full model fits the data better as opposed to the nested model. The selection of this model was done using adjusted r squared statistics and the Akaike information criterion (AIC) or Bayesian information criterion (BIC).

Table 7: Model selection

Variable	Odds Ratio	95% C.I	P value
Marital status	1.72	0.48-6.11	0.398
Age	0.97	0.61-1.56	0.924
Education level	0.69	0.27-1.75	0.443
Income	0.87	0.57-1.34	0.539
Residence	1.01	0.51-1.98	0.979
Gestational age	1.27	0.56-2.86	0.566
Symptoms	0.90	0.50-1.61	0.728
Previous UTI	1.12	0.28-4.66	0.879
Kidney infection	1.15	0.49-2.66	0.744
UTI Medication	0.12	0.27-0.59	0.008*
Familiarity to antibiotics	2.28	0.21-25.14	0.501
Current antibiotics	1.35	0.45-4.06	0.599
Duration of use	0.33	0.09-1.13	0.078
HIV Status	0.11	0.02-0.45	0.001*

**** Statistically significant P-value (P<0.05)***

5.0 CHAPTER FIVE: DISCUSSION

Our study found the prevalence of urinary tract infection to be 60%, with the main causative agents being *E.coli*, *Klebsiella* and *Staphylococcus aureus* having proportions of 59, 21 and 17 respectively. These results are in consensus with studies conducted in; Libya (Mohammed *et al.,* 2016, Ethiopia, Kibret and Abera 2014), these studies both had *E.coli* and *Klebsiella* as the highest isolated bacteria. However, our results do not agree with other studies conducted in Erbil, Iraq, by Al-Naqshbandi *et al.,* 2019; this study had the highest causative agents being *Acinetobacter baumannii* and *Enterococcus faecalis*.

The prevalence of AMR was found to be 53% with the highest resistant drugs being; ampicillin, nalidixic acid and norfloxacine. The least resistant were found to be ciprofloxacin, chloramphenicol and nitrofurantoin. Our results of AMR are similar to other studies which include; a study conducted in Iraq, (Al-Naqshbandi *et al.,* 2019) indicated that nitrofurantoin as well showed low levels of resistance with a percentage of 30 which is similar to our study. This study however varies with our study in that its highest resistant drugs were ethrythromycin, ciprofloxacin and cefotaxime compared to our study which indicated that these drugs had the least levels of resistance.

Our study further determined predictor variables of AMR; this however showed no evidence of statistical association between our independent variables and the outcome variable. All the socio-demographic variables had p-values greater than 0.05, this is similar to a study conducted in Ethiopia, (Gizachew *et al.,* 2019) where they did not find any sociodemographic predictor variables for AMR, however they found predictor variables for clinical and bacterial variables.

Urinary tract infections (UTIs) are the most widely spread infections seen in hospital settings and the second commonest infections seen in the general population (Valiquette, 2001). The main findings of this study were; prevalence of urinary tract infections, in this study we investigated a total of 203 pregnant women, of which 123 were positive for UTI hence elucidating that UTI had a prevalence of sixty percent (60%). These results are much the same as a study that was carried out in Ebonyi state, Nigeria which had a prevalence of 55%, and a study carried out in India (Ahmed *et al.,* 2016) which had a prevalence of 61%. Similarity in these results is firmly established from the similarity in methodology.

These results are however not in consensus with other studies, for instance in a study carried out in Niger the prevalence was found to be 75% (Adabara *et al.,* 2012) which is quite high when compared to most studies including our current study, attribution to this is the small sample size of 100 which they had. The low socio-economic status of the subjects as observed in an earlier study (Adabara *et al.,* 2012) which plays an important

role in susceptibility to diseases and access to health care services may also be responsible for the high prevalence rate recorded. Our prevalence is however quite alarming as this high incidence highlights the size of the serious problem which necessitates a rapid interference especially that UTI is incriminated in many adverse implications on pregnant women, which include; poor maternal and perinatal outcomes, sepsis, caesarean delivery and preterm birth etc.

The prevalence of bacterial UTI in association with age was highest between the age groups (20-24) and (30-34) which constituted a proportion of 21% and 29.6% respectively, this was similar to a study that was carried out in India (*Ahmed et al.,* 2016) and a study carried out in Nigeria (Onuoha *et al.,* 2014) .This may be the case because women in these age groups are sexually active and symptomatic UTI has a higher prevalence due to the stated fact, furthermore this is basically a reproductive age group and as earlier stipulated, reproduction is related to UTI.

Prevalence of UTI in relation to gestational trimester was higher in the 1^{st} (41.3%) and 2^{nd} (38.9%) trimesters as compared to the third trimester (20.0%), our results are consistent with a study carried out in India (Thomas *et al.,* 2018) where they found UTI to be more prevalent in the 1^{st} and 2^{nd} trimesters. Our results however do not tally with those of a study conducted in Uganda (Sekikubo *et al.,* 2017) and India (Ahmed *et al.,* 2016) which indicated higher prevalence related to the 2^{nd} and 3^{rd} trimesters with the 1^{st} trimester having the least association. This difference may be attributed to either the vesico-ureteral reflux or change in urine stasis or decrease in urinary oestrogens and progesterone in the different trimesters of pregnancy.

This study shows a higher prevalence of UTI in HIV positive pregnant women as compared to HIV negative pregnant women (71% and 39% respectively). This factor is attributed to the firm understanding that HIV positive patients are more susceptible to several infections seeing that their immune system is compromised especially if not adhering well to the drugs (Marami *et al.,* 2019).

The prevalence of antimicrobial resistance in our study was found to be 53% , furthermore the prevalence of the resistance of the different drugs was determined, which are in decreasing order as follows; Nalidixic acid (88.3%), ampicillin (77.8%), norfloxacine (58.5 %), vancomycin (50%), penicillin (50%), co-trimoxazole (47.6%), erythromycin (44.4%), gentamicin (41.2%), nitrofuratoin (40.5%), ciprofloxacin (37.5%), cefotaxime (20%) and chloramphenicol (20%). These results are related to other studies, for instance, in Libya (Mohammed *et al*., 2016), Nigeria (Onuoha *et al*., 2014) and Uganda (Sekikubo *et al*., 2017) the resistance levels of Nalidixic acid, ampicillin, norfloxacine, ciprofloxacin and nitrofuratoin where similar. However, some variation with other studies like one carried out in Ethiopia (Gizachew *et al*., 2019), this study indicated higher resistance rates of ciprofloxacin and chloramphenicol.

There indicates a high level of resistance for drugs that are commonly prescribed, the high level of resistance could probably be because they have been on the market for a long time thus allowing microorganisms time to develop resistance mechanisms towards the antibiotics. In addition to this, this level of resistance could be attributed to easy access to antibiotics over the counter in developing countries like Zambia. Additionally, the initial use of antibiotics before the laboratories results of antimicrobial susceptibility can be an attribution to the high resistance levels. Consequently, the need for the development and enforcement of antibiotic policies and proper antibiotic stewardship in developing countries cannot be overemphasized.

Our study points out that nitrofuratoin was more susceptible to uropathogens than most prescribed drugs, this is interesting considering that there have been debates to have it phased out. Literature reviews that there is need to resurface old drugs as they would be more effective, some of these drugs being nitrofuratoin and fosfomycin i.e. specifically for resistant uropathogens (Gardiner *et al*., 2019).

With reference to data collected from our questionnaire we discovered that pregnant women are prescribed medication such as metranidazole and ciprofloxacin upon symptoms of UTI without having been screened thus urinalysis test and culture done hence increasing the probability of resistance of these drugs to uropathogens. Furthermore some women even complained of having instances where these medications didn't actually work and when they went back to the hospital were still prescribed the same medication, this hence calls for emphasis to follow protocols by clinicians and avoid short cuts when it comes to patient treatment.

The three most prominent bacteria isolated in our study were, *E.coli* 59(28.78%), *Staph aureus* 17(8.29%) and *Klebsiella pneumoniae* 21(10.24) of which *E.coli* was the most predominant. The least isolated bacteria were; *Shigella* 1(0.49%), *E.agglomerans* 1(0.24%) and *Pseudomonas spp* 3(2.44%). These results are consistent with studies carried out in Ethiopia, (Derbie *et al.,* 2017), Nigeria, (Onuoha *et al.,* 2014) which had isolates *E.coli* and *Klebsiella* with similar percentage proportions. However, our results were contrary with other studies carried out in Brazil (Cunha *et al.,* 2016), India (Ahmed *et al.,* 2016) which showed *Staphylococcus aureus* as the most isolated bacteria. The difference in variation may be due to variation in clinical or underlying conditions of patients since *Klebsiella spp* assume greater prevalence in recurrent infections associated with urological manipulations.

These results are as well similar to a study carried out in Libya (Mohammed *et al.,* 2016). In contrast a study carried out in Nigeria showed that *E.coli* was the highest followed by *staph aureus* (Onoh *et al.,* 2013) the similarities and differences in the type and distribution of uropathogens show a discrepancy from country to country due to many factors such as environmental conditions, health practices, patient conditions,

personal hygiene, number of patients examined and laboratory procedures. That *E.coli* is still the main causative agent of UTI in pregnant women is quite disturbing because of its implication. *E. coli* causes diarrhoea which causes the body to lose lots of fluids, pregnant women with an *E.coli* infection can easily become dehydrated. In rare cases, they may start to bleed heavily and there is even high rate of miscarriage due to the same. (Mothertobaby.org)

In our study we determined current antimicrobial susceptibility trends from the positive urine cultures but will however only discuss the three most isolated bacteria which are *E.coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. *E.coli* was most susceptible to erythromycin(100%), chloramphenicol (80%), cefotaxime (78%) and nitrofurantoin (63%) which is similar to a study conducted in Ethiopia (Derbie *et al.*,2017) but not in consensus with a study carried out in Libya (Mohammed *et al.*,2017). *Klebsiella pneumoniae* showed highest susceptibility to; chloramphenicol (100%), nitrofurantoin (50%) and ciprofloxacin (25%). *Staph aureus* showed highest susceptibility to; cefotaxime (100%), ciprofloxacin (83%), and nitrofuratoin (75%). Most of the bacteria were resistant to Nalidixic acid, Norfloxacine, ampicillin and penicillin.

Among the gram negative isolates, the drugs most resistant to *E.coli* were; ampicillin 2(67%), norfloxacine 14(52%) and nalidixic acid 1(50%) and the least resistant was nitrofuratoin 14(37). The drugs most resistant to *Klebsiella* were; nalidixic acid 14(82%), ciprofloxacin 3(75%) and the least resistant chloramphenicol 0(0%). The drugs most resistant to *Enterobacter spp* were; nalidixic acid 9(90%), norfloxacine 2(67%) and the least resistant was ciprofloxacin 0(0%). The drugs most resistant to *proteus* were; nalidixic acid 4(100%), norfloxacine 3(75%). For the Gram positives drugs including penicillin, cefotaxime, and chloramphenicol showed 100% sensitivity. Among the gram-negative isolates drugs including; chloramphenicol showed maximum sensitivity of 100%.

Our susceptibility pattern results are comparable to those of a study conducted in Zimbabwe (Rukweza *et al*., 2018) where their study indicated nitrofurantoin having a high resistant rate mean while our study indicated that nitrofuratoin had sensitivity of 64%.

The possible reason for the variances might be related to difference in study populations and additionally literatures also stated that antimicrobial resistance rates among common uropathogens continue to evolve and appear to be increasing to many commonly used agents from time to time.

This study also tested for the association of predictor variables (age, education level, marital status, HIV status, residence, previous UTI history and gestational age) with the outcome variable (antimicrobial resistance) using univariate and multivariable analysis models. Our study only found two significant predictor variables of antimicrobial

resistance, these were history of urinary tract medication and HIV status which had P values of 0.034 and 0.020 respectively, these results are not similar to other studies which include; a study carried out in Ethiopia, where they did not find significant association with the socio-demographic variables but however found significant association with the clinical and phenotypic variables (Gizachew *et al.,* 2019). This could be attributed to the difference in sample size and methodology used in both studies. Our results on HIV status being a predictor of antimicrobial resistance could be related with a study conducted at the University of Tennessee at Knoxville in 2019, the study found that persons with a weakened immune system are more vulnerable to opportunistic infections and are therefore frequently prescribed antibiotics to prevent or treat these infections. This increases the exposure of those bacteria to antibiotics, giving them more chances to evolve to become resistant to the medication and contributing to the current serious public health threat of drug resistant diseases. (Ashley *et al.,* 2019).

Conclusion

From our study, we had a UTI prevalence of 60% which is generally much higher than most studies undertaken, this is indicative of the level of menace urinary tract infections are in Zambia. This is furthermore alarming considering the adverse infects of untreated UTI on the mother and fetus, these include; kidney infections, preterm birth, low birth weight as well as miscarriages.

The highest causative agent of urinary tract infections was found to be *E. coli* which is consistent with many other studies, which calls for concern considering it has the highest resistant strains which is resistant to 3rd generation cephalosporins implying that treatment of severe infections rely on carbapenems which are more expensive and might be a challenge for low income countries. The prevalence of antimicrobial resistance was found to be 53% with Nalidixic acid, Norfloxacine and ampicillin being the most resistant and chloramphenicol, ciprofloxacin and nitrofuratoin being the least resistant. Some of these drugs that had high resistance rates are routinely given hence questioning treatment effectiveness; furthermore, AMR is a global health security threat that requires concerted cross sectional action by governments and society as a whole.

Our study found HIV status and UTI medication as statistical predictor variables of antimicrobial resistance. However the study did not record any significant association between AMR and socio-demographic factors which was more so because most indicator variables are genetic and clinical as indicated by our study which found UTI medication and HIV status to be significantly associated with AMR which is comparable to other literature.

We can further conclude that old drugs are more susceptible to resistant uropathogens seeing that nitrofuratoin had a good susceptibility pattern. Our antimicrobial resistance results additionally reflect the need for development of policies that restrict the usage of antibiotics.

Limitations

Our study encountered challenges, one of which was information bias in the administration of the questionnaires. In order to answer some of the questions, participants had to recall past events and their responses were validated using laboratory analysis and use of their medical records as its one of the most credible approaches to validate self-reported data.

The other challenge encountered was unavailability of some of the susceptibility drug discs as the research was not sponsored hence limiting us to the drug discs available to the hospital at every given point. Nonetheless we asked for assistance with susceptibility drug discs from other institutions of which in most cases gave positive feedback.

Recommendations

Study findings show that the prevalence of antimicrobial resistance is high. Therefore in line with the government health policies under the multisectoral National Action Plan on Antimicrobial resistance there is need to strengthen actions to ensure that antibiotics are used appropriately, such as re-enforcing prescription-only policies, implementing surveillance of antimicrobial consumption and implementation of antimicrobial stewardship programs. (WHO 2018)

Finding that in this study social demographic characteristics were not associated with Antimicrobial resistance suggests need for conducting similar studies such as (Gizachew *et al*., 2019) that include examining genotypic and phenotypic characteristics.

Our study like many other studies has shown that old drugs such as nitrofuratoin are less resistant compared to most routinely used drugs hence the need to resurface old drugs as indicated from other studies. (Gardiner *et al.,* 2019)

Furthermore, similar studies should be carried out in different areas of the country in order to have a vivid picture of the prevalence of antimicrobial resistance among pregnant women and additionally extended to the general population considering the fact that not many articles have been published on the same within the country, that is to the best of our knowledge.

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Appendices

Appendix 1: Informed Consent Form

INFORMED CONSENT DOCUMENT FOR PREGNANT WOMEN ATTENDING ANTENATAL CLINIC IN LUSAKA

Study Title: URINARY TRACT INFECTIONS AND ANTIMICROBIAL RESISTANCE AMONG PREGNANT WOMEN ATTENDING ANTENATAL CLINIC AT LEVY MWANAWASA UNIVERSITY TEACHING HOSPITAL- CROSS SECTIONAL STUDY

INTRODUCTION

My name is Kekelwa Inyambo Yeta. I am student at the University of Zambia pursuing a Master of Science in Epidemiology under the school of Public Health. I am conducting a study on Antimicrobial resistance (which is basically the ability of a microorganism like bacteria, viruses and some parasites to stop an antimicrobial such as antibiotics from working against it. As a result, standard treatments become ineffective, infections persist and may spread to others) among pregnant women attending antenatal clinic at Levy Mwanawasa University Teaching Hospital. I am going to give you information and invite you to be part of this study. You do not have to decide today whether or not you will participate in this study. Before you decide, you can talk to anyone you feel comfortable with about the study. This consent form may contain words that you do not understand. Please ask me to stop as we go through the information and I will take time to explain. If you have questions later, you can ask them of me or of another researcher.

PURPOSE OF THE STUDY

The study will help provide more information and knowledge concerning the prevalence of antimicrobial resistance among pregnant women and the general public. The information that will be provided will assist the Ministry of Health and organizations like WHO to combat and monitor antimicrobial resistance and formulate new drugs. This is very important as this information will provide feedback to the Ministry of Health in order to influence decision making and policy formulation.

PROCEDURE

If you agree to be a participant in this study, you will be interviewed by me. This will take approximately 15 minutes. During the interview, I will sit down with you in a place of your choice and no one else but me will be present unless you would like someone else to be there. If you do not wish to answer any of the questions during the interview, you may say so and I will move on to the next question. You will also be required to fill out a questionnaire provided by me or it can be read to you and you can say out loud the answer you want me to write down. If you do not wish to answer any of the questions, you may skip them and move on to the next question. The information you provide is confidential, your name is not being included on the forms, only a number will identify you, and no one else except me will have access to the information.

RISKS AND DISCOMFORTS

There are no physical risks to participating in this study except the time spent on answering these questions, which will take approximately 15 minutes. Some information you may tell me or enter in the questionnaires may be personal or sensitive; I would like to assure you that the information that will obtain from you will not be shared with anyone.

BENEFITS

There are no direct benefits to you, but you will contribute and broaden the spectrum of knowledge concerning antimicrobial resistance, which we hope will benefit all current and future pregnant women. Your participation will also help provide information that will assist relevant authorities to formulate new line of drugs.

PROTECTING CONFIDENTIALITY

TO protect the information, I will get from you. I will do my best to make sure that your name is not included with any information that I collect from you. Your personal information will not be published nor will it be revealed to anyone. The collected information will be used for academic purposes only.

VOLUNTARY PARTICIPATION

You can decide whether you want to take part in this, and you may change your mind later and stop participating even if you agreed earlier. Withdrawing from the study will not bring any problems to you nor will you be punished. No privileges will be taken from you should you decide not participate. The choice that you make will have no bearing on your job or on any work-related evaluations or reports.

CONTACT INFORMATION

If you have questions or problems concerning this study, contact me, Kekelwa I Yeta on cell number; 0976098932. If you have any questions and complaints about the study, call or contact the University of Zambia Ethics Review Board for any ethical queries on 0211256067.

CERTIFICATE OF CONSENT

I declare that I have been informed about the purpose of this study, procedures, possible benefits and risks.

I have read the foregoing information and I have been given the opportunity to ask questions about it and all have been answered to my satisfaction. I consent voluntarily to be a participant in this study.

Print Name of Participant_____ Signature of Participant _____ Date _____ Day/month/year

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the

best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

Print Name of person obtaining the consent_____

Signature of person obtaining the consent_____ Date _____
Day/month/year

A copy of this ICF has been provided to the participant.

Appendix 2: Questionnaire

THE UNIVERSITY OF ZAMBIA

SCHOOL OF PUBLIC HEALTH

DEPARTMENT OF EPIDEMIOLOGY AND BIOSTATISTICS

Research questionnaire ()

Dear Respondent.

I am Kekelwa Inyambo Yeta a student at The University of Zambia-Ridgeway campus undertaking a research on Urinary Tract Infections (UTI) and Antimicrobial Resistance (AMR) among pregnant Women attending Antenatal clinic at Levy Mwanawasa University Teaching Hospital.

The research is being undertaken in partial fulfilment of the award of a Masters' degree offered by The University of Zambia and is therefore purely academic. You have been selected to be part of the sample by way of sampling from the wider population. Please note that this is a voluntary activity where the information you provide will be held with utmost confidentiality. You are advised not to write your name anywhere on this questionnaire. All respondents to this questionnaire are assured that their responses and information so contributed shall not be used for any other purposes other than the one stated above**.** Answer the questions freely and truthfully.

INSTRUCTIONS:

Do **not** write your **name** on this questionnaire.

For questions where options are given, **tick** where appropriate.

Where blank spaces are provided, kindly **write** your own answers.

	Marital status	a) Single b) Married c) Separated d) Widowed e) Divorced		
2.	Age	a) < 20 years b) 20-24 years c) 25-29 years d) 30-34 years e) 35-39 years f) 40 years and over		
3.	Have you been to school?	a) Yes b) No		
4.	if yes What level did you attain?	a) Primary b) Secondary c) Tertiary		
5.	Level of monthly income	a) 1001-1500 b) 1501-2000 c) 2001-3000 d) Above 3000		
6.	Residential area			
7.	Please choose the appropriate answer	a) This is my first pregnancy b) I have been pregnant before.		

8.	Please tick the symptoms you are			
	experiencing	a) Dysuria: **(**Burning or pain on urination) b) Haematuria:(Blood in urine) c) Urgency: (sudden need to urinate) Nocturia: (awakening during sleep to urinate**)**		
9.	How many times an hour do you urinate?	_____		
10.	Do you experience back pain **(**if yes, right side, left side or both?)	_____		
11.	Have you had a previous urinary tract infection (UTI)? If yes, this month or previous months.	a) Yes b) No		
12.	Have you ever had an infection of the kidney?	a) Yes b) No		
13.	Have you taken any medication for current UTI symptoms?	a) Yes b) No		
14.	Have you ever heard of antibiotics?	a) Yes b) No Don't know		
15.	Have you ever used antibiotics to treat a UTI?	a) Yes b) No c) Don't know		
16.	Please provide the name of the antibiotics you have used or are familiar with	_____		

17.	Are you currently on antibiotics?	a) Yes b) No c) Don't know		
18.	For how long have used the antibiotics	a) 7 days b) 14 days		
		c) A month		
19.	Have you had incidences where antibiotics did not get rid of your UTI?	_____		
20.	HIV Status (To be checked from the ANC)	Reactive Non-Reactive		

Thank you

Appendix 3: The University of Zambia Biomedical Research Ethics Committee Approval Letter

THE UNIVERSITY OF ZAMBIA

BIOMEDICAL RESEARCH ETHICS COMMITTEE

Telephone: 260-1-256067
Ridgeway Campus
Telegrams: UNZA, LUSAKA
P.O. Box 50110
Telex: UNZALU ZA 44370
Lusaka, Zambia Fax:
+ 260-1-250753
E-mail: unzarec@unza.zm **Assurance**
No. FWA00000338
IRB00001131 of IORG0000774

8th November, 2018.

REF. No. 002-09-18.

Ms. Kekelwa I. Yeta,
University of Zambia,
School of Public Health,
P.O. Box 50110,
Lusaka.

Dear Ms. Yeta,

RE: "ANTIMICROBIAL RESISTANCE AMONG PREGNANT WOMEN WITH URINARY TRACT INFECTIONS ATTENDING ANTENATAL CLINIC AT LEVY MWANAWASA UNIVERSITY TEACHING HOSPITAL (LMUTH) LUSAKA, ZAMBIA" (REF. NO. 002-09-18)

The above-mentioned research proposal was presented to the Biomedical Research Ethics Committee (UNZABREC) 5th November, 2018. The proposal is approved. The approval is based on the following documents that were submitted for review:

a) **Study proposal**

b) **Questionnaires**

c) **Participant Consent Form**

APPROVAL NUMBER : REF. 002-09-18

This number should be used on all correspondence, consent forms and documents as appropriate.

- **APPROVAL DATE : 8th November, 2018**
- **TYPE OF APPROVAL : Standard**
- **EXPIRATION DATE OF APPROVAL : 7th November, 2019**
  After this date, this project may only continue upon renewal. For purposes of renewal, a progress report on a standard form obtainable from the UNZABREC Offices should be submitted one month before the expiration date for continuing review.
- **SERIOUS ADVERSE EVENT REPORTING**: All SAEs and any other serious challenges/problems having to do with participant welfare, participant safety and study integrity must be reported to UNZABREC within 3 working days using standard forms obtainable from UNZABREC.
- **MODIFICATIONS**: Prior UNZABREC approval using standard forms obtainable from the UNZABREC Offices is required before implementing any changes in the Protocol (including changes in the consent documents).
- **TERMINATION OF STUDY**: On termination of a study, a report has to be submitted to the UNZABREC using standard forms obtainable from the UNZABREC Offices.
- **NHRA:** Where appropriate, apply in writing to the National Heath Research Authority for permission before you embark on the study.
- **QUESTIONS**: Please contact the UNZABREC on Telephone No.256067 or by e-mail on unzarec@unza.zm.
- **OTHER**: Please be reminded to send in copies of your research findings/results for our records.

You're also required to submit electronic copies of your publications in peer-reviewed journals that may emanate from this study.

Yours sincerely,

Dr. S.H Nzala

VICE-CHAIRPERSON

Appendix 4: The National Research Health Research Authority approval letter

THE NATIONAL HEALTH RESEARCH AUTHORITY
Paediatric Centre of Excellence
University Teaching Hospital
P.O. Box 30075
LUSAKA
T: +260 211 250309/+260 95 563276 | **E:** znhrasec@gmail.com | www.nhra.org.zm

23rd November, 2018

Kekelwa I. Yeta
The University of Zambia
School of Public Health
P.O. Box 50110
LUSAKA

Re: Request for Authority to Conduct Research

The National Health Research Authority is in receipt of your request for authority to conduct research titled **"Antimicrobial Resistance among pregnant women With Urinary Tract Infections attending Antenatal clinic at Levy Mwanawasa University Teaching Hospital, Lusaka Zambia."** I wish to inform you that following submission of your request to the Authority, our review of the same and in view of the ethical clearance, this study has been **approved** on condition that:

1. The relevant Provincial and District Medical Officers where the study is being conducted are fully appraised;
2. Progress updates are provided to NHRA quarterly from the date of commencement of the study;
3. The final study report is cleared by the NHRA before any publication or dissemination within or outside the country;
4. After clearance for publication or dissemination by the NHRA, the final study report is shared with all relevant Provincial and District Directors of Health where the study was being conducted, University leadership, and all key respondents.

Yours sincerely,

Dr. Godfrey Biemba
Director/CEO
National Health Research Authority

All correspondences should be addressed to the Director/CEO National Health Research Authority