PROFILING OF ANTIBIOTIC RESISTANCE AMONG UROPATHOGENS ISOLATED FROM PATIENTS ATTENDING KERICHO COUNTY REFERRAL HOSPITAL

GLADYS CHEPKOECH MOSONIK

A Thesis Submitted to the Board of Graduate Studies in Partial Fulfillment of the Requirements for the Conferment of the Degree of Master of Science in Microbiology of the University of Kabianga

UNIVERSITY OF KABIANGA

OCTOBER, 2019

DECLARATION AND APPROVAL

Declaration

I declare that this thesis is my original work and has not been presented for the conferment of a degree or for the award of a diploma in this or any other university:-

Signature__________________ Date: ___________________

Gladys Chepkoech Mosonik,

PGC/MIC/005/15

Approval

This thesis has been submitted for examination with our approval as the University Supervisors:-

Signature__________________ Date: ___________________

Dr. Janeth Kombich, PhD.

Department of Biological Sciences,

University of Kabianga.

Signature__________________ Date: ___________________

Prof. Peter G.N. Njagi, PhD.

Department of Biological Sciences,

University of Kabianga.

COPYRIGHT

No part of this thesis may be produced, stored in any retrieval system or transmitted in any form mechanical, photocopying, recording or otherwise without prior permission from the author or the University of Kabianga

© Gladys chepkoech mosonik, 2019

DEDICATION

This work is dedicated to my daughter Shanice Cheptoo and to my parents Mr. William Mosonik and Mrs. Jane Mosonik. Thank you for your support and encouragements.

ACKNOWLEDGEMENT

Foremost, I want to thank God for granting me good health, knowledge and wisdom to undertake this study. It is through His mercy that I made it through.

I would like to give special credit to my supervisors Dr. Janeth Kombich and Prof. Peter G. N. Njagi for their guide, valuable comments, insightful criticism, patient and steadfast encouragements that made this work a success. Special thanks also to Mr. Samuel Symekher for his guide throughout the write-up and support in data analysis that gave this work a meaning. My appreciation also goes to Dr. Jared Yugi of Biological Sciences department and Dr. Reuben Langat of Mathematics department, University of Kabianga for their continuous encouragements whenever I was stuck.

I would also like to appreciate the lab manager, Kericho County Referral Hospital (KCRH), Mr. Robert Sang for allowing me to undertake the research in the lab. His valuable critiques and technical support made this work a success. My gratitude also goes to all the KCRH lab staffs especially the microbiology lab analysts for their unrelenting support and guide while undertaking the laboratory work.

Special thanks to my classmates and friends Emily Sitienei, Maryline Langat, Julius Koske, Festus Sang and Josphat Tonui for their moral support, advice and encouragements throughout the studies. It's through your encouragements that I made it through.

May God bless you all.

ABSTRACT

Antibiotic resistance among the causative agents of urinary tract infections (UTIs) presents a crisis that hinders efforts for effective management of the infections globally. Understanding the antimicrobial susceptibility profiles of bacteria to antibiotics at the local level is very important in empirical therapy. WHO in their report for early implementation to minimize the spread of antibiotic resistance emphasized on the importance of continuous surveillance of antimicrobial resistance in order to determine the current status. Although antibiotic resistance among uropathogens has been reported in some regions in Kenya, the profile of this antibiotic resistance has not been done in Kericho County. This research intended to find out the bacteria causative agents of UTI among patients attending Kericho County Referral Hospital (KCRH) and the current resistance to the available therapeutic agents. The research was conducted after obtaining authority from the Board of Graduate Studies of University of Kabianga, ethical committee of KCRH and the ethical approval by Institutional Research and Ethics Committee of Moi Teaching and Referral Hospital, Eldoret. The study design was hospital-based cross-sectional and the participants were all outpatients with manifestation of UTI as diagnosed by the clinician and consented to participate. Data collection was done using questionnaires and laboratory analysis. Three hundred urine samples from the eligible participants were inoculated onto the respective agar media and the bacteria growth identified using biochemical tests. Antibiotic sensitivity test was done using Kirby Bauer disk diffusion method. Statistical Package for Social Scientists version 21 software was used to analyse data using frequencies. Pearson correlation was used to test for association between categorical variables. Of the 300 samples received, 60 yielded bacteria isolates giving UTI prevalence of 20%. Urinary tract infection was common in females compared to males. Gram positive cocci were the major causative agents of UTI accounting for 75%. Among these, 41.7% were *Staphylococcus aureus* and *Enterococci faecalis* (33.3%). Gram negative rods accounted for 25% of whom 20.0% were *Escherichia coli,* 3.3% *Proteus* spp. and 1.7% *Klebsiella pneumoniae.* There was no statistically significant association between organisms causing UTI and gender (Pearson correlation=0.872). Antibiotic sensitivity tests were done for the sixty (60) bacteria isolates. The isolates showed various susceptibility levels to the therapeutic agents in the study. Although some bacteria were susceptible to the commonly used antibiotics, resistance was observed towards the antibiotics namely; ampicillin (84.3%) and azithromycin (71.9%). There was a higher resistance (75%) to augmentin by gram negative bacteria as compared to resistance (40%) by gram positive bacteria. Overall, bacteria were moderately resistant (30%) to norfloxacin and least resistant to cefoxitine (13.3%), gentamycin (11.7%) and ciprofloxacin (10%). While most bacteria showed multiple resistance to 3 drugs, some showed resistance to utmost 5 drugs tested in the study. This study found *Staphylococcus aureus* to be the predominant aetiological agent of UTI while *Enterococci faecalis* showed high level of multiple resistance to antibiotics. Significant resistance levels exist against augmentin, azithromycin and ampicillin. In conclusion, cefoxitine, gentamycin and ciprofloxacin are good therapeutic choices for recurrent UTI when culture results are unavailable. The study will support an empiric approach to the management of UTI hence preventing reoccurrence of infection and deterring further development of related complications.

TABLE OF CONTENTS

DECLARATION AND APPROVAL ii
COPYRIGHT iii
DEDICATION iv
ACKNOWLEDGEMENT v
ABSTRACT vi
TABLE OF CONTENTS vii
LIST OF TABLES x
LIST OF FIGURES xi
LIST OF PLATES xii
LIST OF ABBREVIATIONS AND ACRONYMS xiii
DEFINITION OF TERMS xiv
CHAPTER ONE 1
INTRODUCTION 1
1.1 Overview 1
1.2 Background of the Study 1
1.3 Statement of the Problem 4
1.4 General Objective 5
1.5 Specific Objectives 5
1.6 Hypotheses of the Study 6
1.7 Justification of the Study 6
1.8 Significance of the Study 6
1.9 Scope of the Study 7
1.10 Limitations of the Study 7
1.11 Assumptions of the Study 7
CHAPTER TWO 8
LITERATURE REVIEW 8
2.1 Introduction 8
2.2 Urinary Tract Infections 8
2.2.1 Epidemiology of uropathogens 9
2.2.2 Pathogenicity of uropathogens 9
2.2.3 Antibiotics and mode of action 11
2.2.4 Treatment of UTI 12
2.2.5 Antibiotic resistance and its mechanisms 12

2.2.6 Predisposing factors for antibiotic resistance ... 14
2.2.7 Detection of antibiotic resistance ... 15
2.2.8 Status of antibiotic resistance in Kenya ... 16
2.2.9 Global approaches to antimicrobial resistance ... 17
2.3 Identification of Knowledge Gap ... 18
CHAPTER THREE ... 20
MATERIALS AND METHODS ... 20
3.1 Introduction ... 20
3.2 Research Design ... 20
3.3 Location of the Study ... 20
3.4 Target Population ... 20
3.5 Sample Size and Sampling Procedures ... 21
3.5.1 Inclusion criteria ... 22
3.5.2 Exclusion criteria ... 22
3.6 Data Collection Instruments ... 22
3.6.1 Validity ... 23
3.6.2 Reliability ... 23
3.7 Urine Specimen Sampling and Analysis ... 24
3.7.1 Inoculation of urine sample ... 24
3.7.2 Dipstick screening ... 25
3.7.3 Microscopy examination ... 25
3.7.4 Gram staining ... 26
3.7.5 Biochemical identification of uropathogens ... 26
3.7.6 Analytical profile index principle ... 30
3.7.7 Antibiotic Susceptibility Test (AST) ... 33
3.8 Data Analysis and Presentation ... 33
3.9 Ethical Considerations ... 34
CHAPTER FOUR ... 35
RESULTS AND DISCUSSION ... 35
4.1 Introduction ... 35
4.2 Presentation of Results ... 35
4.2.1 Demographic characteristics of the patients with urinary tract infection ... 35
4.2.2 Uropathogens identified in urine sample from patients with UTI ... 37
4.2.3 Antibiotic resistance profile of uropathogens isolated from patients with UTI ... 38
4.3 Discussion of Results ... 41

CHAPTER FIVE **50**
SUMMARY, CONCLUSIONS & RECOMMENDATIONS **50**
5.1 Introduction 50
5.2 Summary 50
5.3 Conclusions 50
5.4 Recommendations 51
5.5 Suggestions for Further Research 52
REFERENCE **53**
APPENDICES **68**
Appendix 1: Consent Form 68
Appendix 2 : Questionnaire 70
Appendix 3: Antibiotic inhibition zone diameter as per CLSI guideline 73
Appendix 4: Approval of the research proposal by Board of Graduate studies 74
Appendix 5: Approval of the proposal by IREC. 75
Appendix 6: Ethical approval by Research and ethics committee of KCRH 76

LIST OF TABLES

*Table 4.*1: *Occurrence of UTI in the study population (N=300)* 36

Table 4.2: Age-matched cases with urinary tract infection 36

Table 4.3:Resistance of uropathogens to antibiotics 39

Table 4.4: Bacteria resistance to antibiotics 41

LIST OF FIGURES

Figure 4.1: Overall proportion of uropathogens isolated from patients with UTI 37

Figure 4.2: Overall antibiotic resistance 40

LIST OF PLATES

Plate 3.1: Analytical Profile Index @) Enterobacteriaceae strip 32

LIST OF ABBREVIATIONS AND ACRONYMS

AR Antibiotic Resistance

AST Antibiotic Susceptibility Test

BA Blood Agar

BD Becton Dickson & Company

CDC Center for Disease Control

CDDEP Center for Disease Dynamics, Economics and Policy

CLED Cystein Lactose Electrolyte Deficiency

CLSI Clinical Laboratory Standards Institute

ECDC European Center for Disease Control

GARP Global Antibiotic Resistance Partnership

KCRH Kericho County Referral Hospital

MAC MacConkey

MDR Multi-drug Resistance

MH Mueller Hinton Agar

MIC Minimum Inhibitory Concentration

UTI Urinary Tract Infection

WHO World Health Organization

DEFINITION OF TERMS

Bacteriuria : Bacterial infection of the ureter.

Cystisis : Bacterial infection of the bladder.

Dip stick : Reagent-impregnated strip which uses leukocyte estearase and nitrite to detect bacteria in urine sample.

Pyelonephritis : Bacterial infection of the kidney.

Uropathogens : Microbial pathogens that invade urinary tract.

Urinary tract infections : Presence of uropathogens in the urinary tract.

Zone of inhibition : Zone of clearance around an antibiotic in a bacterial lawn impregnated with antibiotic hence used to deduce organism's susceptibility to an antibiotic.

CHAPTER ONE

INTRODUCTION

1.1 Overview

This chapter entails the background of the study, statement of the problem, objectives, hypothesis, justification, significance and the scope of the study.

1.2 Background of the Study

Uropathogens are microbial pathogens that invade the urethra and bladder (cystitis), or ureter (bacteriuria) and pelvis of the kidney (pyelonephritis) causing urinary tract infection (UTI). Urinary tract infection comes second after respiratory infections in most communities and hospital settings affecting people of all ages. Its prevalence was estimated to be 150 million persons per year (Sewify *et al.,* 2016). Recurrence of the infection especially in vesico-ureteral reflux may cause long term sequelae such as end-stage renal disease, hypertension and renal scar (Foxman, 2014). It is a common infection in women and has been reported in 20% pregnant women (Wamalwa *et al.,* 2013). Bacteria pathogens have been recognized as the major causative agents of UTI. These bacteria are grouped into gram positive and gram negatives depending on the nature of their cell wall. Most gram negative bacteria particularly *Esherichia coli* colonize the intestines few hours after birth and constitutes part of the normal flora of the intestines. These gram negative bacteria have been known as the primary uropathogens hence most antibiotics prescribed are beta lactams and fluoroquinolones. Literature strongly suggests that gram positive uropathogens are often overlooked due to limited culture based assays

that are used to identify them in an ordinary hospital setting (Kafil & Mobarez, 2015; Kline & Lewis, 2016).

Antibiotics are often used to prevent recurrence and subsequent permanent renal damage in cases of UTI. The treatment is normally done empirically based on the information on antibiotic resistance (AR) pattern. Effective antibiotic treatment is essential as preventive and curative measure, protecting patients from potentially fatal diseases. However, an increase in uncontrolled use of antibiotics has led to an emergence of AR, thereby complicating antibiotic therapy (Marshal & Levy, 2011). Multinational organisations such as US Centre for Disease Control (CDC), World Health Organisation (WHO), and European Centre for Disease Prevention and Control (ECDC) have documented the diseases caused by multidrug resistance organisms as a global threat to public health (Maron, 2016; WHO, 2012). Uropathogen resistance to antibiotics in communities has also risen especially to carbapenems, quinolones and third generation cephalosporins (Rawat and Nair, 2010). These micro-organisms acquire the resistance genes either through mutation or by transfer of mobile genetic elements carrying resistant genes (Ulstad *et al.,* 2016). This implies that antimicrobial resistance can occur irrespective of the presence of the antimicrobial agents.

Management of UTI is becoming ineffective due to rise in antimicrobial resistance among bacteria in particular those belonging to the family *Enterobacteriaceae,* mostly *E. coli* and *Klebsiella pneumoniae*. The European Centre for Disease Prevention and Control has also reported a wide spread increase in AR of *E. coli* and *K. Pneumoniae* to carbapenems and multi-drug resistant *Staphylococcus aureus* (Glasner *et al*., 2013). Epidemiological studies done in different regions of the world indicate that multi-drug

resistant bacteria are responsible for 23-51% of community/ hospital acquired infections which include but not limited to urinary tract infection. (Sorlozano *et al*., 2016; Severin *et al*., 2010; BenDavid *et al*., 2012).Emergence of this antimicrobial resistance is due to non-compliance to drug prescription (Ndihokubwayo *et al*., 2013), over the counter prescription and presence of sub-standard drugs in the market (Mitema & Kikuvi, 2004). Epidemiological and AR patterns of uropathogens vary inter-regionally. Antibiotic resistance patterns are varying continually due to different antibiotic treatment regime (Den Heijer *et al*., 2010). Susceptibility of bacterial pathogens to antimicrobials also vary regionally within the same country (Gobernado *et al*., 2007). This is because in most cases of UTI, antibiotic prescription is initiated empirically before culture results are observed and thus AR to uropathogens might occur due to inappropriate antibiotic choice. The World Health Organisation and European Union have emphasized on the importance of studying the trends of antimicrobial resistance and the strategic plan to combat it since it is a public health concern (WHO, 2014). The regional study of AR of uropathogens is therefore critical in providing surveillance data to clinicians to enable them make informed decision on the choice of antibiotics to administer.

In Kenya, few studies have been done on AR associated with UTI. A study conducted on *E. coli* from community-acquired urinary tract infections by Kariuki *et al.,* (2007) demonstrated a high resistance of the bacteria to fluoroquinolones and extended-spectrum beta-lactam antibiotics. This work has been corroborated by other researchers (Nelson *et al*., 2016; Cheruyot, D., 2016; Wamalwa *et al*., 2013, Oundo *et al*., 2008). A study conducted on antibiotic susceptibility of uropathogenic *E. coli* in urine samples in Kericho District Hospital demonstrated an emerging resistance of the pathogen to the

commonly prescribed antibiotics (Cheruyot, D., 2016). It was noted that there is insufficient information on the regional antibiotic susceptibility patterns of overall bacterial spectra since most studies focus on antibiotic susceptibility of specific bacteria, particularly *E. coli*. This study aimed at isolation, identification and determination of antibiotic susceptibility of uropathogens in urine samples from patients with UTI in Kericho County Referral Hospital. This study provided information that was key in guiding administration of antibiotic to patients with UTIs.

1.3 Statement of the Problem

Urinary tract infections are common in most communities and hospital settings and AR has led to the recurrence of the infection (Ahsan *et al*., 2011; Bensman, 2012; Nelson *et al.,* 2016). Infections with multi-drug resistant bacterial strains can occur in a single infection or as an outbreak. Delayed detection of the AR leads to recurrence of the infection, further health complications, increased costs of treatment, prolonged hospital stay and sometimes the infection becomes fatal. Recurrence of the infection increases the risk of bladder cancer and still births in pregnant women (Vermeulen *et al.,* 2015). Therefore, there is need for information on the current trend of AR to aid clinicians in antibiotic therapy and deter further health complication.

Treatment of UTIs is mostly done empirically by administering antibiotics based on current trends of AR. However, misuse of antibiotics has been documented which could be as a result of many factors that includes over the counter prescription, hence contributing to an increase in the rate of antimicrobial resistance (Mitema, 2010; Indalo, 1997). As a result of this common practice, antimicrobial resistance associated with

urinary tract infection has been increasing gradually worldwide (Martinez-Brocal *et al.*, 2014).

Resistance of one bacteria species to an antibiotic give rise to the likelihood of development of resistance to other antibiotics. This association has led to widespread resistance to commonly prescribed antibiotics (Golkar & Pace, 2014). Antibiotic susceptibility pattern varies regionally depending on the strain of the bacteria and the drugs in the market in a particular region. There is limited information on the regional study of antibiogram pattern of uropathogens, therefore this study intended to add to the pool of data for the regional antibiotic susceptibility of the bacteria causing UTIs in Kericho County Referral Hospital. This further contributed to making informed decision in the selection of antibiotics and treatment regime for a given uropathogen.

1.4 General Objective

The main objective of this study was to determine the antibiotic resistance pattern of uropathogens causing UTI among patients attending Kericho County Referral Hospital (KCRH).

1.5 Specific Objectives

The specific objectives of the study were to:

1. Determine the demographic characteristics of patients with UTI attending KCRH.
2. Identify uropathogens in urine samples from patients with UTI attending KCRH.
3. Determine the antibiotic resistance profiles of uropathogens isolated from patients with UTI attending KCRH.

1.6 Hypotheses of the Study

There is no statistically significant antibiotic resistance in bacteria isolated in urine samples from patients with UTI in KCRH.

1.7 Justification of the Study

Rapid emergence of antibiotic resistant bacteria reduces the efficacy of first line antibiotics that have long been used for treatment of a wide range of ailments. Presence of antibiotic resistant uropathogens therefore presents a crisis and potential burden to the global health care systems and patients (Gupta & Bhadelia, 2014). 'Antimicrobial resistance is one of the most urgent health risks of our time and threatens to undo a century of medical progress,' (WHO, 2019). The Global Antimicrobial Resistance Surveillance System (GLASS), in their report for early implementation (WHO, 2018), stressed on the importance of continuous surveillance of antimicrobial resistance in order to find the current status and monitor the trends to aid in action plan to minimize the spread.

Thus, knowing the magnitude of drug resistance is critical as the changing rate of AR has impact in empirical therapy. This study was intended to foster complete recovery, thus preventing reoccurrence of the infection, development of health complications and further development of multi-drug resistance.

1.8 Significance of the Study

The findings from this study provided the bacterial spectra of UTIs in Kericho County region and the AR associated with them. This provided references to related research studies and added to the pool of data for regional antimicrobial resistance.

The antibiotic susceptibility pattern was done on the antibiotics that were available in the market, hence this will aid in guiding the clinicians involved in empirical therapy. This study will further contribute to improved effectiveness of health delivery programmes and preservation of the effectiveness of the current antibiotics. This is through use of antibiotics regime that delay development of resistance by uropathogens.

1.9 Scope of the Study

This study was performed at the clinical laboratory of KCRH involving three hundred clinically suspected cases of UTI who consented to participate in the study. The research design was hospital-based cross-sectional and was performed for three months, from March to May, 2017. Kericho County Referral Hospital clinical laboratory supported this research by sharing the available facilities and equipment including, incubators, microscopes and freezers among others. The reagents for use were at the cost of the researcher.

1.10 Limitations of the Study

The study was limited to:

i. Patients who chose to seek medical attention at KCRH.

ii. Urinary tract infection caused by bacteria species.

1.11 Assumptions of the Study

There were no assumptions made in the research.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

This chapter entails literature from journals related to this study that were useful in identifying the research gap.

2.2 Urinary Tract Infections

Urinary tract infection is one of the most prevalent bacterial infections in any rural community with varying frequencies among age-groups and sexes (Ahsan *et al.,* 2011). The infection can either occur as symptomatic or asymptomatic. Clinically, it is classified as complicated UTI and uncomplicated UTI. Uncomplicated UTI is characterized by dysuria, pyuria and frequent urination. It affects healthy individuals who have no structural or neurological urinary tract defect (Hooton, 2012). The risk factors associated with uncomplicated UTI are sexual activity, diabetes, prior UTI, genetic susceptibility, vaginal infections, female gender and obesity (Ndihokubwayo *et al.,* 2013).

Complicated UTIs is an infection of genitourinary tract that has structural or functional abnormalities and is associated with instrumentation such as indwelling urethral catheters. It occurs when the urinary tract functioning is compromised by health issues such as renal failure, immunosuppression, neurological diseases, pregnancy and indwelling catheters. In USA, 70%-80% of these infections have been reported to be acquired in hospitals through indwelling catheters (Lo *et al*., 2014).

Community acquired infections are mainly uncomplicated UTI and colonizes mainly the bladder causing cystitis (Wagenlehner *et al.,* 2011).

2.2.1 Epidemiology of uropathogens

Uropathogens are pathogens that cause urinary tract infection. They include various micro-organisms including gram negative and gram positive bacteria and fungi. Some of these microorganisms are found in the gastrointestinal tract but they can also invade extraintestinal organs. Most of them are commensals but some are pathogenic. They are transmitted through faecal-oral route, sexual contact and unhygienic practices (Löhr *et al.,* 2013). Invasion of the pathogen depends on its virulence factors, inoculums size, presence of the antibiotic resistant gene in the pathogen and host's immune defence mechanisms.

It is estimated that 80% of the UTI occur in women with 40% to 50% experiencing one asymptomatic UTI and half of them experiencing recurrence within one year (Foxman, 2014). The major causative agent of both complicated and uncomplicated UTI is *E. coli* (UPEC) (Linhares *et al.,* 2013; Alabi *et al.,* 2013). However, other findings have mentioned *Citrobacter freundii* to be the major aetiological agent of UTI in Sierra Leone (Leski *et al.,* 2016) and *E. coli* in Nigeria (Okesola *et al*., 2009). Both complicated and uncomplicated UTI have been found to be caused by UPEC, *Klebsiella* spp., *Staphylococcus saprophiticus*, *E. faecalis*, Group B *Streptococcus* (GBS)*, Proteus mirabilis, S. aureus*, *Pseudomonas aeruginosa*, *Providensia spp*. and *Candida* spp. (Foxman, 2014; Levison & Kaye, 2013; Chen *et al*., 2013).

2.2.2 Pathogenicity of uropathogens

Uropathogens possess virulence factors that include ability to colonize, adhere to the epithelial cells, produce toxins and invade tissues. They acquire AR plasmid mediated genes that enable the organism to evade the activity of the antibiotics. Most of them

possess antigenic features on their cell surface including O antigen present in the cell wall, H antigen present in the flagella and K antigen present in the capsular (Sorlozano *et al.,* 2016). O antigen is heat stable while K and H antigens are heat labile. O antigens are complex macromolecules composed of core polysaccharides, lipid A and O antigen. The lipopolysaccharide is a potent endotoxin which is released when the cell lyses during the infection. The lipid A induces the endotoxin activity while the O side chain is responsible for the antigenic activity and is serotype specific (Raetz & Whitfield, 2002).

The K antigens are pilus-like protein component of the polysaccharide capsule which is associated with colonization. It also blocks the agglutination of bacteria by specific O antisera by preventing the antibodies and complement binding to the bacteria. It also prevents the recognition and phagocytosis of the bacteria. Some *citrobacter* spp. serotypes and *salmonella typhi* produce Vi (virulence) type K antigen (Guentzel, 1996).

The H antigens (flagellar proteins) are present mostly in *E. coli, Citrobacter* spp., and *Proteus* spp. hence motile (Nielubowicz & Mobley, 2010). *Klebsiella* species do not have H antigens and are non-motile. Sex pili (fimbriae) are responsible for the transfer of genetic material from one bacterium to another by conjugation. It is through this pili that antimicrobial resistance plasmid is transferred from one bacteria to another in a colony. The type 1 fimbriae provide a mechanism of adherence to the bladder cells (Rendón *et al.,* 2007).

Some uropathogens produce soluble and cell bound haemolysin, siderophores (aerobactin and enterochelin) and pilial antigens whose activity is surface adherence to the target cell surface. The haemolysin kills the host cell by forming trans mebraneous pores in host cell

membrane and releases the haemoglobin-bound iron from the red blood cells. The bacteria then releases siderophores both aerobactin and enterochelin types to strip out the iron from the iron binding protein (transferin and lactoferin). The lysis effect of heamolysin also affect the white blood cells since beta-heamolysin inhibit phagocytosis and chemotaxis of neutrophils, and alpha-haemolysin lyse lymphocytes (Stapleton, 2005)

The enzyme urease produced by *Proteus* spp. and *Klebsiella* spp. hydrolyses urea to ammonia and carbon dioxide resulting in alkalinisation due to ammonia. The alkalinisation causes super saturation of magnesium and phosphate and subsequent crystallization to form struvite and apartite stone respectively. These stones are harmful to the epithelial cell of the urinary tract causing induced urinary tract infection. Bacteria within these stones are resistant to antimicrobial therapy. (Guentzel, 1996).

2.2.3 Antibiotics and mode of action

An antibiotic refers to a drug/ medicine used to kill or inhibit growth of bacterial or other organisms through antibiosis. These drugs are essential in public health for protection of patients against infections that would otherwise be fatal. They have also been applied in other areas such as surgery as a preventive measure against hospital acquired infections due to cross contamination.

Antibiotics exert bactericidal effects on bacteria by inhibiting its cell metabolism pathways using these mechanisms; (1) inhibition of bacterial cell wall synthesis, these are the β-lactam (penicillin and cephalosporin), carbapenem (imipenem) and glycopeptides (vancomycin) antibiotics, (2) inhibition of protein synthesis, these are the aminoglycosides (streptomycin), streptogramins (pristinamycin), macrolides

(erythromycin), tetracyclines (tetracycline, doxycyclin), chloramphenicol and oxazolidinones (linezolids), (3) disruption of cytoplasmic membrane, these are the lipopeptides (daptomycin), (4) inhibition of nucleic acid for example RNA synthesis which includes ansamycins (geldanamycin, rifamycin) and DNA synthesis, which includes quinolones (ciprofloxacin), (5) anti-metabolites, these are the sulphonamides (prontosil, sulfisoxazole) (Tenover, 2006; Cloete, 2003; Woodin & Morrison, 1994).

2.2.4 Treatment of UTI

Urinary tract infection is generally treated with β-lactam antibiotics, fluoroquinolones and trimethoprim/ sulfamethoxazole (Molina-Lopez *et al*., 2011). Thus, some clinicians use empirical treatment which is the common mode of antibiotic therapy in the early treatment of UTI (Moura *et al*., 2009; Roca *et al*., 2015) with most clinicians prescribing broad spectrum antibiotics.

Effective treatment has progressively become a challenge due to increasing development of antibiotic resistance and paucity in development of new antimicrobials. Currently, increasing level of antimicrobial resistance is becoming a challenge in both developed and developing countries and might become more devastating due to limited access to second line drugs like vancomycin and carbapenems (Gupta & Bhadelia, 2014; WHO, 2019).

2.2.5 Antibiotic resistance and its mechanisms

Antibiotic resistance refers to inability of the antibiotic to inhibit or kill bacteria at a clinically achievable concentration. This AR may have existed naturally in the bacteria (chromosomic genetic support) or acquired through mutation that is plasmidic,

chromosomic or transponson genetic support. Acquired resistance increases with antibiotic use. nosocomial infection can be acquired by a population in a given species while undergoing treatment (Woodin and Morrison, 1994). The predominance of AR among uropathogens is majorly due to their ubiquity in the environment, wide host range and their ease of acquiring and transferring antibiotic resistant plasmid that confer resistance to most antibiotics.

Bacteria confer resistance to antibiotics by; (1) preventing antibiotics from reaching to its target by impairing cell membrane permeability, (2) preventing antibiotics from binding to its target site. Bacteria achieve this by modifying the target site to form a supplementary target with less affinity to the antibiotic and, (3) inactivating the antibiotic before reaching the target site by producing antimicrobial-inactivating enzymes (Georgios & Egki*,* 2014). Acquisition of this AR may occur through mutation in the chromosome gene or through horizontal gene transfer, that is conjugation, transformation, transduction and through transponsons.

Antibiotic resistance in gram negative bacteria has been attributed to acquisition of AR genes such as extended spectrum beta-lactamase and carbapenemase enzymes that hydrolyses penicillins, cephalosporins and carbapenems antibiotics. Carbapenems are the strong and last line of β- lactam antibiotics (Poulou *et al*., 2014). Microorganisms are capable of transmitting these genes conferring AR (R plasmid) to susceptible organisms through cell-cell contact by conjugation.

Multi drug resistant gram negative bacteria including but not limited to *Klebsiella pneumoniae* and *E. coli* pose life-threatening infections all over the world (Arana *et al*., 2017).

2.2.6 Predisposing factors for antibiotic resistance

The rising level of AR among the bacterial pathogens causing hospital or community acquired-infection is a threat to global health. Recent studies have indicated the contributing factors to antibiotic resistance to be; (1) empirical treatment, (2) indiscriminate use of inexpensive antibiotics by outpatients, (3) use of over the counter drugs, (4) under-dosing and (5) use of counterfeit drugs (Horcajada *et al*., 2013; Roca *et al*., 2015). However, malpractices in drug technology, health systems and personal behaviour have led to drug resistance. Malpractices in drug industry include; long drug half-life, cross-resistance, monotherapy and length of treatment and complexity. In health systems, malpractices include; unregulated prescription, poor quality, weak infection control, poor surveillance and lack of rapid diagnostic tools. Personal behaviour such as; poor adherence, self-medication, unclear diagnosis and industry promotion are some of the malpractices that contribute to the rise of AR (Beitha, 2008).

Kariuki and Dougan, (2014) in their review of the AR in the Sub-saharan Africa noted that the burden of AR was attributed to poor hygiene, unreliable water supply, civil conflicts and increasing number of the immunocompromised patients which aided the evolution of bacteria pathogens resistant to antibiotics and subsequent spread in the community.

Use of antibiotics in agriculture as growth boosters and subsequent spread to people has also contributed to development of AR (Marshall & Levy, 2011; Ajak T.A.D., 2017). This has led to the use of new and expensive broad-spectrum drugs.

2.2.7 Detection of antibiotic resistance

Antibiotic resistance is detected by performing antibiotic susceptibility test through phenotypic or molecular (genotypic) method. Phenotypically, AR is detected by using disc diffusion method, double disc synergy test, boronic acid technique, combination meropenem disc technique, imipenem-EDTA sygnergy test and Hodge technique (Cockerill & Clinical and Laboratory Standards Institute, 2010; Wayne, 2011).

2.2.7.1 Disc diffusion technique

Disc diffusion method, also known as Kirby Bauer method is a technique of testing antibiotic sensitivity based on the principle of inhibition of bacterial growth on Mueller Hinton agar (Bonev *et al*., 2008). This is performed using several antimicrobial agents in order to accurately manage the treatment of infections and to detect AR.

In this technique, a suspension of bacteria isolate is spread uniformly on Mueller Hinton agar and antibiotic-impregnated discs placed on the cultured media and incubated at 37^0C for 24 to 48 hours. The antibiotic will then diffuse through the media causing the bactericidal effect on the susceptible bacteria (Bauer *et al.,* 1966). This is visible as clear zones around the antibiotic discs known as zones of inhibition. Susceptibility of the organism to the antibiotic is determined by measuring the zones of inhibition using a ruler and interpretation done as per the guidelines recommended by Clinical Laboratory Standards Institute (CLSI, 2008).

2.2.7.2 Minimum inhibitory concentration

Minimum inhibitory concentration (MIC) is the smallest concentration of antibiotic that inhibits the growth of an organism. Minimum inhibitory concentration can be determined using liquid media (broth dilution) or solid media (diffusion). In broth dilution, dilutions at various concentration of the test antimicrobial is added onto the vials containing broth culture of similar organism and incubated at 37^0C overnight. Growth is observed in form of turbidity in the vial. The vial containing minimum concentration of antimicrobial (highest dilution) and has no growth is taken as the minimum inhibitory concentration of the antimicrobial to that particular organism (Cockerill & Clinical and Laboratory Standards Institute, 2010). In solid media, E-test and disc diffusion methods are used, where the length at which the antibiotic can diffuse through solid media (usually Mueller Hinton agar) and kill microorganism (zones of inhibition) is measured to determine susceptibility of an organism to a particular antibiotic (Clinical and Laboratory Standards Institute, 2008).

2.2.8 Status of antibiotic resistance in Kenya

Emergence of AR amongst infectious bacteria has made treatments difficult. It has led to the use of broad-spectrum antibiotics which are expensive and capable of killing the normal flora of the body. The antimicrobial resistance global report on surveillance by the WHO indicated high resistance in bacterial pathogen implicated in common hospitals, community and food chain related infection in all WHO regions (WHO, 2018). It has also warned and called for urgent co-ordinated action that the world is heading to a post-antibiotic era where treatable infections would potentially be deadly. In Kenya, various studies have been done on AR of bacteria. A study that was done by Kariuki *et al.*, (2005)

on non-typhoidal salmonella isolated from patients in Kenya demonstrated an emergence of AR. Other studies that have been done to demonstrate AR in Kenya include a study done by Kariuki *et al.,* (2007) who studied *E. coli* from community acquired infections found twelve of the 17 *E. coli* that were resistant to ampicillin, co-amoxyclav, cefotaxime, ceftriaxone, ceftazidime and gentamicin and nalidixic acid, Oundo *et al., (*2008) who studied Entero-aggregative *E. coli* isolated from food handlers found 65.5% of the bacteria isolates which were multidrug resistant (MDR) with resistance varying from 6.9% for cefuroxime to 72.4% for co-trimoxazole, Nabbugodi *et al.*, (2015) on enteric pathogens from urinary and non-urinary isolates from Kenyatta National Hospital which demonstrated sensitivity rate of ceftriaxone, ceftazidime and ciprofloxacin to be above 70%. Co-amoxiclav, gentamicin, cefuroxime, minocycline and piperacillin showed moderate to high activity. The press release in July, 2019 by the Daily Nation, "the death of 11 infants at Kenyatta National Hospital due to multi-drug resistant *Klebsiella* spp." (Okeyo, V.**,** 2019), showed that the AR rate is rising to a dangerously high level and that there is need for urgent national strategy and action plan for antimicrobial resistance.

2.2.9 Global approaches to antimicrobial resistance

Antibiotic resistance has risen to a level of becoming global threat and of particular concern in developing countries. This challenge has been noted in different parts of the world and has compromised the management of infectious diseases in health care facilities (Roca *et al.,* 2015; WHO, 2019). The Global Antibiotic Resistance Partnership (GARP) has noted the need to address antimicrobial resistance by preparing a National Strategy and Action Plan for antimicrobial resistance. This includes a national antibiotic surveillance system and laboratory network collaborating with WHO-.South East Asian

Regional office (WHO, 2018). For effective 'one health' approach, WHO established coordination with various international health sectors in various fields including human, fisheries, veterinary and agriculture (WHO, 2015). Global Antimicrobial Resistance Surveillance System advocates for continuous antimicrobial resistance surveillance so as to get the current status that will aid in decision making towards reducing further spread. This study was based on the call for active surveillance and established the current AR in pathogens causing UTI. Uganda, through Uganda National Academy of Sciences (UNAS) with support from Center for Disease Dynamics, Economics and Policy (CDDEP), also established a National Action Plan 2018-2023 whose implementation is expected to control the development of antimicrobial resistance (CDDEP, 2018). Currently, WHO has launched AWaRe (Access, Watch and Reserve) tool to regulate drug prescription and sales so as minimize the spread of antibiotic resistance, antibiotic-related adverse events and cost of treatment (WHO, 2019).

2.3 Identification of Knowledge Gap

Increasing rate of AR and paucity in development of new antibiotics is a threat to global health care. The WHO Global Strategy for Containment of Antibiotic Resistance recommends laboratory-based surveillance as a strategy to contain AR and for the assessment of the impact of intervention (WHO, 2014; Perovic and Schultz, 2016). Frequent monitoring of the antibiotic susceptibility pattern at local, national and international levels is an important key to prevention-control strategy of the spread of multi drug resistant organisms. Empirical therapy is the common mode of treatment in most public hospitals. This is because culture and drug sensitivity tests take long to obtain results, thus allowing the infection to spread further. Prescription of drugs to

which organisms have developed resistance to, leads to recurrence of the infection and may lead to further complications. Empirical treatment should therefore be guided by regional AR profiles in the provision of effective treatment. In Kericho County, AR profile for urinary tract infections have not been done before. Therefore, this study sought to provide an empirical support in the management of urinary tract infections.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

This study was carried out at the clinical laboratory of Kericho County Referral Hospital (KCRH) which is situated in Kericho County, Ainamoi constituency. The hospital provides health service to people in Kericho County which has a population of 752,396 and an area of 2,479km^2 (Kenya National Bureau of Statistics census of 2009). The hospital also serves patients parts of the neighbouring counties namely Bomet and Kisumu. According to the Ministry of Health report of 2015, the hospital receives approximately 10,000 outpatients and 1,450 inpatients per month. It has a bed capacity of 250 patients. The research study covered the isolation and identification of the uropathogens among outpatients attending KCRH and determined their antibiotic susceptibility profile.

3.2 Research Design

The study design was hospital-based cross sectional design, and no follow-up of the participants was required.

3.3 Location of the Study

The study was performed at the clinical laboratory of KCRH. The laboratory is located within the hospital and serves all patients requiring laboratory investigations at KCRH.

3.4 Target Population

The study involved all outpatients with clinically suspected cases of UTI who consented to participate in the study.

3.5 Sample Size and Sampling Procedures

A pilot study was done by visiting the laboratory daily for two weeks to determine the number of samples that were received on each day from patients presenting UTI. This was used to approximate the number of samples received per day. Thereafter, the observation was used to set the duration and interval of systematic random sampling in order to meet the required sample size. It was also done to find out the availability of equipments and the reagents needed for data collection. Test run was also done during pilot study to ascertain the accuracy of analysis by the researcher.

Samples involved in this study were urine from outpatients who were clinically suspected to have UTI. Clinical samples that were tested were collected in routine clinical workflow from the eligible participant who consented to participate in the study. Systematic random sampling technique was applied in this study. In this case, the participants were recruited in groups of five patients. A pilot study had showed that at least twenty five patients visit the laboratory each with a request for the test on UTI per day. Since the research was carried out in a period of three months, the target sample population size was divided by the number of days in order to get the sample size per day. This was then divided by the approximated number of patients presenting UTI per day in order to get the interval at which systematic random sampling was done. The sample size was determined using formula for estimating minimum sample size for prevalence studies (Hajian-Tilaki K, 2011).

$$n = \frac{z^2 * p\,(1-p)}{d^2}$$

where;

n is the minimum sample size,

p is the prevalence of UTI in the previous studies which is 26.7% (Nabbugodi *et al.,* 2015),

z is the value corresponding to 95% confidence interval, and

d is the significance level, 0.05.

$$\mathbf{n} = \frac{1.96^2 * 0.267\ (0.733)}{0.05^2} = 300$$

n=300, Therefore, 300 was the sample size required for the study.

3.5.1 Inclusion criteria

Patients with UTI who gave written consent or ascent.

3.5.2 Exclusion criteria

i. Patients with UTI who did not consent.
ii. Patients with UTI who had used antibiotics in the last two weeks were excluded from the study.

3.6 Data Collection Instruments

During data collection, eligible participants were issued with structured questionnaires which they were to fill so as to obtain the demographic data for the first objective. Laboratory analysis was done to detect and identify the pathogen in the urine samples in order to obtain the second objective. Organism(s) that were identified were subjected to antibiotic susceptibility test using the commonly prescribed antibiotics to test for the AR so as to obtain the third objective.

3.6.1 Validity

Expiry period of the reagents were confirmed before the start of analysis. Analysis was also done alongside their quality control so as to ensure that the procedures followed yielded valid results. In case of variance in the quality control results, the analysis was repeated. Trouble shooting was done should the variance reoccur.

The machines that were used in the study were made sure that they were calibrated and run twice using a quality control so as to ascertain their reproducibility and precision.

Incubator temperatures were checked using a thermometer throughout the incubation period so as to ascertain the accuracy of the results.

Working bench were sanitized using 70% Ethanol and 10% Sodium hypochlorite to prevent cross contamination.

Reagents and materials that were used were already sterilized by manufacturer. Those that were not sterile were autoclaved before using to prevent contamination that may yield false results.

3.6.2 Reliability

Standard operating procedures were used in routine analysis for consistency and to minimize sample handling errors.

Reagents for use were obtained from the same manufacturer in order to maintain consistency of the chemical contents of the reagents.

Anonymous test samples with known results were used to optimize runs prior to actual tests run.

3.7 Urine Specimen Sampling and Analysis

A sterile wide mouthed plastic urine sampling container was provided to the eligible participants. The participants were then given clear instructions on how to collect a clean catch urine specimen and asked to avail the sample to the laboratory immediately.

3.7.1 Inoculation of urine sample

Inoculation is the process of culturing the sample onto a media and subsequent incubation so as to allow its optimum growth. Culturing allows rapid cell division of microorganism to form a large colony that is visible with naked eyes. This was the initial step so as to prevent contamination of the sample. Ten microlitres (µl) of the urine sample were cultured aseptically onto Blood agar base agar media (BA), MacConkey agar (MAC) and Cystein Lactose Electrolyte Deficient (CLED) agar media (Oxoid ltd, Basingstoke, UK) using a standard bacteriological loop by streaking. These microorganisms are broadly classified into gram positive and gram negative based on the nature of their cell wall. Blood agar base media is a nutrition enhanced media used to grow fastidious and non-fastidious organisms. Cystein Lactose Electrolyte Deficient is a non-inhibitory medium used to isolate bacteria from urine samples. MacConkey agar is a selective media that allows growth of gram negative bacteria and inhibit growth of gram positive bacteria. Culturing of urine samples was done inside a bio-safety cabinet. The cultures were then incubated aerobically at 35^0C and observed after 24 hours for growth.

A culture that had a single pure bacterial growth of greater than 10^4 colony forming units (CFU/ml) was considered significant and indicative of UTI. Bacterial colonies were

further observed for their physical characteristics including colony morphology, odour, colony elevation, swarming and presence of haemolysis in the respective media. The bacterial colonies of similar characteristics were sub-cultured on CLED to obtain a pure culture and gram stained to distinguish the bacteria (Wilson & Miles, 1975).

3.7.2 Dipstick screening

Dipstick is a test strip impregnated with chemical reagents that indicate the presence of analytes in a fluid by colour change. The dipstick strip was dipped onto the urine sample and left for one minute then removed for observation. Detection of bacterial infection was based on the presence of leukocytes and nitrate reduction by bacteria. A positive reaction indicated by appearance of a pink colour, depicted the presence of leukocyte estearase and indicated a bacterial infection. The intensity of the colour directly relates to the bacterial count (Cheesbrough, 2006).

3.7.3 Microscopy examination

Microscopy is a technique used to enlarge very tiny invisible objects. Urine sample was swirled gently and left to stand for three to five minutes to obtain a supernatant and the deposit. Microscopy was done to observe the presence of pus cells or leukocytes hence an indication of bacterial infection. The supernatant was poured off so as to remain with the deposit. A Pasteur pipette was used to place a drop of the deposit onto the sterile glass slide. The sample was then observed using a light microscope for identification and quantification of bacteria and leukocytes at power X10 and then X40 objective lens. More than one leukocyte indicated urinary tract infection while non infected urine had very few or no bacteria or leukocytes (Vandepitte *et al*., 2003).

3.7.4 Gram staining

Gram staining is a method of bacteria identification used to broadly classify them into gram positive and gram negative by detecting the nature of peptidoglycan in their cell wall. The bacterial colonies were gram stained following the procedure described by Claus, 1992. A drop of normal saline was placed on a sterile glass slide and a colony picked from the culture plate using a sterile bacteriological inoculation loop and spread circularly and uniformly on the slide to make a very thin layer. The smear was then air-dried, fixed using absolute methanol and flooded with Gram Crystal violet stain for one minute. Upon rinsing with clean tap water, the smear was flooded with Gram iodine solution and allowed to stand for one minute. Few drops of Gram decolorizer was then added upon rinsing and left to stand for 5 seconds, then rinsed again. The smear was then flooded with a Gram safranin counter stain and allowed to stand for 30 seconds, washed off, then blotted with bibulous paper to remove the excess water. Both positive control (*S. aureus* ATCC 25923 and *E. coli* ATCC 25922) and negative control (blank sterile slide) slides were stained alongside the samples. The Gram negative bacteria appeared pink in colour while the gram positive bacteria appeared purple/ violet. Results were reported as gram positive or gram negative cocci, bacilli, clusters or diplococci depending on the shape of the bacteria.

3.7.5 Biochemical identification of uropathogens

Gram positive bacteria colonies were tested for catalase and coagulase. Gram positive *Streptocococci* and other cocci that did not appear in clusters were tested for catalase and coagulase reaction, and then subjected to Analytical Profile Index (API). Analytical Profile Index 20 Strep Biomereux was used for final identification of gram positive

Streptocococci bacteria (Hemraj *et al*., 2013). Identification of Gram negative bacteria was done by series of biochemical tests including, Tripple Sugar Iron (TSI), simmon's citrate agar, Methyl-red Vorges-Proskauer (MR-VP), motility-indole-lysine (MIL), catalase, and oxidase and where these did not give conclusive results, API® 20E Biomereux method was used.

3.7.5.1 Catalase test

Catalase is an enzyme present in aerobic bacteria that neutralizes toxic forms of oxygen. It mediates breakdown of hydrogen peroxide into oxygen and water. To test for catalase reaction, a small inoculum of bacteria was placed on a clear slide containing hydrogen peroxide and mixed. Immediate effervescence indicated catalase positive reaction and none for a negative reaction (Hemraj *et al*., 2013). *Staphylococcus aureus* ATCC 25923 was used as positive control and sterile distilled water was used as negative control.

3.7.5.2 Coagulase test

Coagulase is a protein enzyme that converts fibrinogen to fibrin. It is found in the surface of *S. aureus*. It reacts with prothrombin in the blood to form a complex which enables protease to convert fibrinogen into fibrin resulting in clotting of blood. To test for coagulase reaction, a small inoculum of bacteria was placed on a clear slide containing human plasma and swirled softly for 10 seconds. Visible clumping indicated a positive reaction while no clumping indicated a negative reaction (Hemraj *et al*., 2013). *Staphylococcus aureus* ATCC 25923 was used for positive control and sterile distilled water used for negative control. Gram positive cocci that appeared in clusters during microscopy, and were catalase positive and coagulase positive were identified as *S.*

aureus. Those that appeared coagulase negative were reported as coagulase negative *S. aureus*.

3.7.5.3 Oxidase test

Oxidase test is used to test for bacteria that produce cytochrome oxidase. A disk impregnated with reagent (*N,N*-dimethyl-*p*-phenylenediamine) was held asceptically using forceps and allowed to come in contact with a bacteria colony in the culture plate. The disk turned colour when it reacted with cytochrome oxidase to indicate a positive test. cytochrome oxidase is usually present in aerobic organisms. *Pseudomonas aeruginosa* ATCC27853 was used as positive control and sterile distilled water as negative control (Cheesbrough, 2006).

3.7.5.4 Motility Indole Lysine test

Motility indole lysine (MIL) is a broth media used to identify organism based on motility and their ability to produce indole, lysine decarboxylase and lysine deaminase.

Morphologically identical sub-cultured colonies were stabbed onto the MIL medium in a test tube and incubated at 35^0C for 18-24 hours. Motility and lysine results were read first, and then 3-4 drops of Kovacs reagents added to test for the Indole reaction. Growth away from the stab line showed a positive motility test while growth along the stab line showed a negative result. Formation of a purple band and a purple butt indicated a positive Lysine decarboxylase test while a negative result showed a narrow purple band and a yellow butt. Lysine deaminase positive test was indicated by a deep red band with a yellow butt while a negative test indicated by a purple band with a yellow butt. A positive Indole test was indicated by a colour change in the reagent layer from yellow to red after

addition of Kovac's reagent, while a negative test showed a yellow to a bright yellow colour. Positive controls that were used were *Proteus mirabilis* ATCC 25933, *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 13883 (Vandepitte *et al.*, 2003).

3.7.5.5 Tripple Sugar Iron

Tripple sugar iron (TSI) agar use agar media to test for the ability of the organism to utilize three sugars, lactose, sucrose and glucose fermentatively and produce hydrogen sulphide. Using a sterile standard bacteriological loop, 2-3 colonies were picked from a pure culture and stabbed through the centre of the media to the bottom (butt) and streaked on the slant of the TSI media, then incubated at 35^0C for 18-24 hours. Fermentation of any of the sugar led to subsequent production of acid which was indicated by a colour change in the phenyl red indicator from red to yellow in both the slant and the butt. Hydrogen sulfide production was also indicated by a black colour in the media. The results were reported as either alkaline or acid butt and/or alkaline or acid slant. Positive controls were *E. coli* 25922, *Shigella flexneri* 12022 and *Salmonella typhi* 700931 (Hemraj *et al*., 2013).

3.7.5.6 Simmon's citrate test

Simmon's citrate test is used to test the ability of an organism to utilize citrate as a source of energy. Simmon's citrate agar has ammonium dihydrogen phosphate which is the sole source of nitrogen and sodium citrate which is the sole source of carbon. Dipotassium phosphate is the source of buffer. Organisms with capability of utilizing ammonium dihydrogen phosphate and sodium citrate grew resulting in accumulation of alkaline by-

products. This shifted the pH of the medium to basic hence turning bromothymol blue indicator from green to blue. Positive control strains used were *Escherichia coli* 25922, *Shigella flexneri* 12022 and *Salmonella typhi* 70093 (Hemraj *et al*., 2013).

3.7.5.7 Methyl Red- Voges Proskauer Test

This test uses methyl red indicator to detect acidity as a result of fermentation of glucose. However, with further incubation for two to five days, the MR positive organisms keep on producing more acids which resulted in a low Ph and formation of a red color.

Methyl red negative organisms, such as *K. pneumoniae*, further metabolize the fermentation products through decarboxylation producing acetoin and results in an alkaline reaction. Addition of 40% potassium hydroxide, in presence of atmospheric oxygen, converted acetoin to diacetyl. To test for VP reaction, alpha-naphthol and creatine was added onto Diacetyl which reacted to form a red compound. This indicated a positive Voges-Proskauer (VP) test. The VP test was used presumptively to differentiate *E. coli* (25922) (VP-negative) from the *K. pneumoniae* (13883) (VP-positive) (Hemraj *et al*., 2013).

3.7.6 Analytical profile index principle

Analytical profile index (API) is a method of identification of bacteria using a biochemical test strip. The strip consists of different dehydrated substrates for each test in twenty mini-test cupules. Inoculated bacteria will react to them and give different colors which are used in identification. Analytical profile index 20E®Biomereux was used for enteric pathogens and API® 20 Strep was used for the identification of *Streptococci* and

diplococci bacteria. The principle of API is testing the ability of the organism to assimilate or ferment the substrate. Substrate utilization could be spontaneous or revealed by addition of reagents. It is indicated by a colour change produced when the substrate is fermented hence changing the pH within the substrate. The change in pH is detected by an indicator which then changes the colour. Growth of the bacteria is indicated by the assimilation of the substrate and hence a positive result. The test results are then entered into an online database for the identification of the organism (Hussein, 2010).

3.7.6.1 Analytical Profile Index 20E®Biomereux

A single colony from a pure culture were suspended in sterile distilled water and a sterile Pasteur pipette used to fill each cupule with the bacteria suspension. Oil was added onto LCD, ADH, ODC, H_2S and URE cupules. The strip was labelled and incubated at 37^0C for 18-24 hours after which, ferric chloride was added to TDA, Kovacs reagent added to IND and one drop of 40% KOH (VP1) and α-naphthol (VP2) added to VP cupules and left for 10 minutes before reading the results. The results of each biochemically testing cupule was recorded as positive or negative (+ or -) using the API colour chart. The results were then entered into the API web software for bacteria identification. *Escherichia coli* ATCC 25922 was used as positive control and uninoculated test strip used for negative control (Hussein, 2010). The identified colonies were stored in Trypticase Soy Broth (TSB) with 25% glycerol at -70^oC pending antibiotic susceptibility test.

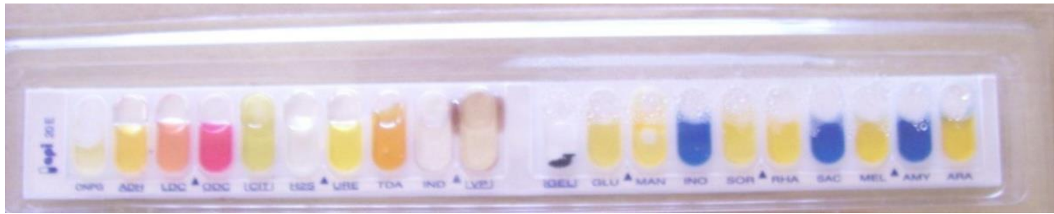

Plate 3.1: Analytical Profile Index @) Enterobacteriaceae strip

3.7.6.2 Analytical Profile Index 20® Strep Biomereux

Bacteria colonies from a pure culture was suspended in 2ml sterile distilled water until a suspension of 0.5 McFarland was achieved. A sterile Pasteur pipette was used to fill each cupule from VP to LAP cupule with the bacteria suspension, taking note of the extent at which each cupule was to be filled as described by the manufacturer (Biomereux). ADH cupule was only filled to the tube. To the API strep media, 0.5 ml of the bacteria suspension was added and mixed to homogenize taking care not to create bubbles. The suspension was then distributed to the remaining cupules from Rib cupule to GLYG cupule. Liquid paraffin was overlayed to cupules as indicated in the test kit and incubated at 36^{0}C for 4 hours to obtain initial profile. After incubation, a drop of VP1 and VP2 reagents were added to VP cupule, a drop of NIN reagent to HIP cupule and a drop of ZYM 1 followed by ZYM 2 reagents to cupules from PYRA to LAP cupule and then left for ten minutes before reading the results. Positive control used was *Enterococci faecalis* ATCC 8043 and uninoculated test strip used for negative control. The resulting profile was entered into the APIweb software for bacteria identification. The identified colonies were stored in Trypticase Soy Broth (TSB) with 25% glycerol at -70°C pending antibiotic susceptibility test (Hussein, 2010). Figure 3.2 shows the researcher working in the laboratory.

3.7.7 Antibiotic Susceptibility Test (AST)

Antibiotic susceptibility test was done for bacterial isolates identified from urine culture using Kirby Bauer disk diffusion method (Bauer *et al.,* 1966) as per the guidelines recommended by Clinical Laboratory Standards Institute (CLSI, 2008). Commonly prescribed antibiotics and commercially available discs (Oxoid ltd) were used for antimicrobial susceptibility testing.

The antibiotics that were used with their concentration were norfloxacin (5µg), ciprofloxacin (5µg), gentamycin (10µg), cefuroxime (30µg), azithromycin (15µg), amoxillin clavulanic acid (30µg) and ampicillin (20µg), (Liofilms.r.l Rosete (TE), Italy). A bacterial isolate suspension of 0.5 McFarlands was prepared and cultured on Mueller Hinton agar (MH) (Liofilms.r.l Rosete (TE), Italy) by uniformly swabbing and spreading the bacterial suspension onto the MH solid agar media using a sterile swab. The above antibiotics were then placed aseptically in each cultured plate using sterile forceps and incubated aerobically at 37^0C for 18-24 hours. Control strain used was *E. coli* ATCC 25922. The zone of inhibition around each antibiotic was measured using a ruler and compared with the zone diameter interpretative criteria recommended by CLSI to determine the antibiotic susceptibility (Wayne, 2011). The results were reported as susceptible, intermediate or resistant to a particular drug following the table in the table in appendix 3.

3.8 Data Analysis and Presentation

Obtained laboratory results were recorded in a tabulated form in a laboratory book and a soft copy saved in an access-controlled computer. The laboratory procedures and results were recorded in a laboratory record book and kept in a locked cabinet.

The data obtained were entered in statistical package for social scientists (SPSS) version 21 software for coding and analysis. The access to the data was controlled using passwords.

Descriptive statistics were used to analyse demographic characteristics and the rate of AR. Pearson correlation was used to test for association between organism and gender.

3.9 Ethical Considerations

The study was conducted in accordance to the ethical standards of Helsinki declaration on harmonization guideline on Good Clinical Laboratory Practices. The protocol and informed consent were reviewed and approved by Board of Graduate Studies of University of Kabianga, Research and Ethical committee of KCRH and Institutional Research and Ethics Committee (IREC) of Moi Teaching and Referral Hospital, Eldoret.

Written informed consent or ascent was obtained from every recruited patient and given a unique identification code for confidentiality of the patient's records. Patient's records were kept under lock and key cabinet and information saved in the computer was access-controlled. Authority for access was only allowed to the researcher and the supervisor.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Introduction

This chapter presents the results on the data collected and discusses the inference from the findings of the study.

4.2 Presentation of Results

The results from the analysed data are presented in line with the objectives of the study as described below.

4.2.1 Demographic characteristics of the patients with urinary tract infection

A total of 300 samples were received for laboratory analysis for detection of urinary tract infection. Among these, 60 samples yielded bacteria isolates giving UTI prevalence of 20% (Table 4.1). Of the total population, females consisted 67% and males 33%. Of those with confirmed with UTI, the majority were females (n=48; 80%), as compared to males (n=12; 20%).

Table 4.1

Occurrence of UTI in the study population (N=300)

Study participants	*Presence of UTI n (%)*	*Absence of UTI n (%)*	*Total participants N (%)*
Females	48 (16)	153 (51)	201 (67)
Males	12 (4)	87 (29)	99 (33)
Total	60 (20)	240 (80)	300 (100)

The age range of the study population was 1 to 72 years. Majority of participants with UTI were in the age-group of the range 21-30 years constituting 57% (n=34) (Table 4.2).

Table 4.2

Age-matched cases with urinary tract infection

Age	*Females (n)*	*Males (n)*	*Total N (%)*
1 to 10	1	0	1 (1.7)
11 to 20	3	1	4 (6.7)
21 to 30	27	7	34 (56.7)
31 to 40	8	1	9 (15)
41 to 50	4	1	5 (8.2)
51 to 55	2	1	3 (5)
61 and above	3	1	4 (6.7)
TOTALS	48	12	60 (100)

4.2.2 Uropathogens identified in urine sample from patients with UTI

Determine the antibiotic resistance profiles of uropathogens isolated from patients with From the 300 samples received, 60 (20%) yielded bacteria isolates. In description of aetiology of bacteria isolated from patients with UTI, bacteria were grouped into gram negative rods (GNR) and gram positive cocci (GPC). Of the isolates detected, majority were GPC which constituted 73% (n=45), while 27% (n=15) were GNR (Fig. 4.1). Of the GPC, majority were *S. aureus* (n=25; 41.7%), followed by *E. faecalis* (n= 20; 33.3%). Of the GNR, majority were *E. coli* (n=12; 20.0%) followed by *Proteus* spp. (n=2; 3.3%) and *K. pneumoniae* (n=1; 1.6%). There was no statistically significant association between organism causing UTI and gender (Pearson correlation= 0.872).

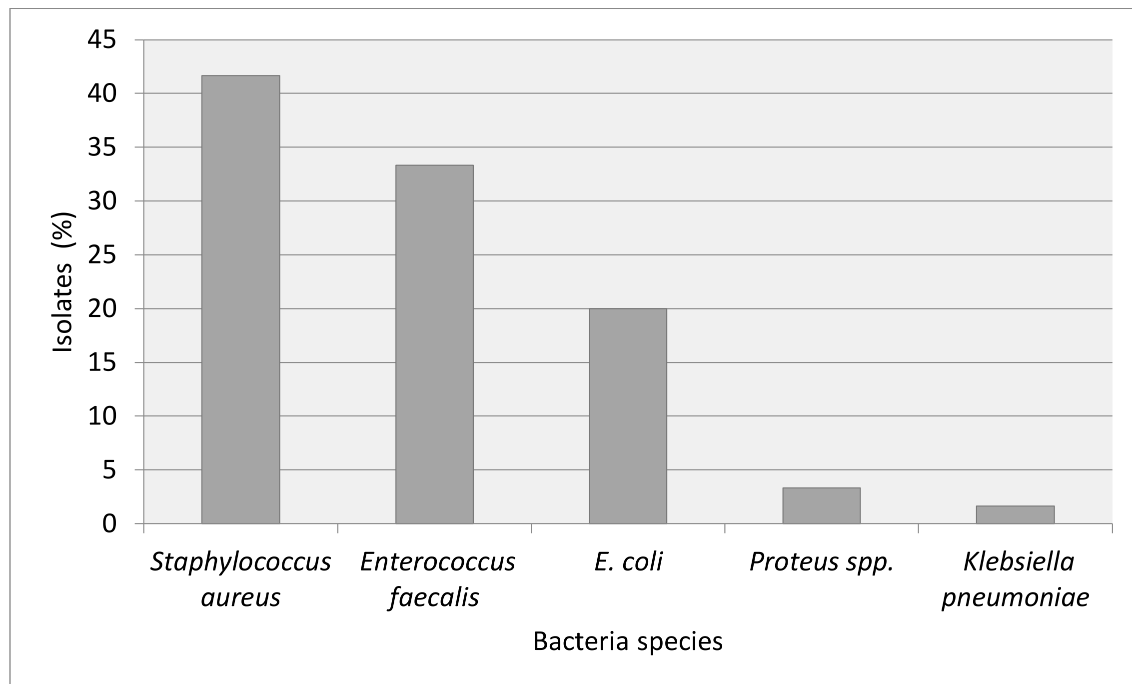

Figure 4.1: Overall proportion of uropathogens isolated from patients with UTI

4.2.3 Antibiotic resistance profile of uropathogens isolated from patients with UTI

Antibiotic sensitivity tests were done for the sixty (60) bacteria isolates. These isolates showed varying trends of susceptibility and resistance to therapeutic agents. Overall, the respective AR pattern of uropathogens were as shown in Table 4.3. Among the gram positive bacteria, *S. aureus* showed high resistance to ampicillin (68%), norfloxacin (64%) and azithromycin (56%). *Staphylococcus aureus* demonstrated low resistance to gentamycin (32%), cefoxitine (20%) and ciprofloxacin (12%). *Enterococcus faecalis* showed 70% resistance to ampicillin and azithromycin. Lower resistance to norfloxacin was observed in *E. faecalis* (45%) as compared to *S. aureus* (64%). However, least resistance to gentamycin (10%), cefoxitine (30%) and ciprofloxacin (30%) was also observed in *E. faecalis.*

Among gram negative bacteria isolates, *E. coli* demonstrated resistance to ampicillin (83%) and augmentin (75%) while *Proteus* spp. (100%) and *K. pneumoniae* (100%) were fully resistant to ampicillin and augmentin. Resistance to azithromycin was also observed in *K. pneumoniae* (100%) and *E. coli* (83.3%) while *Proteus* (50%) showed moderate resistance. *Escherichia coli* was moderately resistant to norfloxacin (45%), while *Proteus* spp. and *K. pneumoniae* showed no resistance. *Escherichia coli* also showed least resistance to gentamycin (16.7%), cefoxitine (30%) and ciprofloxacin (30%).

Table 4.3

Resistance of uropathogens to antibiotics

Antibiotic	*Susceptibility Level*	*S. aureus N=25 n (%)*	*E. faecalis N =20 n (%)*	*E. coli N =12 n (%)*	*Proteus spp. N =2 n (%)*	*K. pneumoniae N =1 n (%)*
Augmentin	Intermediate	5(20)	5(25)	1(8)	0	0
	Susceptible	9(36)	9(45)	2(17)	0	0
	Resistant	11(44)	6(30)	9(75)	2(100)	1(100)
Gentamycin	Intermediate	0	0	1(8)	1(50)	0
	Susceptible	17(68)	18(90)	9(75)	1(50)	1(100)
	Resistant	8(32)	2(10)	2(17)	0	0
Ciprofloxacin	Intermediate	5(20)	3(15)	3(25)	0	0
	Susceptible	17(68)	11(55)	8(67)	2(100)	1(100)
	Resistant	3(12)	6(30)	1(8)	0	0
Norfloxacin	Intermediate	2(8)	2(10)	0	1(50)	1(100)
	Susceptible	7(28)	9(45)	7(58)	1(50)	0
	Resistant	16(64)	9(45)	5(42)	1(50)	0
Cefoxitine	Intermediate	3(12)	2(10)	1(8)	0	0
	Susceptible	17(68)	12(60)	10(83)	2(100)	1(100)
	Resistant	5(20)	6(30)	2(17)	0	0
Ampicillin	Intermediate	1(4)	2(10)	1(8)	0	0
	Susceptible	7(28)	4(20)	1(8)	0	0
	Resistant	17(68)	14(70)	10(83)	2(100)	1(100)
Azithromycin	Intermediate	1(4)	0	2(17)	1(50)	0
	Susceptible	10(40)	6(30)	0	0	0
	Resistant	14(56)	14(70)	10(83)	1(50)	1(100)

Overall significant AR among uropathogens was observed in azithromycin, augmentin and ampicillin. There was moderate resistance to norfloxacin and least resistance to cefoxitin, gentamycin and ciprofloxacin, (Fig. 4.2). *Staphylococcus aureus*, *Enterococcus faecalis* and *E. coli* showed at least resistance to all antibiotic tested in the study. However, *K. pneumoniae* and *Proteus* spp. showed no resistance to cefoxitine, gentamycin, ciprofloxacin and norfloxacin.

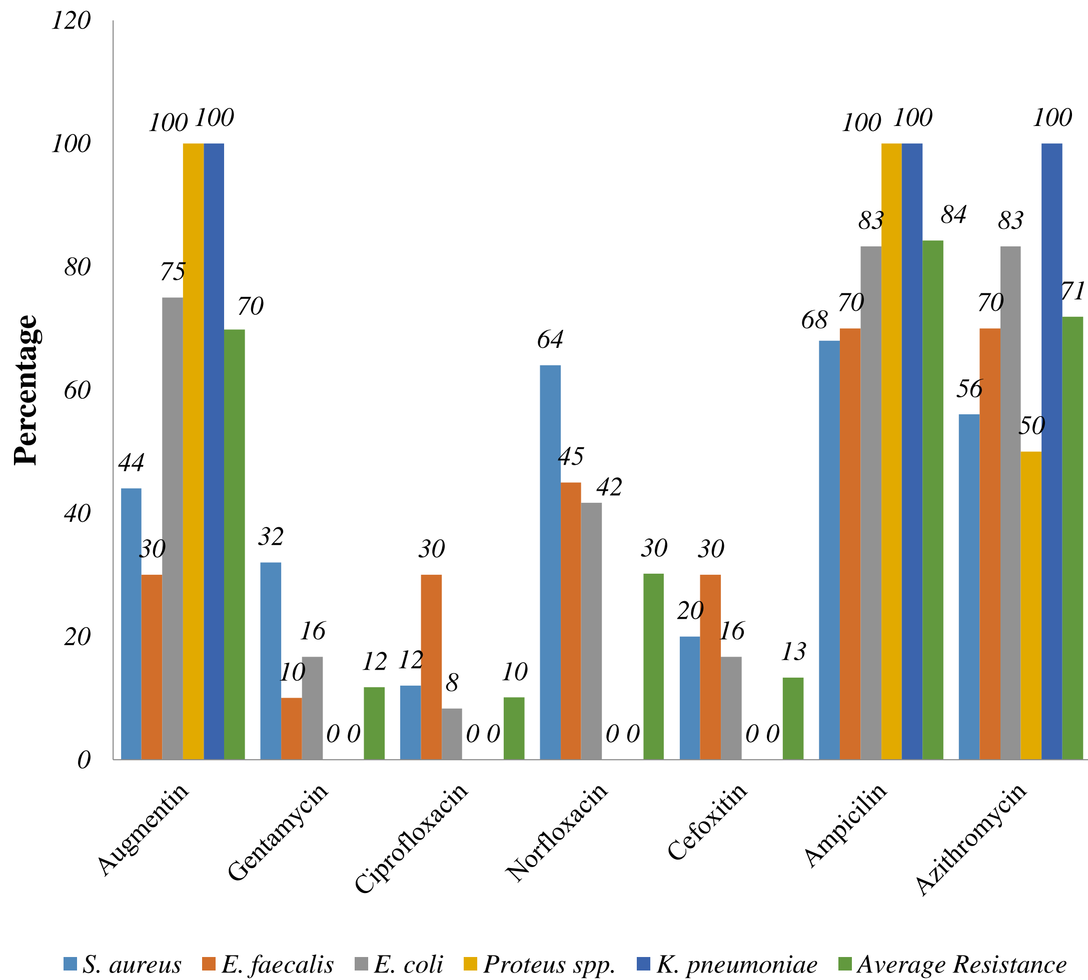

Figure 4.2: Overall antibiotic resistance

Multi-drug resistance was observed among the clinical isolates when each organism was considered for multiple resistance to each of the antibiotics. Among the commonly isolated organisms, at least all showed multiple resistance to utmost 5 drugs tested in the study (Table 4.4). *Enterococci faecalis* showed highest resistance to most antibiotics (n=4; 20%), followed by *S. aureus* (n=3; 12%) while *E. coli* showed the lowest (n=1; 8.3%).

Table 4.4

Bacteria resistance to antibiotics

Organism	*Multiple Resistance*			
	R2	*R3*	*R4*	*R5*
S. aureus (n=25)	4(16%)	11(44%)	3(12%)	3(12%)
E. faecalis (n=20)	6(30%)	4(20%)	1(5%)	4(20%)
E. coli (n=12)	3(25%)	4(33.3%)	4(33.3%)	1(8.3%)

* R2=Resistance to two antibiotics; R3=Resistance to three antibiotics; R4=Resistance to four; R5=Resistance to five antibiotics.

4.3 Discussion of Results

Isolation rate of 20% of bacteria showed that symptoms of UTI alone cannot be an adequate guide for diagnosis and subsequent antibiotic therapy in cases of urinary tract infections. This is because certain sexually transmitted diseases that mimic UTI are caused by organisms other than bacteria which includes protozoa, fungi and virus infections. Interstitial cystitis, cancer of the bladder and prostate problems, pyelonephritis and genital herpes also present symptoms that are similar to UTI. Isolation rate of 20% in this study compares closely to 20.6% obtained from a similar study that was done in Kisii

Teaching and Referral Hospital (Mageto *et al.,* 2018). It therefore confirms that the low isolation rate cannot be attributed to improper sample handling. This finding is also in agreement with other studies that were done in Tanzania which obtained a prevalence of 23.3% (Mwambete *et al*., 2017) and in Ethiopia at 11.5% (Kebede *et al.,* 2016). A study conducted in India obtained a slightly higher prevalence of 32.1% (George *et al*., 2015).

A higher number of females had UTI compared to males. This difference in UTI prevalence between men and women could be due to a variety of factors. In women, the proximity of the anal canal to genitalia provides a high risk of contamination of the latter and easy transmission of various pathogens. Research have shown that women are more predisposed to UTIs and have a 50% chance of experiencing at least one episode of UTI during their lifetime (Wamalwa *et al.,* 2013).

In this study, the age-group between 21-30 year-old had the highest prevalence of UTIs. This is indicative of the possibility of the role played by sexual activity in the transmission of UTI. Several other studies have suggested a similar relationship placing being female in the age-group of 19-39 to be at the highest risk of contracting UTI (Beyene & Tsegaye, 2011; Fred *et al.*, 2015). This presents a risk of ascending infection that can lead to kidney damage, renal failure and still births in pregnant women. If UTI is not treated it can lead to obstetric complications, poor perinatal and maternal complications such as caesarean delivery and intrauterine growth restriction (Haider *et al*., 2010). Recurrent UTI leads to further health complication such as renal scar, permanent renal damage, hypertension, and end-stage renal disease if associated with vesicoureteral reflux (VUR) (Foxman *et al.,* 2014).

The major aetiological agents of UTI isolated were gram positive bacteria; *S. aureus* (41.7%) and *E. faecalis* (33.3%), followed by gram negative bacteria; *E. coli* (20%), *Proteus* spp. (3.3%) and *K. pneumoniae* (1.7%). Research conducted on prevalence, aetiological agents and AR in UTI in Kenyatta National Hospital (Nabbugodi *et al*., 2015) found *E. coli* (40%), *Staphylococcus* spp. (25%), *Klebsiella* spp. (15%), *E. faecalis* (10%) and *Proteus* spp. (10%) which is consistent findings in the current study. Another study conducted on AR among uropathogens in Kenyatta University Health care found higher prevalence (61.3%) of *E. coli* isolates from urine, others being *Staphylococcus* spp. (9.3%), *Enterobacter* spp. (3.7%), *Klebsiella* spp. (3.3%), *Citrobacter* spp. (2.5%) and *Proteus* spp. (2.1%) (Kimando *et al*., 2010). This can be explained by time and environmental variations, social habits of the community, the personal hygiene standard and education. *Staphylococcus aureus* is a normal flora of the skin of human and a common opportunistic pathogen causing soft tissue infections. When it finds its entry into the body, it causes abscess in deep organs, toxin-mediated diseases, infections of the respiratory tract, urinary tract infection and post-surgical wound. *Enterococci* species are bacteria that are widely distributed in nature as harmless commensals colonizing the intestines of mammals and birds, and are also opportunistic pathogen (Teixeira *et al*., 2013). *Enterococci* spp. Are bacteria that contaminate surfaces and medical equipment, enabling it to be transmitted to patients through healthcare workers. *Escherichia coli* and *Proteus* spp. are also commensals of the intestines of humans, animals and birds. Unhygienic practices including back to front self cleaning after defecation, promote the entry of these organisms to urinary tract in females. These organisms express a number of

virulence factors which includes biofilm formation, motility, adhesion, nutrient acquisition and immunoavoidance.

The 4 classes of antibiotics that were used were quinolones: norfloxacin and ciprofloxacin; aminoglycosides: gentamycin; macrolides: azithromycin; β-lactam antibiotics: amoxillin clavulanic acid, ampicillin and cefoxitine which is a fourth generation cephalosporin. In Kenyan health facilities, augmentin, ampicillin and azithromycin are mostly prescribed for treatment of UTI because of their availability in intravenous and oral formulation. The various bacterial pathogens isolated in this study demonstrated resistance to these commonly prescribed antibiotics. Bacteria resistant to ampicillin and azithromycin were common in the two groups of bacteria. Significant difference in AR was observed where gram negative bacteria showed significant resistance to augmentin while gram positive showed significant resistance to norfloxacin.

Considering each organism, *S. aureus* demonstrated high resistance to azithromycin (56%), norfloxacin (64%) and ampicillin (68%), confirming the previously reported resistance rates of these drugs of over 70% in Kenya, Ethiopia and Tanzania (Kariuki S. M., 2011; Kebede *et al.,* 2016 & Mwambete *et al*., 2017). The rate of resistance of *S. aureus* to ampicillin was in consistence with a study conducted on prevalence, aetiological agents and AR in UTI in Kenyatta National Hospital which recorded 80% resistance to ampicillin (Nabbugodi *et al.,* 2015). While *S. aureus* showed multi-drug resistance to utmost 5 drugs tested, majority of them were resistant to at least three drugs. Studies have shown that *S. aureus* have ability to produce beta lactamase enzymes which hydrolyse beta-lactam antibiotics (Rağbetli *et al.,* 2016). Therefore, high resistance to ampicillin observed in this study could be due to the acquisition of these enzymes.

Enterococcus faecalis recorded 70% resistance to both ampicillin and azithromycin and moderate resistance (45%) to norfloxacin. This multi-drug resistance is in agreement with a study that was done in Iran by Kafil & Mobarez, (2015) which recorded resistance (85%) of *E. faecalis* to ampicillin, augmentin (75%) and ciprofloxacin (30%). Resistance rate to ampicillin and ciprofloxacin is also in consistence with a study that was done on AR among uropathogens in Egypt which recorded ampicillin (62%), ciprofloxacin (30%), although 56% resistance to gentamycin was higher than the findings of this study at 11.74% (Hashim & Amin, 2015). A higher rate of multi-drug resistance was observed in *Enterococci faecalis* which showed the highest percentage of multiple resistance of up to 5 that were drugs tested in the study. The ability of *Enterococci* spp. to form biofilm on abiotic surfaces gives it important virulent and AR property (Anderson *et al.,* 2016). The nature and properties of *Enterococci* spp. which include biofilm formation, eases the transfer of genes that allows them to rapidly acquire antibiotic resistance gene and genetic elements that enhance their ability to invade and infect patients (Kafil *&* Mobarez*,* 2015). Studies that have been conducted on *Enterococci* in relation to urinary tract infection demonstrated the bacteria to have *esp* gene which is an important gene for biofilm formation and gene transfer (Hashem *et al.,* 2015). These bacteria also exhibit decreased susceptibility to β-lactam antibiotics due to acquisition of penicillin binding protein (PBP) which hydrolyze β-lactam antibiotic and is coded for by *pbp5* genes (Guzman *et al.,* 2016). When the bacteria acquire and disseminate these AR genes, it leads to spread of high-level resistant clone of *Enterococci* spp. to beta lactam antibiotic, which was also observed in this study.

Escherichia coli showed high resistance to augmentin and ampicillin. The findings compare closely to a study conducted in Ethiopia by Derese *et al.,* (2016) which showed emerging *E. coli* from urine sample was resistant (75%) to augmentin. In Kenya, this bacteria was found to be resistant (83%) to ampicillin (Thiong'o L.N., 2012). Antibiotic resistance in *E. coli* from urine samples was initially observed by Kariuki *et al.,* (2007) and since then, other studies have reported multi-drug resistance of *E. coli* from various sample sources including animal faeces (Kikuvi *et al.,* 2013), food handlers (Oundo *et al.,* 2008) and water (Wambugu *et al.,* 2015). World Health Organization national data from 5 regions reported at least 50% resistance by *E. coli* and *Klebsiella* spp. to fluoroquinolones (ciprofloxacin, ofloxacin and norfloxacin) (WHO, 2015). *Escherichia coli* showed multiple resistances to utmost 5 drugs tested in this study. A study conducted in Sudan by Hamdan *et al.,* 2015 found multiple resistant of *E. coli* to 4 drugs. Overall, the most common antibiotics in the resistance blocks were azithromycin, ampicillin and augmentin.

Proteus species were fully resistant (100%) to augmentin and ampicillin. However, resistance to azithromycin was 50% and no resistance was found to gentamycin, chloramphenicol and cefoxitine. This multi-drug resistance was in agreement with a study done in Kisii by Mageto *et al.,* 2018 which recorded AR by *Proteu*s spp. to ampicillin (75%), nitrofurantoin (75%), cotrimoxazole (75%) and gentamycin (0%). Similar resistance level in *Proteus* spp. was also reported for augmentin (100%), chloramphenicol (0%), ampicillin (100%) and gentamycin (0%) (Derese *et al*., 2016). *Klebsiella pneumoniae* was also fully resistant (100%) to azithromycin, augmentin, ampicillin, and no resistance to norfloxacin, cefoxitine, ciprofloxacin and gentamycin.

Hamdan *et al.,* (2015) in Sudan found *Klebsiella* spp. Isolated from urine samples resistant to augmentin 72.4%, norfloxacin 34.9%, gentamycin (3.9%), cefoxitine (37%) and ciprofloxacin (10.5%). In addition to these findings being similar to the current study, there was another study in India (George *et al*., 2015), in which 100% resistance by *Klebsiella* spp. to ampicillin, augmentin (100%), cefoxitin (20%) and ciprofloxacin (40%) was reported. Although the findings on *Proteus* spp. and *Klebsiella pneumoniae* in the current study could be attributed to the low prevalence of isolation, it is a wake-up call on the level of resistance associated with these and other organisms.

Escherichia coli and *Klebsiella* spp. produce extended spectrum beta-lactamase (ESBL) enzyme that confer resistance to β-lactam antibiotics. These bacteria were found to acquire beta-lactamase enzymes such as *bla*OXA-48 and *bla*CTX-M gene which hydrolysed penicillins, carbapenems and fluoroquinolones at high level but not expanded spectrum cephalosporins (Poirel *et al.,* 2004). In this study, there was high resistance of these gram negative bacteria to beta lactam antibiotics-augmentin and ampicillin. It was likely that the high level of resistance of these clinical isolates to beta-lactams was attributed to acquisition of peculiar beta-lactamases and modification of outer membrane proteins. To combat ESBL producing bacteria, cefoxitin which is a new, cephalosporin-like antibiotic with high resistance to hydrolysis by beta-lactamase enzymes, have been used in the treatment of UTI. Compared to studies within the country, the majority of these bacteria have developed resistance to antibacterials except to third-generation cephalosporin- cefepime and cefoxitine (Mageto *et al.,* 2018; Fred *et al.*, 2015; Cheruyot, D., 2016; Kariuki S. M., 2011).Variation in the aetiological agents associated with community acquired UTI and their antibiotic susceptibility showed the need for the study

to be done regularly in order to capture any emerging resistance and understand epidemiology of urinary tract infections.

Review of other literature in Kenya and other regions of the world revealed that AR is normally higher in cases where there is high usage of antibiotics hence directly associated with misuse of antibiotics (Ajak T.A.D., 2017; Ulstad *et al.,* 2016; Kariuki *et al.,* 2007). High resistance rate to antibiotics is a reflection of widespread uncontrolled use of these antibiotics combined with failure to follow ethics in antibiotic use which vary from place to place (Kariuki S.M., 2011; Ajak T.A.D., 2017). The newer fluoroquinolones; ciprofloxacin and norfloxacin, showed good efficacy for the isolates with resistance rates of 20%. Gentamycin is an aminoglycoside which showed high efficacy (78%) for all isolates. This drug has been in use for more than 50 years and still retains its potency. Gentamycin has a limited use probably because of the injection route of administration which is not desirable to many patients. This limited use has delayed the emergence and spread of resistance to the drug. Only 10% showed resistance to cefoxitine showing that the drug may be considered an alternative for the treatment of recurrent UTIs.

Bacterial resistance to commonly used antibiotics can be attributed to self-medication, failed treatment, poor or wrong choice of antibiotics, symptomatic treatment and possible withdrawal from effective antibiotics. This AR is worrying especially the resistance to the few remaining effective antibiotics (Ventola C.L., 2015). With paucity in the development of new antibiotics, the world is heading to an era where diseases that were once treatable would potentially be deadly (Torome, T.K., 2015).

Continuous development of AR in currently susceptible population suggests that some bacteria mutate to the resistant state. This study confirms an increasing prevalence of

infections caused by multi-drug resistant bacteria which complicate the empirical treatment of UTI, calling upon policy makers to fast-track and enforce policies that guide the use of antibiotics in the country. WHO launched the AWaRE (Access Watch and Reserve) tool to whose adherence to is aimed at minimizing the spread of antimicrobial resistance, antibiotic related adverse effects and drug cost (WHO, 2019).

CHAPTER FIVE

SUMMARY, CONCLUSIONS & RECOMMENDATIONS

5.1 Introduction

This chapter summarizes the study, presents the conclusion and the recommendation drawn from the study.

5.2 Summary

The aetiological agents of UTI found in patients seeking treatment in KCRH were *S. aureus*, *E. faecalis*, *E. coli*, *Proteus* spp. and *K. pneumoniae*. Antibiotic resistance was observed towards the commonly used antibiotics namely; ampicillin (84.3%), azithromycin (71.9%) and augmentin (70%). However, some bacteria were susceptible to these commonly used antibiotics. Moderate resistance was observed in norfloxacin (30%) except *S. aureus* which showed 64% resistance. There was less resistance to cefoxitine (13.3%), gentamycin (11.7%) and ciprofloxacin (10%).

5.3 Conclusions

Urinary tract infection was common in females than in males and particularly common in the age-group of the range 21-30 years. Prevalence of UTI was 20%. Twenty percent isolation rate showed that self-medication and empirical treatment based on the symptoms of UTI is unreliable and that contributes to AR.

Azithromycin and ampicillin had the highest resistance to antibiotics and hence can be prescribed when the case of UTI is not recurrent.

Cefoxitin, ciprofloxacin and gentamycin had least resistance to antibiotics hence are good therapeutic agents for treatment of UTI. They can be the drug of choice in cases of recurrent UTI.

In a situation where there is no feasibility of doing laboratory work, like in the dispensaries, augmentin and norfloxacin can be used as a drug of choice for the treatment..

The study confirmed existence of both single and multiple AR by the various bacteria causing UTI. It thus, contributes towards data on current antimicrobial resistance in Kenya. This will help the policy makers in the fight towards reducing antimicrobial resistance.

5.4 Recommendations

Whole clinical picture should be put into perspective and laboratory evaluation done before treatment of UTI.

Since there was variation in AR among uropathogens, antibiotic susceptibility tests should be done periodically in order to capture further emergence of the resistance. A surveillance system is therefore recommended to be put in place so as to monitor the trends and emergence of antimicrobial resistance. This will ensure continuous research into its prevention.

The study recommends more studies to be done in other regions of Kenya using more antimicrobials so as to get a diverse antimicrobial susceptibility that will aid in treatment of UTI.

There is need for sensitization on importance of seeking medication and knowledge on control measures of UTI to all youths.

Pharmacist should not prescribe strong antibiotics for self medication because of possible misuse while there is paucity in developing new drugs.

5.5 Suggestions for Further Research

More research needs to be done on non-bacterial aetiological agents that cause UTI and their antimicrobial resistance. This will give a diverse antimicrobial susceptibility profile which will help in treatment of UTIs.

REFERENCE

Ahsan, B., Abdulmaleki, N., Mohamadi, H., & Kalantar, E. (2011). A surveillance study of antimicrobial susceptibility in 11 hospitals in Kurdistan Province. *African Journal of Microbiology Research*, *5*(20), 3157-3161.

Ajak, T. A. D. (2017). Status of antimicrobial resistance in bacteria isolated from Kenyan chickens (Unpublished Doctoral Dissertation, University of Nairobi).

Alabi, A. S., Frielinghaus, L., Kaba, H., Kösters, K., Huson, M. A., Kahl, B. C., & Schaumburg, F. (2013). Retrospective analysis of antimicrobial resistance and bacterial spectrum of infection in Gabon, Central Africa. *BMC Infectious Diseases*, *13*(1), 1.

Anderson, A. C., Jonas, D., Huber, I., Karygianni, L., Wölber, J., Hellwig, E. & Al-Ahmad, A. (2016). Enterococcus faecalis from food, clinical specimens, and oral sites: Prevalence of virulence factors in association with biofilm formation. *Frontiers in microbiology*, *6*, 1534.

Arana, D. M., Rubio, M., & Alós, J. I. (2017). Evolution of antibiotic multiresistance in Escherichia coli and Klebsiella pneumoniae isolates from urinary tract infections: A 12-year analysis (2003–2014). *Enfermedades infecciosas y microbiologia clinica*, *35*(5), 293-298.

Bauer, A. W., Kirby, W. M. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, *45*(4), 493.

Beitha, A. (2008). Mapping Factors that Drive Drug Resistance (with a Focus on Resource-Limited Settings): A First Step towards Better Informed Policy. *Center for Global Development, 2*(1).

Ben-David, D., Kordevani, R., Keller, N., Tal, I., Marzel, A., Gal-Mor, O., & Rahav, G. (2012). Outcome of carbapenem resistant Klebsiella pneumoniae bloodstream infections. *Clinical Microbiology and Infection*, *18*(1), 54-60.

Bensman A, Dunand O., & Ulinski T. (2012). Urinary tract infection. In: Avner ED, Harmon WEB, Niaudet P, Yoshikawa N (6th ed.). *Pediatric Nephrology*. Springer-Verlag, Berlin Heidelberg, 1299–310.

Beyene G., &Tsegaye W. (2011). Bacterial uropathogens in urinary tract infection and antibiotic susceptibility pattern in Jimma University specialized hospital, southwest Ethiopia. *Ethiopian Journal of Health Sciences,* 21:141–146.

Bonev, B., Hooper, J., & Parisot, J. (2008). Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method. *Journal of antimicrobial chemotherapy*, *61*(6), 1295-1301.

Center for Disease Dynamics, Economics and Policy, (2019). Uganda releases AMR National Action Plan; AMR role elevated at WHO. Retrieved on July, 2019 from https://cddep.org/blog/posts/weekly-digest-mar-12-2019/.

Cheesbrough, M. (2006). *District Laboratory Practice In Tropical Countries*. Cambridge University Press.

Chen, Y. H., Ko, W. C., & Hsueh, P. R. (2013). Emerging resistance problems and future perspectives in pharmacotherapy for complicated urinary tract infections. *Expert opinion on pharmacotherapy*, *14*(5), 587-596.

Cheruyot, D. (2016). Antibiogram patterns of uropathogenic Escherichia coli and socio-demographic factors of patients attending Kericho County Hospital (Doctoral dissertation, Mount Kenya University).

Claus, D. (1992). Standardized gram staining procedure. *World Journal of Microbiology and Biotechnology*, *8*(4), 451-452.

Cloete, T. E. (2003). Resistance mechanisms of bacteria to antimicrobial compounds. *International Biodeterioration & Biodegradation*, *51*(4), 277-282.

CLSI- Clinical and Laboratory Standards Institute (2008). Performance standards for antimicrobial susceptibility testing; Eighteenth informational supplement vol. 28 No.1 M100-s18, ISBN1-56238-653-0. *National Committee for Clinical Laboratory Standards.*

Cockerill, F. R., & Clinical and Laboratory Standards Institute. (2010). Performance standards for antimicrobial susceptibility testing: twentieth informational supplement. *National Committee for Clinical Laboratory Standards.*

Okeyo, V. (2019, July 12). 11 babies at KNH newborn unit die of suspected bacterial infection. *Daily Nation Newspaper*. Retrieved on 12th July, 2019 from https://www.nation.co.ke/news/11-babies-at-KNH-die-of-suspected-bacterial-infection/1056-5192778-55i9qez/index.html

Den Heijer, C. D. J., Donker, G. A., Maes, J., & Stobberingh, E. E. (2010). Antibiotic susceptibility of unselected uropathogenic Escherichia coli from female Dutch general practice patients: a comparison of two surveys with a 5 year interval. *Journal of Antimicrobial Chemotherapy*, *65*(10), 2128-2133.

Derese, B., Kedir, H., Teklemariam, Z., Weldegebreal, F., & Balakrishnan, S. (2016). Bacterial profile of urinary tract infection and antimicrobial susceptibility pattern among pregnant women attending antenatal Clinic in DilChora Referral Hospital, Dire Dawa, eastern Ethiopia. *Therapeutics and clinical risk management*, *12*(251).

Foxman, B. (2014). Urinary tract infection syndromes: occurrence, recurrence, bacteriology, risk factors, and disease burden. *Infectious Disease Clinics of North America*, *28*(1), 1-13.

Fred, N. W., Gichuhi, J. W., & Mugo, N. W. (2015). Prevalence of urinary tract infection, microbial aetiology, and antibiotic sensitivity pattern among antenatal women presenting with lower abdominal pains at Kenyatta National Hospital, Nairobi, Kenya. *The Open Access Journal of Science and Technology*, *3*.

George, C. E., Norman, G., Ramana, G. V., Mukherjee, D., & Rao, T. (2015). Treatment of uncomplicated symptomatic urinary tract infections: Resistance patterns and misuse of antibiotics. *Journal of family medicine and primary care*, *4*(3), 416.

Georgios, M., and Egki, T., (2014). Phenotypic and Molecular Methods for the Detection of Antibiotic Resistance Mechanisms in Gram Negative Nosocomial Pathogens. https://www.intechopen.com/.../phenotypic-and-molecular-methods-for-the-detection-of.-antibiotic-resistance-mechanisms-in-gram-negative-nosocomial-pathogens.

Glasner, C., Albiger, B., Buist, G., TambićAndrašević, A., Canton, R., Carmeli, Y., & Livermore, D. M. (2013). Carbapenemase-producing Enterobacteriaceae in

Europe: a survey among national experts from 39 countries, February 2013. *Eurosurveillance*, *18*(28), 20525.

Gobernado, M., Valdés, L., Alós, J. I., García-Rey, C., Dal-Ré, R., García-de-Lomas, J., & Spanish Surveillance Group for Urinary Pathogens. (2007). Antimicrobial susceptibility of clinical *Escherichia coli* isolates from uncomplicated cystitis in women over a 1-year period in Spain. *Revista Española de Quimioterapia, 20*(1), 68-76.

Golkar, Z., Bagasra, O., & Pace, D. G. (2014). Bacteriophage therapy: a potential solution for the antibiotic resistance crisis. *The Journal of Infection in Developing Countries*, *8*(02), 129-136.

Guentzel, M. N. (1996). *Medical Microbiology* (4th ed.). Galveston (TX): University of Texas Medical Branch press.

Gupta, K, & Bhadelia, N. (2014). Management of urinary tract infections from multi-drug resistant organisms. *Infectious Disease Clinics of North America*, *28*(1), 49-59.

Guzman Prieto, A. M., van Schaik, W., Rogers, M. R., Coque, T. M., Baquero, F., Corander, J., & Willems, R. J. (2016). Global Emergence and Dissemination of Enterococci as Nosocomial Pathogens: Attack of the Clones?. *Frontiers in microbiology*, *7*(2), 788.

Haider, G., Zehra, N., Munir, A. A., & Haider, A. (2010). Risk factors of urinary tract infection in pregnancy. *The Journal of the Pakistan Medical Association*, *60*(3), 213.

Hajian-Tilaki, K. (2011). Sample size estimation in epidemiologic studies. *Caspian journal of internal medicine*, *2*(4), 289.

Hamdan, H. Z., Kubbara, E., Adam, A. M., Hassan, O. S., Suliman, S. O., & Adam, I. (2015). Urinary tract infections and antimicrobial sensitivity among diabetic patients at Khartoum, Sudan. *Annals of clinical microbiology and antimicrobials*, *14*(1), 26.

Hashem, Y. A., Yassin, A. S., & Amin, M. A. (2015). Molecular characterization of Enterococcus spp. clinical isolates from Cairo, Egypt. *Indian journal of medical microbiology*, *33*(5), 80.

Hemraj, V., Diksha, S., & Avneet, G. (2013). A review on commonly used biochemical test for bacteria. *Innovare J Life Sci*, *1*(1), 1-7.

Hooton, T. M. (2012). Uncomplicated urinary tract infection. *New England Journal of Medicine*, *366*(11), 1028-1037.

Horcajada, J. P., Shaw, E., Padilla, B., Pintado, V., Calbo, E., Benito, N., & Rodríguez-Baño, J. (2013). Healthcare-associated, community-acquired and hospital-acquired bacteraemic urinary tract infections in hospitalized patients: a prospective multicentre cohort study in the era of antimicrobial resistance. *Clinical Microbiology and Infection*, *19*(10), 962-968.

Hussein S. (2010). Bacterial identification by the Analytical Indexing system. *American Society for microbiology.* http://www.microbelibrary.org/library.

Indalo, A.A. (1997). Antibiotic sale behavior in Nairobi: A contributing factor to antimicrobial drug resistance. *East Africa Medical Journal, 74*(1), 171 -173.

Kafil, H. S., & Mobarez, A. M. (2015). Spread of Enterococcal Surface Protein in Antibiotic Resistant Entero-coccus faecium and Enterococcus faecalis isolates from Urinary Tract Infections. *The Open Microbiology Journal*, *9*, 14–17. http://doi.org/10.2174/1874285801509010014.

Kariuki, S., Revathi, G., Kariuki, N., Muyodi, J., Mwituria, J., Munyalo, A., ... & Hart, C. A. (2005). Increasing prevalence of multidrug-resistant non-typhoidal salmonellae, Kenya, 1994–2003. *International journal of antimicrobial agents*, *25*(1), 38-43.

Kariuki, S. M., (2011). Antibiotic Use and Resistance in Kenya-GRAP. https://cddep.org/.../situation_analysis_and_recommendations_antibiotic_use_and res.

Kariuki, S. M., and Dougan, G., (2014). Antimicrobial resistance in Sub-Saharan Africa: an underestimated emergency. *Annals of the New York Academy of Science*, 5(1), 43-55.

Kariuki, S., Revathi, G., Corkill, J., Kiiru, J., Mwituria, J., Mirza, N., & Hart, C. A. (2007). Escherichia coli from community-acquired urinary tract infections resistant to fluoroquinolones and extended-spectrum beta-lactams. *The Journal of Infection in Developing Countries*, *1*(03), 257-262.

Kebede, D., Menkir, D., & FeyissaBedada, A. (2016).Prevalence and Antibiotic Susceptibility of Bacterial Uropathogens among Patients of Urinary Tract Infection Visiting Hasasa Health Center, Gedab Hasasa Woreda, West Arsi Zone, Ethiopia (Doctoral dissertation, Haramaya University).

Kenya National Bureau of Statistics, census of 2009. Retrieved on June, 2019 from https://www.knbs.or.ke/download/kericho-count/.

Kikuvi, G. M., Ole-Mapenay, I. M., Mitema, E. S., & Ombui, J. N. (2013). Antimicrobial resistance in Escherichia coli isolates from faeces and carcass samples of slaughtered cattle, swine and chickens in Kenya.

Kimando, J. M., Okemo, P. O., & Njagi, E. N. M. (2010). Resistance to antibiotics in urinopathogenic bacteria isolated in patients attending Kenyatta University Health Clinic, Nairobi. *East African medical journal*, *87*(3), 115-119.

Kline, K. A., & Lewis, A. L. (2016). Gram-positive uropathogens, polymicrobial urinary tract infection and the emerging microbiota of the urinary tract. *Microbiology spectrum*, *4*(2).

Leski, T. A., Taitt, C. R., Bangura, U., Stockelman, M. G., Ansumana, R., Cooper, W. H., & Vora, G. J. (2016). High prevalence of multidrug resistant Enterobacteriaceae isolated from outpatient urine samples but not the hospital environment in Bo, Sierra Leone. *BMC Infectious Diseases*, *16*(1), 1.

Levison, M. E., & Kaye, D. (2013). Treatment of complicated urinary tract infections with an emphasis on drug-resistant gram-negative uropathogens. *Current Infectious Disease Reports*, *15*(2), 109-115.

Linhares, I., Raposo, T., Rodrigues, A., & Almeida, A. (2013). Frequency and antimicrobial resistance patterns of bacteria implicated in community urinary tract

infections: a ten-year surveillance study (2000–2009). *BMC Infectious Diseases*, *13*(1), 1.

Lo, E., Nicolle, L. E., Coffin, S. E., Gould, C., Maragakis, L. L., Meddings, J., & Yokoe, D. S. (2014). Strategies to prevent catheter-associated urinary tract infections in acute care hospitals: 2014 update. *Infection Control & Hospital Epidemiology*, *35*(2), 32-47.

Löhr, I. H., Rettedal, S., Natås, O. B., Naseer, U., Øymar, K., & Sundsfjord, A. (2013). Long-term faecal carriage in infants and intra-household transmission of CTX-M-15-producing Klebsiella pneumoniae following a nosocomial outbreak. *Journal of Antimicrobial Chemotherapy*, *2*(1)502.

Mageto, V. M., Gatwiri, M. S., & Njoroge, W. (2018). Uropathogens antibiotic resistance patterns among type 2 diabetic patients in Kisii Teaching and Referral Hospital, Kenya. *The Pan African Medical Journal*, *30*(1).

Maron, D. F. (2016): Superbug explosion triggers UN General Assembly (UNGA) meeting.*Scientific American*. https://www.scientific american.com/article/superbug-explosion-triggers-u-n-general-assembly-meeting/.

Marshall, B. M., & Levy, S. B. (2011). Food animals and antimicrobials: impacts on human health. *Clinical Microbiology Reviews*, *24*(4), 718-733.

Martinez-Brocal, A., Miranda-Casas, C. & Gutiérrez-Fernández, J. (2014). Evolution of the resistance to antibiotics of bacteria involved in urinary tract infections: a 7-year surveillance study. *American Journal of Infection Control*, *42*(10), 1033-1038.

Mitema, E. S. The role of unregulated sale and dispensing of antimicrobial agents on the development of antimicrobial resistance in developing countries. *Springer, NewYork*. (2010).

Mitema, E. S., & Kikuvi, G. M. (2004). Surveillance of the overall use of antimicrobial drugs in humans over a 5 year period (1997–2001) in Kenya. *Journal of Antimicrobial Chemotherapy*, *54*(5), 966-967.

Molina-López, J., Aparicio-Ozores, G., Ribas-Aparicio, R. M., Gavilanes-Parra, S., Chávez-Berrocal, M. E., Hernández-Castro, R., & Manjarrez-Hernández, H. Á. (2011). Drug resistance, serotypes, and phylogenetic groups among uropathogenic Escherichia coli including O25-ST131 in Mexico City. *The Journal of Infection in Developing Countries*, *5*(12), 840-849.

Moura, A., Nicolau, A., Hooton, T., & Azeredo, J. (2009). Antibiotherapy and pathogenesis of uncomplicated UTI: difficult relationships. *Journal of Applied Microbiology*, *106*(6), 1779-1791.

Mwambete, K. D., & Malaba, P. (2017). High prevalence of antibiotic resistance among bacteria isolated from pregnant women with asymptomatic urinary tract infections in Dar es Salaam, Tanzania. *Research Journal of Health Sciences*, *5*(2), 65-73.

Nabbugodi, W. F., Gichuhi, J. W., & Mugo, N. W. (2015). Prevalence of urinary tract infection, microbial aetiology, and antibiotic sensitivity pattern among antenatal women presenting with lower abdominal pains at Kenyatta National Hospital, Nairobi, Kenya. *Open Access Journal of Science and Technology*, *3*(1).

Ndihokubwayo, J. B., Yahaya, A. A., Desta, A. T., Ki-Zerbo, G., Odei, E. A., Keita, B., & Nkhoma, W. (2013). Antimicrobial resistance in the African Region: Issues, challenges and actions proposed. *Health Monitor*, 27(1).

Nelson, C. P., Hoberman, A., Shaikh, N., Keren, R., Mathews, R., Greenfield, S. P., & Carpenter, M. A. (2016). Antimicrobial resistance and urinary tract infection recurrence. *Pediatrics*, *137*(4).

Nielubowicz, G. R., & Mobley, H. L. (2010). Host–pathogen interactions in urinary tract infection. *Nature Reviews Urology*, *7*(8), 430-441.

Okesola, A. O., & Oni, A. A. (2009). Antimicrobial resistance among common bacterial pathogens in south western Nigeria. *American-Eurasian Journal of Agriculture and Environmental Sciences, 5*(3):327, 330.

Oundo, J. O., Kariuki, S. M., Boga, H. I., Muli, F. W., & Iijima, Y. (2008). High incidence of enteroaggregative Escherichia coli among food handlers in three areas in Kenya: A possible transmission route of travellers‘diarrhoea. *Journal of Travel Medicine15* (1): 31-38.

Perovic, O., and Schultz, C. (2016). Stepwise approach for implementation of antimicrobial resistance surveillance in Africa. *African Journal of Laboratory Medicine, 5*(3): 482-488.

Poirel, L., Héritier, C., Tolün, V., & Nordmann, P. (2004). Emergence of oxacillinase-mediated resistance to imipenem in Klebsiella pneumoniae. *Antimicrobial Agents and Chemotherapy*, *48*(1), 15-22.

Poulou, A., Grivakou, E., Vrioni, G., Koumaki, V., Pittaras, T., Pournaras, S., &Tsakris, A. (2014). Modified CLSI extended-spectrum β-lactamase (ESBL) confirmatory

test for phenotypic detection of ESBLs among Enterobacteriaceae producing various β-lactamases. *Journal of Clinical Microbiology*, *52*(5), 1483-1489.

Raetz, C. R., & Whitfield, C. (2002). Lipopolysaccharide endotoxins. *Annual Review of Biochemistry*, *71*(1), 635.

Rağbetli, C., Parlak, M., Bayram, Y., Guducuoglu, H., & Ceylan, N. (2016). Evaluation of Antimicrobial Resistance in Staphylococcus aureus Isolates by Years. *Interdisciplinary Perspectives on Infectious Diseases*.

Rawat, D., & Nair, D. (2010). Extended-spectrum β-lactamases in Gram Negative Bacteria. *Journal of global infectious diseases*, *2*(3), 263.

Rendón, M. A., Saldaña, Z., Erdem, A. L., Monteiro-Neto, V., Vázquez, A., Kaper, J. B., & Girón, J. A. (2007). Commensal and pathogenic Escherichia coli use a common pilus adherence factor for epithelial cell colonization. *Proceedings of the National Academy of Sciences*, *104*(25), 10637-10642.

Roca, I., Akova, M., Baquero, F., Carlet, J., Cavaleri, M., Coenen, S., & Kahlmeter, G. (2015). The global threat of antimicrobial resistance: science for intervention. *New Microbes and New Infections*, *6*, 22-29.

Severin, J. A., Mertaniasih, N. M., Kuntaman, K., Lestari, E. S., Purwanta, M., Lemmens-Den Toom, N., & Goessens, W. H. (2010). Molecular characterization of extended-spectrum β-lactamases in clinical Escherichia coli and Klebsiella pneumoniae isolates from Surabaya, Indonesia. *Journal of Antimicrobial Chemotherapy*, *65*(3), 465-469.

Sewify, M., Nair, S., Warsame, S., Murad, M., Alhubail, A., Behbehani, K., & Tiss, A. (2016). Prevalence of urinary tract infection and antimicrobial susceptibility

among diabetic patients with controlled and uncontrolled glycemia in Kuwait. *Journal of Diabetes Research,* 5(1).

Sorlozano, A., Jimenez-Pacheco, A., del Castillo, J. D. D. L., Sampedro, A., Tandogdu, Z., & Wagenlehner, F. M. (2016). Global epidemiology of urinary tract infections. *Current Opinion in Infectious Diseases*, *29*(1), 73-79.

Stapleton A. (2005). Novel Mechanism of P-Fimbriated Escharichia coli virulence in pyelonephritis. *Journal of American Social Nephrology 5(*16), 3458-3460.

Teixeira, L. M., & Merquior, V. L. C. (2013). *Molecular Typing in Bacterial Infections,* Humana Press, Totowa, *5*(4), 17-26).

Tenover, F. C. (2006). Mechanisms of antimicrobial resistance in bacteria. *The American Journal of Medicine*, *119*(6), 3-10.

Thiong'o, L. N. (2012). Molecular characterization of multi-drug resistant *Escherichia coli* isolated from urine samples in Mater Hospital, Nairobi, Kenya, Msc. Thesis-Jomo Kenyatta University of Agriculture and Technology.

Torome, T. K. (2015). Isolation and characterisation of antibiotic producing Bacillus organisms in Lake Bogoria. MSc, Thesis-Jomo Kenyatta University of Agriculture and Technology.

Ulstad, C. R., Solheim, M., Berg, S., Lindbæk, M., Dahle, U. R., & Wester, A. L. (2016).Carriage of ESBL/AmpC-producing or ciprofloxacin non-susceptible Escherichia coli and Klebsiella spp. in healthy people in Norway. *Antimicrobial Resistance & Infection Control*, *5*(1), 57.

Vandepitte, J., Verhaegen, J., Engbaek, K., Rohner, P., Piot, P., & Heuck, C. C. (2003). *Basic laboratory procedures in clinical bacteriology* (2nd Ed.). World Health Organization.

Ventola, C. L. (2015). The antibiotic resistance crisis: part 1: causes and threats. *Pharmacy and Therapeutics*, *40*(4), 277.

Vermeulen, S. H., Hanum, N., Grotenhuis, A. J., Castano-Vinyals, G., Van Der Heijden, A. G., Aben, K. K., &Kiemeney, L. A. (2015). Recurrent urinary tract infection and risk of bladder cancer in the Nijmegen bladder cancer study. *British Journal of Cancer*, *112*(3), 594-600.

Wagenlehner, F. M., Hoyme, U., Kaase, M., Funfstuck, R., Naber, K. G., & Schmiemann, G. (2011). Uncomplicated urinary tract infections. *Deutsches Ärzteblatt International. 108*(24), 415-423.

Wamalwa, P., Omolo, J., & Makokha, A. (2013). Prevalence and risk factors for Urinary Tract Infections among pregnant women. *Prime Journal of Social Sciences*, *2*(12), 525-31.

Wambugu, P., Habtu, M., Impwi, P., Matiru, V., & Kiiru, J. (2015). Antimicrobial susceptibility profiles among Escherichia coli strains isolated from Athi river water in Machakos County, Kenya. *Advances in Microbiology*, *5*(10), 711.

Wayne, P. A. (2011). Performance standards for antimicrobial susceptibility testing. Clinical and laboratory standards institute. *31*(1)

Wilson, G. S., & Miles, A. (1975). *Principle of Bacteriology, Virology and immunity* (6thEd.). London, Edward Arnold Ltd., 887-900.

Woodin, K. A., & Morrison, S. H. (1994). Antibiotics: mechanisms of action, Pediatrics in review. *American Academy of Pediatrics,15* (11), 440–7.

World Health Organization. (2012). Evolving threats of antimicrobial resistance options for action WHO Cataloguing -in-Publication data. Retrieved on June, 2019 from https://www.who.int/biologicals/WHO_TRS_964_web.pdf

World Health Organization. (2014). Antimicrobial resistance: global report on surveillance. World Health Organization.

World Health Organization. (2015). Global Action Plan For Antimicrobial resistance. Retrieved on April 12 2018 from *www.wpro.who.int/entity/drug_resistance/resources/global_action_plan_eng.pdf.*

World Health Organization. (2018). Global antimicrobial resistance surveillance system (GLASS) report: early implementation 2017-2018. Retrieved on July, 2019 from https://apps.who.int/iris/bitstream/handle/10665/279656/9789241515061-eng.pdf

World Health Organization. (2018). Antimicrobial resistance global report on surveillance 2018. Retrieved on May, 2019 from https://www.who.int/antimicrobial-resistance/global-action-plan/surveillance/GLASS-infographic-(web).pdf.

World Health Organization. (2019). In the face of slow progress, WHO offers a new tool and sets a target to accelerate action against antimicrobial resistance. Retrieved on July, 2019 from https://www.who.int/news-room/detail/18-06-2019-in-the-face-of-slow-progress-who-offers-a-new-tool-and-sets-a-target-to-accelerate-action-against-antimicrobial-resistance.

APPENDICES

Appendix 1: Consent Form

Am a student at University of Kabianga undertaking a study on profiling of antibiotic resistance of uropathogens among patients with UTI seeking treatment in Kericho County Referral Hospital (KCRH).

Purpose of the study: To determine the level of antibiotic resistance in bacteria pathogens causing UTI.

Procedure to be followed: A sterile container will be provided to you to collect urine sample. You are required to collect the midstream urine taking care not to contaminate the sample. You will bring the sample to the lab immediately after collection for analysis.

Risks involved: The procedure for urine sample is not invasive and does not cause pain or harm.

Benefits: The results will provide the appropriate antibiotic that will be used for your treatment. My phone number is 0729833410 in case you will need the results of your test sample.

Confidentiality of records: The sample and your personal records will be coded and not disclosed. Only the coded numbers will be used in thesis report and publication.

Participation: It is important for you to know that you can decline to participate in the study.

Consent: I have read the above information carefully and I was allowed to seek clarification. I have understood that there are no risks associated with urine sample collection.

Signature ....date..........................

Age ....gender.......................

Study number provided.........................

I, the undersigned have fully explained the relevant details of this study to the patient.

Signature................................date......................

Appendix 2 : Questionnaire

Sample Identification : ________________

Age: ______________________

Gender: ___________________

Location: _________________

Previous antibiotic used: ________________

Last date used: _______________

<u>Objective 2: Data analysis results</u>

Microscopy

Sample Identification	Presence or absence of deposits (leukocytes)

Inoculation and culture identification of bacteria

<table>
<tr><td>MACCONKEY AGAR (LF, NLF)</td><td>CLED AGAR (LF, NLF)</td><td>BLOOD AGAR (Haemolysis, swarming)</td><td colspan="2">REMARKS</td></tr>
<tr><td></td><td></td><td></td><td colspan="2">EPU 001a:

EPU 001b:</td></tr>
<tr><td colspan="5">Subculture results</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td></tr>
</table>

LF: Lactose Fermenters, NLF: Non-Lactose Fermenters

Gram stain results

Sample Identification	Gram stain (Gram positive or negative, cocci or bacilli)
001 (a)	
001 (b)	

Biochemical identification of bacteria

Biochemical test		Results -(positive, negative)	Remarks
TSI	001a		
	001b		
MIL	001a		
	001b		
MR-VP	001a		
	001b		
S- citrate	001a		
	001b		
Coagulase	001a		
	001b		
Catalase	001a		
	001b		
Oxidase	001a		
	001b		

Analytical Profile Index (API) Identification results

Final organism(s) identified by API using APIweb software: ________________________

Objective 3: Antibiotic susceptibility testing (AST)

	Antibiotics							Remarks
Test isolate	Gentamycin	Ciprofloxacin	Ampicillin	Cefoxitine	Ofloxacin	Azithromycin	Amoxilin Clavulanic Acid	

Appendix 3: Antibiotic inhibition zone diameter as per CLSI guideline

Antibiotics	Resistant	Intermediate	Sensitive
Ciprofloxacin (5µg)	≤15	16-20	≥21
Gentamycin (10µg)	≤12	13-14	≥15
Ampicillin (10µg)	≤13	14-16	≥17
Chloramphenicol (30ug)	≤12	13-17	≥18
Augmentin (30µg)	≤13	14-17	≥18
Cefoxitine (30µg)	≤14	15-17	≥18
Azithromycin (30ug)	≤11	12-14	≥15

Appendix 4: Approval of the research proposal by Board of Graduate studies

UNIVERSITY OF KABIANGA

ISO 9001:2008 CERTIFIED

OFFICE OF THE DIRECTOR, BOARD OF GRADUATE STUDIES

30TH MAY, 2018

Ref: PGC/MIC/005/15
Gladys Chepkoech Mosonik
Biological Sciences Department,
University of Kabianga,
P.O Box 2030- 20200,
KERICHO.

Dear Ms. Mosonik,

RE: CORRECTED PROPOSAL

This is to acknowledge receipt of two copies of your corrected Proposal entitled **"Profiling of Antibiotic Resistance among Uropathogens Isolated from Patients Attending Kericho County Referral Hospital."**

You are now free to commence your field work on condition that you obtain a research permit from NACOSTI.
Please note that, you are expected to publish at least one paper in a peer reviewed journal before final examination (oral defence) of your Masters thesis.

Thank you.

Yours Sincerely,

PROF. J. K. KIBETT
DIRECTOR, BOARD OF GRADUATE STUDIES.

c.c 1. Dean, SST
2. HOD, Biological Sciences
3. Supervisors

Appendix 5: Approval of the proposal by IREC.

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471//2/3

MOI UNIVERSITY
COLLEGE OF HEALTH SCIENCES
P.O. BOX 4606
ELDORET

Reference: IREC/2017/23
Approval Number: 0001977

9th November, 2017

Gladys Chepkoech Mosonik,
University of Kabianga
Dept. of Biological Sciences,
P.O. Box 2030-20200,
ELDORET-KENYA.

Dear Ms. Mosonik,

RE: FORMAL APPROVAL

The Institutional Research and Ethics Committee has reviewed your research proposal titled:-

"Antibiotic Resistance Associated with ESBL Production among Enterobacteriaceae Isolated from Urine Samples in Kericho County Referral Hospital, Kenya."

Your proposal has been granted a Formal Approval Number: ***FAN: IREC 1977*** on 9th November, 2017. You are therefore permitted to begin your investigations.

Note that this approval is for 1 year; it will thus expire on 8th November, 2018. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date.

You are required to submit progress report(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change (s) or amendment (s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

Sincerely,

For

PROF.E.O.WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc CEO - MTRH Principal - CHS Chairman - COBES

Appendix 6: Ethical approval by Research and ethics committee of KCRH

COUNTY GOVERNMENT OF KERICHO

KERICHO DISTRICT HOSPITAL

Telegrams: "MEDICAL", Kericho
Telephone: Kericho (0734) 758102
e-mail: kerichodistricthospital@yahoo.com
When replying please quote
Ref: IREC/ 010/17

Medical Superintendent
Kericho District Hospital
P. O Box 11
KERICHO

22nd September 2017

Dear Gladys Chepkoech Mosonik

RE: RESEARCH APPROVAL

Reference is made in regard to your request dated 13th September 2017 seeking for permission to carry out a research in our facility.

This is therefore to inform you that your request to carry out a research entitled ***"Profiling of Antibiotic Resistance among Uropathogens Isolated from Patients Attending Kericho County Referral Hospital "***has been approved.

You are expected to observe Ethics during this process of data collection and ensure feedback on the results of the study is relayed back to the Research &Ethics Committee.

All concerned departments and individuals in the facility are requested to facilitate you in this process. However, consent **must** be sought from the affected patients

Thanks in advance

Yours Faithfully

D.K.Limo

For, Chairman

Research & Ethics Committee

Kericho District Hospital

MEDICAL SUPERINTENDENT
KERICHO DISTRICT HOSPITAL
P. O. Box 11, KERICHO - 20200
TEL: 052 - 31177 / 31191