PRVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF COTRIMOXAZOLE RESISTANT BACTERIAL UROPATHOGENS FROM HIV PATIENTS ATTENDING MASENO MISSION HOSPITAL, WESTERN KENYA

BY

MUSASIA PHILIP SAKWA

MSC/PH/00072/2015

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN MEDICAL MICROBIOLOGY

SCHOOL OF PUBLIC HEALTH AND COMMUNITY DEVELOPMENT

DEPARTMENT OF BIOMEDICAL SCIENCE AND TECHNOLOGY

MASENO UNIVERSITY

JULLY, 2023

DECLARATION

Declaration by Candidate

I hereby declare that this Thesis is my original work; it has never been partly or wholly presented to any institution for award of certificate, diploma or degree to the best of my knowledge.

Philip Sakwa Musasia

MSc/PH/00072/2015

Sign…..…..…….. Date…..……

Declaration by Supervisors

The work in this thesis has been carried out under our supervision as university supervisors

Dr. Bernard Guya

School of Public Health and Community Development

Maseno University, Kenya

Sign…..…..…….. Date…..……

Dr. Lilian Ogonda

School of Public Health and Community Development

Maseno University, Kenya

Sign…..…..…….. Date…..……

ACKNOWLEDGEMENT

This work could not have been possible without the support of certain institutions and individuals. I thank my two supervisors Dr. Bernard Guya and Dr. Lilian Ogonda of school of public health and community development of Maseno University for their untiring efforts in providing valuable guidance throughout the period of this study. Their commitment and positive criticism have helped to shape this document into what it currently is.

I am highly indebted to Maseno mission hospital management who gave me access to their facility and provided suitable working environment. I specifically acknowledge the head of comprehensive care clinic Ms. Veronica Mwai who introduced me to the patients and her members of staff not forgetting madam Lavender and Truphosa for their cooperation and assistance in sample collection.

Special thanks go to entire microbiology department of school of medicine Maseno University for making the laboratory available to me despite their busy schedule. In particular, I appreciate the chairperson Mss. Scholastica Korir for granting me access to laboratory facilities, Dr. Geoffrey Arasa & Dr. Masanta for their academic advice and support and not forgetting Ms. Tuvei and Muring who assisted in technical laboratory procedures and sample analyses.

Unreserved gratitude goes to my study respondents who supported this work by offering appropriate responses and the much needed samples without which this would not have been realised.

In a special way, I thank my family members particularly my wife Franciscah, children Calvin, Angellah, Joy and Carlos for their moral support and for giving me humble time during the study period. I can't forget to mention my workmates Ms. Malesi and Ms. Atieno who at times were called upon to stand in for me.

There are many other people and institutions who assisted in one way or another in actualizing this study. Because it is not possible to mention every one by name, kindly accept this joint appreciation.

DEDICATION

To my late parents,

Mr. Reuben Musasia Amadalo Kayiuyi who single handedly saw me through formal education and Mama Zilper Munalitsi Musasia for starting me well off the academic journey.

You positively influenced my academic line and entire life as well.

You never lived to see this though.

To you two, this work is dedicated.

ABSTRACT

Urinary tract infections (UTI) are inflammatory disorders within the urinary system due to presence of pathogenic microorganisms. Taking advantage of lowered immunity in Human Immuno-deficiency Virus (HIV) patients, bacterial UTI globally contribute up to 60% of opportunistic infections (OI). Previous HIV related (OI) studies concentrated on malaria and sexually transmitted diseases ignoring UTI. Owing to its wide interceptive range, cotrimoxazole was recommended for prophylaxis by world health organization and has been standard against opportunistic infections. However, with prolonged use, bacterial resistance is being reported. Uncertainty of UTI management following rising reports of bacterial resistance to commonly used drugs in AIDS era is real. This hospital based descriptive study investigated prevalence and antibiotic susceptibility patterns of cotrimoxazole resistant bacterial uropathogens from HIV patients attending Maseno mission hospital in western Kenya where treatment is blindly based on chance and history during clinic visits without laboratory testing. Specifically described bacterial UTI prevalence, determined their response to cotrimoxazole and established antibiotic susceptibility patterns. At interval of six, 354 participants were systematically selected from population of 2000. Mid-stream urine was cultured on cysteine lactose electrolyte deficiency agar and samples obtaining $\geq 10^5$ colony forming units determined by colony counters were biochemically characterized before being subjected to cotrimoxazole broth dilution sensitivity testing. Isolates not responding to cotrimoxazole were subjected to disc diffusion susceptibility testing in accordance with Clinical Laboratory Standards Institute's guidelines. Tables, graphs and charts were used to present generated data which was analyzed alongside structured questionnaire captured demographic information. Prevalence of UTI among HIV patients was 117(33%) where 75.4% originated from females and 25.6% males with *Escherichia coli* (54.7%) being most encountered. Chi square analysis of bacterial responses to cotrimoxazole evaluated at 95 % confidence level was statistically significant at $P<0.05$ $(x)^2$ $(5)=14.3$, $p< 0.005$ with an average susceptibility of 46.6%. Significantly, study results will serve as reference point for future researchers and medics, formed policy reevaluation for prophylaxis program and enhanced better OI management by establishing susceptibility patterns and suitable antibiograms. Study recommended for constant monitoring profile aetiological bacteria, periodic surveillance of for prophylactic drug and practicing scientifically determined treatment recommending most susceptible gentamicin (80.3%) empirical antibiotic for region with 53.4% resistance

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND ACRONYMS

HIV	:	Human Immuno Deficiency Virus
AIDS	:	Acquired Immunodeficiency Syndrome
ART	:	Anti retro Viral Therapy
HAART	:	Highly Active Anti- Retroviral Therapy
UTI	:	Urinary Tract Infections
E. coli	:	*Escherichia coli*
CCC	:	Comprehensive Care Clinic
CLED	:	Cysteine Lactose Electrolyte Deficiency
CLSI	:	Clinical & laboratory Standards Institute
MDR	:	Multi Drug Resistance
WHO	:	World Health Organization
χ**2**	:	Chi- square
UN	:	United Nations
OI	:	Opportunistic Infections
UNAIDS	:	Joint United Nations Program on HIV/AIDS
MIC	:	Mean inhibitory concentration

OPERATIONAL DEFINITION OF TERMS

Urinary tract infection (UTI) : Bacterial infection in the urinary tract producing $\geq 10^5$ Colony forming units

Multi drug resistant (MDR) : Resistance in ≥ 2 drugs.

Seropositive patients : Anyone who has tested positive for HIV.

Immuno-compromised : Anyone who has been exposed to HIV.

Cotrimoxazole sensitivity : Ability of cotrimoxazole to inhibit UTI in Immuno-compromised.

Cotrimoxazole non response : The inability of cotrimoxazole to inhibit UTI as a prophylactic drug

Sterile mid-stream sample : Urine sample without any environmental bacterial contamination.

CHAPTER ONE
INTRODUCTION

1.1 Background of Study

Urinary tract infections (UTI) are globally among the most frequently encountered infestations in practice with over 150 million isolations yearly (Zeyaullah et al., 2015). They present with typical systemic signs and symptoms. Varieties of aetiological organisms being implicated can complicate UTI diagnosis and affect the treatment course. Sometimes polymicrobial aetiologies in titers of >100,000 colony forming units (CFU)/ml are isolated from a single urine sample. Therefore, the need to establish causative agents in a given case is key to effective antimicrobial therapy as opposed to blind antibiotic administration as practiced in most developing countries. Previous studies have continuously isolated gram negative bacteria (75.6%) more compared to gram positives (24.4%) (Kimberly *et al., 2016*) where members of *Enterobacteriaceae* family are leading culprits with *Escherichia coli* being most common isolate from urine samples. Other commonly encountered gram negative bacteria include *Proteus mirabilis, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Lekha *et al.,* (2016) reported 88% isolates being as a result of *Enterobacteriaceae* with *Escherichia coli* accounting for 80.3% of them. Gram positive bacteria though not as common, have been implicated in the form of *Staphyllocus* family which includes *S. aureus* and *S. epidermidis* (Megan *et al.,* 2015).

Urinary tract infections cause significant morbidity in vulnerable groups of people including HIV seropositive patients where they mask signs and symptoms leading to asymptomatic disease and have as such been isolated in higher prevalence (Ngowi *et al.,* 2021).The virus slows down systemic host defense against infections by diminishing CD4 counts, Vijayan *et al*., (2017) thus precipitating for even less common uropathogens to opportunistically turn into complicated infections. HIV further causes variety of viral renal syndromes including bladder-areflexia and

hypo-reflexia that favour bacterial growth when they disintegrate into neurologic complications that encourage urinary stasis Yang *et.al,* (2017). Resultant opportunistic infections have been reported to be accounting for up to 60% of AIDS defining illness (Marimuthu & Narasingam 2018).

The discovery and understanding of HIV identified the need for control of opportunistic infections. Being broad spectrum covering varieties of bacterial, fungal and protozoan infections, cotrimoxazole was recommended for use in prophylaxis (WHO guidelines 2014). Since then, interceptive therapy has been a central care practice in preventive management of opportunistic infections in seropositive patients. However, being cheaper and easily available as "an over the counter" remedy, cotrimoxazole has been as well used unsparingly in management of common infections thus resulting into resistance development (Marwa *et al.,* 2015). Cotrimoxazole's interceptive relevance is heavily dependent on periodic evaluations of effectiveness, a practice that globally has none or very limited published information. As early as 2008 there arose a genuine concern of whether such preventive measures were more cost effective against (OI) than the alternative 'wait to effectively treat when cases occur' (Rodh*e et al.,* 2009). Most patients are received at CCCs with complaints related to opportunistic UTI despite increased availability and accessibility of prophylaxis drugs.

Previous researchers have even attributed massive antibiotic failures in the aids era to long time cotrimoxazole use in prophylaxis (Marwa *et al.,* 2015). Management of bacterial UTI continues to face challenges with the absence of reliable scientifically determined reference antibiograms on the local scene thus leaving chance and history as the main guiding principle for drug choice. This has commonly been identified as a leading cause for resistance being reported towards commonly used drugs particularly among Enterobacteriaceae family (Park *et al.,* 2017).This calls

for more involvement of laboratory procedures in urinary tract infection management an exercise that has been overlooked particularly in non-urban regions of most developing countries. Diagnostic procedures though a bit expensive guide treatment by determining of reliable drugs for appropriate antibiotics therapy failure of which eventually leads to resistance development. This is particularly true when offered empirical treatment is based on antibiograms developed elsewhere when it is clear that patterns of susceptibility are regional and time bound as Prasada *et al.,* (2019) was able to demonstrate in his five year study on resistance development.

Another treatment guiding principle that is linked to antimicrobial insusceptibility is assigning antibiotics in relation to disease severity related i.e. whether the case is routine, uncomplicated, or complicated. Joanna (2020), in a study on UTI treatment noted the use of nitrofurantoin (32%) and trimethoprim-sulphamethoxazole (26%) were globally preferred remedies for uncomplicated cases. However, when it came to routine and more complicated infections ciprofloxacin (35%) and levofloxacin (2%) were drugs of choice in developed countries was price of medicine was anon issue. But in most developing countries, clinicians are forced to opt for cheaper medications which may compromising treatment as well as negatively affect susceptibility. Disparities in antibiotic therapeutics are responsible for the differing regional susceptibility patterns that are of major concern in management of opportunistic infections that are behind increasing worldwide antibiotic failures in AIDS era. If urinary tract infections are not properly managed on the local scene, multi-drug resistant bacteria which tend to circulate more in seropositive patients (Kemajou *et al* 2016) are likely to cross over to the rest of the communities. But with good strategies in place, seropositive patients have the chance of having near normal lives, long enough to realize their life time goals.

1.2 Statement of the Problem

Urinary tract infections being globally among the most frequently encountered infectious diseases are important cause of morbidity among HIV seropositive patients accounting for up to 60% of AIDS defining illness. Cotrimoxazole which has been central in interceptive therapy against opportunistic infections as recommended by world health organisation has of late been showing resistance and its long time daily prophylaxis use combined with routine therapeutic application in routine management of common infections is being attributed to massive antibiotic failures witnessed in this aids era. Patients on the local scene are more these has left exposed to opportunistic bacterial urinary tract infections given that blind treatment usually based only on likelihood and history relying on antibiograms developed elsewhere is administered. With absence of antimicrobial stewardship and direct involvement of laboratory in management of UTI, the threat of multi drug resistant bacteria which are very expensive to treat is real. These group of dangerous bacteria that are responsible for high urinary tract infection morbidity and mortality circulate among seropositive patients from where they are likely to spread to the rest of the community.

1.3 Broad Objective

To investigate bacterial urinary tract infection prevalence and antibiotic susceptibility patterns of cotrimoxazole resistant bacterial pathogens in HIV seropositive patients attending Maseno mission hospital western Kenya.

1.3.1 Specific Objectives

The specific objectives were to;

i. Describe the prevalence of bacterial urinary tract infections from HIV immunosuppressed patients attending Maseno mission hospital.

ii. Determine bacterial UTI response to prophylactic cotrimoxazole among HIV patients attending Maseno mission hospital**.**

iii. Establish antibiotic susceptibility patterns for cotrimoxazole resistant uropathogens isolated from HIV patients attending Maseno mission hospital.

1.4 Study Questions

i. What is the prevalence of bacterial uropathogens amongst HIV seropositivity patients attending Maseno mission hospital?

ii. How did bacterial uropathogen isolates from HIV seropositive patients attending Maseno mission hospital respond to Co-trimoxazole prophylaxis?

iii. What are the susceptibility patterns of Cotrimoxazole resistant bacterial uropathogen isolates from HIV seropositive patients attending Maseno mission hospital to commonly used antibiotics?

1.5 Significance of the study

Study results provided useful information to researchers, medical practitioners and study population. An overall prevalence of 33% was established with *Escherichia coli* emerging as the most encountered bacterium with 54.7%. This expected to be a point of reference for future researchers. All UTI positive participants were treated in accordance with the ministry of health guidelines in line with Kenya government development goal of universal health care (UHC) of affordable health services aimed at achieving a healthier working citizenry as one of the pillars of big four agenda.

High cotrimoxazole resistance (89.7%) rate established was brought to policy makers to inform on better approaches to preventive management of opportunistic infections in line with Millennium Development Goals (MDG) of lowering communicable disease burden to minimal by the year 2030.

Despite the regional low susceptibility rate (46.6%) established, gentamicin, ciprofloxacin, and nitrofurantoin were established across most bacteria as reliable antimicrobials and formed part of most antibiograms established for future references. However, gentamicin (86.7%) being the most effective drug on average across most pathogens was recommended the empirical drug of choice for reference in the area. This is expected to lower treatment costs in terms of reduced laboratory testing as part of the government big four agenda of affordable healthcare under sustainable development goals (SDGs).

CHAPTER TWO
LITERATURE REVIEW

2.1 Urinary Tract Infections Prevalence

Urinary tract consists of the kidneys, ureters (tubes that carry urine from the kidneys to the bladder), bladder, and urethra (the tube that carries urine from the bladder out of the body). When microbes infect any of these structures, infestations typically present with signs and symptoms which include frequent urination with pain, feeling of urinating even when the bladder is empty, fever, passing of cloudy urine with or without blood and pressure or cramping in the lower abdomen. Urinary tract infections are the most common infectious diseases with global prevalence of at about 150million people per year (Talha *et al.,* 2022). Incidences vary geographically with cases being more common in developing countries. In sub Saharan Africa for instance Mwang'onde *et al.,* (2022) reported prevalence for various countries as follows, Ghana 15.9%, Nigeria 12.3%, Zambia (HIV pregnant women 16.5%, Ethiopia (pregnant women) 15.0%, Uganda (age 8 month to 95years) 32.2%, Tanzania (children 2-6 months 39.13% and Kenya 26.7% (Mwang'onde *et al*., 2022).

Urinary tract infections are of great medical significance as they are associated with easy circulation within communities (*Olowe et al.,* 2015), affecting quality of life and lowering productivity. They may be difficult to medically manage particularly when clinical diagnosis becomes problematic as witnessed in seropositive patients where they occur asymptomatically with systemic inflammations only (Ayoyi *et al* 2017). Despite these challenges, previous researchers on opportunistic infections in HIV concentrated on sexually transmitted diseases and malarial ignoring UTI as there is minimal published information about it.

Disease density is determined to a great extent by the immunity of the infected and this is why some groups of people are more predisposed than others. HIV seropositive patients, the elderly

because of slowing down immunity following long lives and infants who are yet to fully develop full defense system (Nelson & Good 2015) have been associated with high UTI prevalence. Factors that have been linked to high UTI prevalence include gender, (where females due to their genital anatomy and its proximity to the anus are more exposed) as demonstrated by Kanu *et al.,* (2016) who isolated 51% UTI cases from females compared to 17% from males. Lifestyle and behaviour have been cited as factors for prevalence where by more sexually active people are associated with higher UTI prevalence e.g. Odoki *et al.,* (2019) in a Ugandan study recorded highest UTI prevalence of 28/86 (32.6%) from the age group 20–29 linking it to sexual activities. UTI investigations have yielded to different types of organisms being isolated from urine samples worldwide (Behzadi, P. 2019). These include Fungi like (*Candida spp),* viruses such as adenoviruses & herpes simplex virus which may infect the urethra, making urination painful and emptying of the bladder difficult. Other culprits include parasites like (*schistosoma haematobium)* which cause schistosomiasis affecting kidneys, ureters, and bladder, *Trichomonas vaginalis* causing Trichomoniasis that is behind a copious greenish yellow, frothy discharge from the vagina in women, occasionally infecting the bladder or urethra and can as well infect the urethra in men though with no symptoms and Filariasis, which is caused by a threadworm infection, obstructing lymphatic vessels causing lymph fluid to enter urine (chyluria) and is also known to cause enormous swelling of tissues (elephantiasis), which, in men, may involve the scrotum.

Despite the large bionetwork of microorganisms being associated, are almost exclusively due to bacterial inflammations Mireles *et al.,* (2015), with gram-negative bacteria being encountered more (75. 6%) compared to gram-positive (24.4%) (Megan *et al.,* 2015). Bacterial infections mostly infect the lower urinary tract usually the bladder especially of young women. Young

women also often get bacterial kidney infections, but less commonly than bladder infections. Between ages of 20 and 50, bacterial are more common among women than men in whom infections are mostly urethritis or prostatitis. Amongst older people of 50 years and above, bacterial UTIs are equally common in both men and women (Talha H. 2022). *Escherichia coli* is many a times reported the most common bacterium isolated as captured by Lekha *et al.,* (2016*) at* (49.5%) and Maniga *et al.,* (2015) 23%.

2.1.1 Urinary tract infections in HIV infestation

HIV has been known to harm nephrons by infecting the kidneys thus making them work less efficiently. In addition, some medicines used in suppressing the virus may equally injure the nephron if not monitored carefully (National Kidney Foundation 2016) resulting into urologic abnormalities of renal syndromes that include bladder-areflexia and hypo-reflexia. These conditions usually disintegrate into neurologic complications that encourage urinary stasis a condition that greatly favour bacterial growth. This perhaps gives the best explanation behind high UTI prevalence in seropositive patients where they account up to 60% of AIDS defining illness (Marimuthu *et al.,* 2018). Even with revelations, UTI has never been a priority during clinic visits. Even when suspected, patients are blindly treated with laboratory testing due to lack of screening policies particularly in most rural areas in most developing countries.

Due to nonspecific presentations of UTI, diagnosis can be complicated as signs and symptoms of Clinical presentations resemble common ailments. Guiding principles diagnosis recommends evaluation of clinical signs & symptoms alongside Laboratory tests that should be directed at species identification. Turbidity of specimen and cloudiness with positive dipstick though suggestive, on their own cannot confirm an infection. Cultural confirmation by use of non-contaminated mid-stream urine aseptically captured directly into sterile specimen bottles while voiding is preferred (Sinawe *et al.,* 2022). Samples are considered positive if productivity of

$\geq 10^5$ colony forming units of a typical urinary tract causing organism per µl of mid-stream urine is obtained upon culture (Agata *et al.,* 2018). Culture method of pathogens identification is advantageous to other methods because it involves direct isolation of causative agents by way of characterization using appropriate biochemical tests in relation to gram stain reactions of the organisms under study. This is the surest way of confirming of suggestive abnormalities from urinalysis. However, even though high level (36.1%) abnormalities of altered urine constituents reported by Opiyo *et al.,* (2010) from urine samples of HIV seropositive patients at Jaramogi Odinga Oginga Teaching and Referral Hospital-JOOTRH (within the same region) were strongly suggestive of uropathogenesis, no local follow up urine culture study has been published to date.

2.2 Cotrimoxazole Prophylaxis in Urinary Tract Infections

Following the discovery of HIV and study based more understanding of the virus, World Health Organization (WHO) recommended preventive therapy for seropositive patients. Instantly, prophylaxis became a milestone and has remained central in management of opportunistic infections. Considering affordable low pricing, high tolerability and wide interventional range against bacteria, fungi and protozoa cotrimoxazole was picked as interceptive drug of choice. This was based on outcomes of a Zambian study that demonstrated major reductions in mortality, morbidity and in-patient cases upon use of cotrimoxazole prophylaxis (WHO guidelines of 2014)**.** Giving room for further studies in examining whether lower doses of 480 mg and below could still provide the same efficacy, prophylactic dosage was initially set at 960mg (800mg sulfamethoxazole + 160 mg trimethoprim). Even though earlier studies found no difference between the two regimens in respect to death, guideline development group recommended maintaining daily dose of 960mg. However, there are no publications showing that research work on possibility of using lower dosage regimen in prophylaxis have been conducted.

With increased accessibility to prophylaxis thanks to international donations and political willingness, the numbers of enrolled patients in ART/cotrimoxazole programmes have gone up. Kenya national aids control council report that adult patients aged >15 on the program stood at 627,900 in 2010 compared to 1,338,200 in 2017 (Kenya HIV estimates report 2018). However, the lack of patient monitoring strategies that would ensure proper medicine uptake while at home have led to cotrimoxazole misuse. Non-adherence continuous to greatly contribute to prophylaxis failure as a result of resistance development as was reported by Marwa *et al.,* (2015) who posted a 75% cotrimoxazole resistance rate from HIV patients. Surveillance studies designed to pick resistance as they emerge and inform concerned authorities on policy making that should be aimed at strengthening of the program of prophylaxis are locally lacking. None properly functioning preventive therapeutic program has directly opened up ways for UTI to continue flourishing in seropositive patients.

The need to monitor and evaluate cotrimoxazole response to common bacterial uropathogens is key in control of (OI). Single drug monitoring exercises have has been on-going throughout the history of antibiotics and several technics are available for use. These include commercially available modern and faster techniques (Baquer *et al.,* 2021) in the form of E-Test strips (AB BIODISK from bio Me´rieux). However, due to high pricing, broth dilution method which is one of the earliest drug performance testing techniques is preferred and is still commonly employed in establishing ideal minimum inhibitory drug levels that can hinder bacterial growth (Miftahussurur *et al.,* 2022).

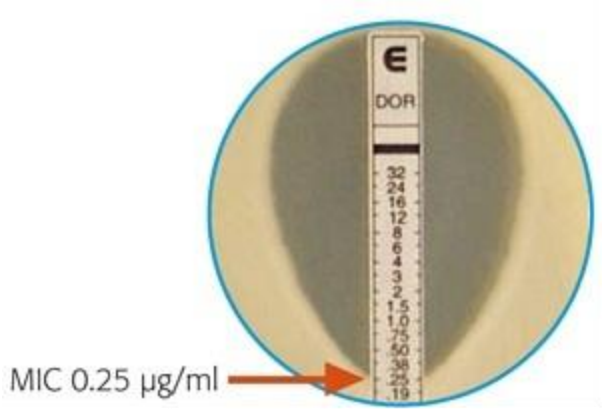


Figure 2.1: E-testing technique showing minimum inhibitory concentration at 0.25ug/ml.

2.3 Antimicrobial Susceptibility and Bacterial Resistance

The fact that previous studies on seropositive patients did not give UTI in HIV much attention is linked to now emerging strains of multi-drug resistant bacteria (Mireles *et al.,* 2015). This group of patients is therefore doubted to be an important source of unsusceptible microbes. Massive antibiotic failure witnessed AIDS era has partly been linked to circulation of counterfeit drugs in the form of 'over the counter remedies' that are readily available without prescription (Bryce *et al* 2016). Resultant misuse and/or under dosage encourage common pathogens to develop resistance to routine antibiotics and even newer drugs Dadi *et al., (*2019). The ever increasing prevalence of multi- drug resistant bacteria among HIV seropositive patients that are difficult to treat has become a global public health concern (Mireles *et al.,* 2015) more so in resource limited areas of most developing countries leading high morbidity and mortality rates arising from escalated cost of treatment (Paul 2018). Antimicrobials routinely employed in treatment of common ailments are affected most. These include Cotrimoxazole, cephalosporins, semi-synthetic penicillins and fosfomycin trometamol. However, all is not lost as some pathogens still respond well to most common routine drugs as reported by Lekha *et al.,* (2016) in whose study amikacin and nitrofurantoin were effective against most pathogens. This calls for antibiotic stewardship aimed at ensuring that correct medication for each isolated pathogen as scientifically

determined locally via laboratory procedures is appropriately administered. Alternatively, local development of periodic antibiograms provides long lasting solution for both geographical and time bound susceptibility effects (Truong *et al.,* 2021). This is a missing aspect in bacterial OI management locally.

Tendency of susceptibility patterns changing with time has always been used in determining the type of antimicrobials in management of cases heavily relying on history and chance. This strategy has been cited a factor in massive drug failures. For instance, Dat *et al*., (2022) from a seven day study of five Vietnamese provinces on antibiotic use demonstrated a 63.6% empirical based treatment where 1112 out of 1747 patients were initiated on treatment without undergoing susceptibility testing on suspected causative agents. Drug choices relied heavily on previous susceptibility trends. Given that sulphonamides had started showing resistance to common pathogenic isolates, most clinicians resorted to broad spectrum antibiotics for treatment of UTI. More investigations on effect on time on susceptibility by Prasada *et al.,* (2019) in a five-year period study (2013-2017) reported increased resistance for uropathogenic *E. coli* to cephalosporins from (51-58%), Cotrimoxazole (52-59%), Piperacillin tazobactam (9.4-23%), Carbapenems (0-5.9%). High rates of resistance towards Fluoroquinolones, Sulfamethoxazole, and Trimethoprim have been reported with previous studies attributing it to cheap pricing leading over exposure. Other drugs associated with frequency related resistance are ampicillin and nitrofurantoin both at 25% susceptibility for *P. mirabilis* (Mageto *et.* 2018).

Distinct susceptibility patterns have emerged for gram negative and positive bacteria. Generally gram positive bacteria in most cases respond more to antimicrobial agents than their counterparts Alhumai *et al.,* (2021). In this Saudi Arabian multi-hospital healthcare system in study, an overall susceptibility rate of 52.6% was reported for gram-positive bacteria compared to (49.5%)

for gram negatives. Researchers have attributed this to structural difference between the two groups of bacteria. Borrowing from Maniga et al., (2015) who recommended gentamicin drug of choice in management of UTI at Gucha sub-county following its high susceptibility, antibiotic application in this era of ever changing patterns of susceptibility, should include evidence based strategies particularly in regions where laboratory testing may not be readily available. This has proved reliable as dependence on local empirical originations reduces cases of resistant bacteria.

CHAPTER THREE
MATERIALS AND METHODS

3.1 Area study

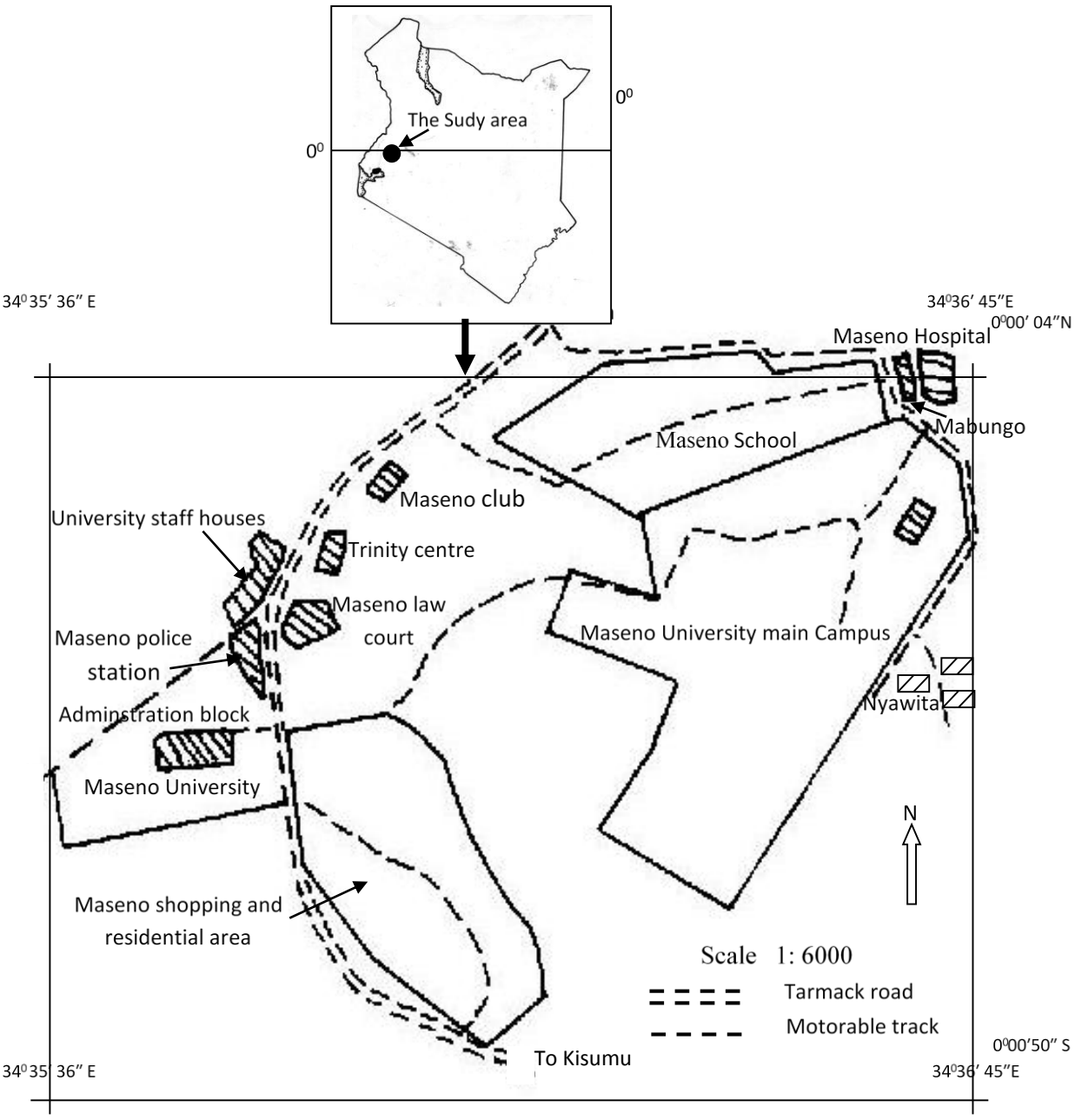


Figure 3.1: Map of Maseno (The Study Area)

The study was conducted at Maseno ACK Mission Hospital at the boundary of Vihiga and Kisumu Counties in the Western Kenya, East Africa (Kenyan census report on Urban Poverty

Estimates for Kenya's Provinces, Districts Divisions and Locations of 2019). The facility is administratively placed Kisumu County and houses a comprehensive care centre (CCC) which clinically carters for HIV patients from surrounding regions.

Geographical co-ordinates for Maseno are 0° &10' 0" South and 3 4°& 36' 0" East (Figure 3.1: map of study area). Maseno covers an area of 580,367 km^2 and is located along Kisumu-Busia highway just 20kilometers north-west of Kisumu town the regional capital of Nyanza region. A 15kilometer Eastward running road connects the town to Vihiga town. Kombewa sub-urban centre is located 10 kilometers west of town. The altitude of the region is about 1,503 meters (4,934 feet) above sea level. The town serves as the headquarters of Kisumu west district which is part of Kisumu Rural Constituency and the larger Kisumu county council (Kenya government census 2019).

Prevalence for HIV in Kisumu County stands at 19.3% which is within the same range as bordering county of Siaya 23.7% (National AIDS control council Report on HIV situation in Kenya 2014). Due to its proximity to Lake Victoria, Maseno experiences generally high temperatures all the year round a condition favorable for bacterial disease causing germs for urinary tract infections. Generally, Maseno has a cosmopolitan population thanks to the many international, national and local institutions found in the region. These include Maseno University, Maseno School, Maseno polytechnic, several primary schools, Agricultural development research centre (ADC), Kenya Forestry Research Institute Kenya (KEFRI), and International Centre for Research in Agro-Forestry -ICRAF). Presence of these establishments has brought in people from all over Kenya as well as internationals as from all over.

3.2 Population of study

The population of study comprised of registered (2000) HIV seropositive patients at Maseno mission hospital's Comprehensive Care Clinic CCC (as per the facility's official records).

3.2.1 Inclusion Criteria

Consenting HIV seropositive patients attending Maseno mission hospital-with or without signs and symptoms of urinary tract infection)

3.2.2 Exclusion Criteria

HIV seropositive patients with underlying immunosuppressive diseases like cancer.

Those on immunosuppressive medication e.g. cancer treatment.

Patients whose initial urinalysis stage results were found to be harboring non-bacterial urinary tract co-infections were excluded from the study due to their confounding effects.

3.3 Study design

It was a hospital based descriptive study of HIV seropositive patients attending comprehensive care clinic at Maseno Mission Hospital who were screened for bacterial urinary tract infections.

3.4 Determination of Sample Size

Basic model formula by Fisher *et al* (1999) as adopted and modified by (Mugenda and Mugenda *2003)* was used to determine the sample size

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

Where,

n = Target population size which is greater than 10,000;

($z = 1.96$) = Value of standard normal distribution curve corresponding to 95% level of significance.

p = 0.5, Adopted prevalence of uropathogens amongst HIV sero positive patients attending Maseno Mission hospital. (This was obtained from (Mugenda and Mugenda 2003) who guided on the use of p value of 50% (0.5) where there is no data available showing percentage of participants with characteristic being investigated.

d = Margin of error which is 5% (0.05)

Therefore, at 95% confidence interval, assuming targeted population was more than 10,000, the desired sample size was worked out as follows.

$$n = \frac{(1.96)^2(0.5)(0.5)}{(0.05)^2}$$
$$= 384$$

But because the population of interest was less than 10,000, finite formula of adjustment was employed as shown below.

Finite adjustment formula $$n_f = \frac{n}{1+\frac{n}{N}}$$

Where:

n_f = The required sample size given that characteristic population is below 10,000;

n = Sample size if target population was more than 10,000 (384); and

N = approximated characteristic population size in the current study (2000)

Hence the sample size in this study was adjusted as shown below,

$$n_f = \frac{384}{1+\frac{384}{2000}} = 322$$

The calculated figure of 322 participants was adjusted upwards by 10% (32) for the purpose of non-responders thus fixing the working sample size was therefore at 354 participants.

3.5 Sampling Techniques

Systematic sampling technique was employed. Based on hospital treatment schedules that all 2000 enrolled patients were to visit the clinic at least once in two months (which translated into an average of 33 per day (2000patients/60 days) , to obtain the calculated working sample, the picking interval was determined by number of sampling days available (60). By dividing the sample size (354) by sampling days (60) the interval determined at 6 i.e. 354/60 = 5.9 rounded off to 6. Sampling starting point was randomly determined by writing on pieces of paper numbers 1 to 6 and folded (6 the sampling interval). While blind folded, one piece of paper was picked. Number (2) which was randomly chosen became the starting point i.e. the 2^{nd} patient that visited the clinic on day one of sampling became the first participant recruited. Thereafter others were systematically selected at an interval of six i.e. (every 6^{th}) starting from patient number two was included into the study i.e. 2^{nd} , 8^{th}, 14^{th},20^{th}…etc. Therefore at an interval of 6 picking 6 participants out of the 33 visiting per day, 59 days during the months of September to November 2020 were used to sample 354 participants required i.e. (59 X 6 =354).

3.6 Training of Research Assistants

Study assistants who were the main link between participants and the laboratory staff were selected from medical staff of the clinic. Importance of patient confidentiality and privacy during and after the study was emphasized. The assistants were trained on procedures of sterile urine collection and handling before delivery to the laboratory**.** Latex and plastic gloves were provided for individual protective handling of the specimen.

3.7 Reliability and Validity

To ensure that collected data would yield information that accurately answered to the specific research questions, the questionnaire's reliability and validity were determined before the study as follows.

3.7.1 Reliability Testing

Test-retest method was carried out by administering copies of the structured study questionnaire to a group of seropositive respondents from a different facility (Siriba dispensary). While keeping the initial conditions constant, the same group of respondents was retested by use of the same questionnaire after ten days with the aim of informing on how consistently the tool would collect data that upon analysis would answer to the specific objectives of the study. The scores from the two tests were correlated for co-efficient of reliability/stability based on Cronbach's alpha index (0.7).

3.7.2 Validity

Construct validity test was performed by a professional statistician who first established purpose of the test before performing a job-test analysis, creating an item pool, reviewing the exam items and finally conducting item analysis of the study questionnaire. An outcome of 90 N (80-100) obtained from seropositive patients from the neighboring Siriba dispensary indicated that the tool designed for the study would be able to measure the concepts it was intended to.

3.8 Ethical Considerations

Approvals for the study were obtained from the Board of Graduate Studies (SGS) (Appendix I-SGS Letter of Approval) and Ethical Review Committee- MUERC (Appendix II-Ethical approval) both of Maseno University. Additional authority was granted by Maseno Mission Hospital (Appendix III: Letter for Permission). All participants gave informed written consent (Appendix IV: Consent Form) before being enrolled. Minors gave verbal assent which was

confirmed by parents/guardians with written consent allowing their participation. For infants written consent was directly sought from parents before being enrolled.

Prior to commencement of the study, all participants were taken through the importance of strict adherence to ARV dosage regimens alongside prophylaxis programs in relation to infectious disease control. The relationship between seropositivity and uropathogenesis was explained to participants as were the importance of the study objectives of surveying the burden of uropathogenesis, investigating resistance to prophylaxis and establishment of susceptibility patterns of uropathogens isolates.

The participants were assured of confidentiality of volunteered information including non-exposure of generated data to unauthorized persons. In achieving this, codes were known only to laboratory staff were improvised. Generated information was kept under lock and key accessible only to the investigator just as were urine samples. The nature of non-invasive procedures used in urine sample collections was explained to participants. Assurance of absence of pain and discomfort was given. The lack of incurring any risk for participating was made known to the participants in fact their right to voluntary participation and freedom of withdrawal without penalization was explained. Generated information was synthesized and used only for the purpose of compiling this thesis.

3.9 Data Collection Process

Bio and laboratory generated data were captured by use of appropriate techniques as follows.

3.9.1 Collection of Bio Data

Bio data of consenting participants was captured through interviews by way of administration of structured questionnaires (Appendix V:Questionnaire-filled by consenting patients. Questions of

which covered socio-demographics and associated variables (age, gender, education level and marital status), HIV diagnosis, history of ARV use and cotrimoxazole prophylaxis.

3.9.2 Laboratory Data Collection

Bacterial strains of isolated uropathogens, their response to prophylactic cotrimoxazole and susceptibility to commonly used drugs were captured on specially designed forms (Appendix VI: laboratory data capture form).

3.10 Collection of Sterile Urine Samples

From each of the participants 5-10mls of fresh midstream urine was collected. The participants were asked to discard part of the stream then directly capture mid-stream sample into a labeled autoclavable sterile universal bottle (from Euromed. Equip. ltd. U.K.) before redirecting the last portion into the toilet. The bottles were tightly capped and samples delivered to Maseno University School of Medicine Microbiology Laboratory without delay (probably within an hour of voiding). Where delay was inevitable, samples were refrigerated till when processing was possible.

3.11 Laboratory Procedures

All samples were processed in accordance with guidelines of clinical laboratories standards institute as follows.

3.11.1 Urinalysis and Microscopy

Potential co-infection confounders were controlled for via urinalysis. Each of the samples was aseptically divided into two portions with the first part being macroscopically examined for parasitic infections including *Trichomonas vaginalis, Candida albicans* and *Schistosoma haematobium.* A commercially available urine stripe (Bio Me´rieux from Spain) per sample was inserted into urine samples and macroscopically examined for biochemical indicators of UTI (presence of red blood cell/haemoglobin, proteins, and pus cells). From each sample 5ml was

transferred into corresponding sterile labeled autoclavable centrifuge tube and span for three minutes at the speed of 3000r/m . Resultant supernatant was disposed and the deposits scanned microscopically for characteristic diagnostic parasitic stages i.e. *Schistosoma haematobium* eggs, *Trichomonas vaginalis* trophozoites and fungal yeast cells of *Candida albicans* using X10 magnification and confirmation with X40 objective. Patients who turned positive for any non-bacterial co-infections were, replaced from the study and contacted for treatment.

3.11.2 Urine Culture

Using calibrated sterile standard wire loops (0.002ml) (GmbH &Co.KG- Germany), urine from the second portion for each sample was inoculated on to Cysteine Lactose Electrolyte Deficiency culture medium (CLED-Oxoid LTD, UK) by streaking. After incubation at 37°C for 24 hours, numbers of bacterial colonies were enumerated using a colony counter (Astor20 colony counter India). Degree of bacteria per ml of urine was determined on all viable samples by multiplying number of colonies obtained by 1/0.002 the dilution factor. Samples obtaining $\geq 10^5$ Cfu/ml were gram stained before being biochemically processed for pathogen identification in accordance with Clinical laboratory Standards Institute (CLSI 2015 guidelines). Gram positive bacteria were biochemically tested for catalase, coagulase, urease, Methyl Red (MR), oxidase, indole, motility, Voges-Proskaur (VP) while gram negative bacteria were subjected to Catalase, Oxidase, Indole, Motility, Triple Sugars Iron Agar (TSI Agar) and Urease. Bacterial were identified based on their biochemical characteristics as recorded in tables (3.3 & 3.4)

3.11.3 Broth Dilution Technique - Determination of Bacterial Response to Cotrimoxazole

Isolated, characterized and identified uropathogens were subjected to a cotrimoxazole two-fold dilution technique for sensitivity testing as follows. Nine sterile capped test tubes were arranged in a rack and labelled (C) concentrated & 1 to 8 in that order. All tubes except (C) were aseptically filled with 1.0ml of Mueller-Hinton broth (Oxoid LTD, UK). Two tablets containing

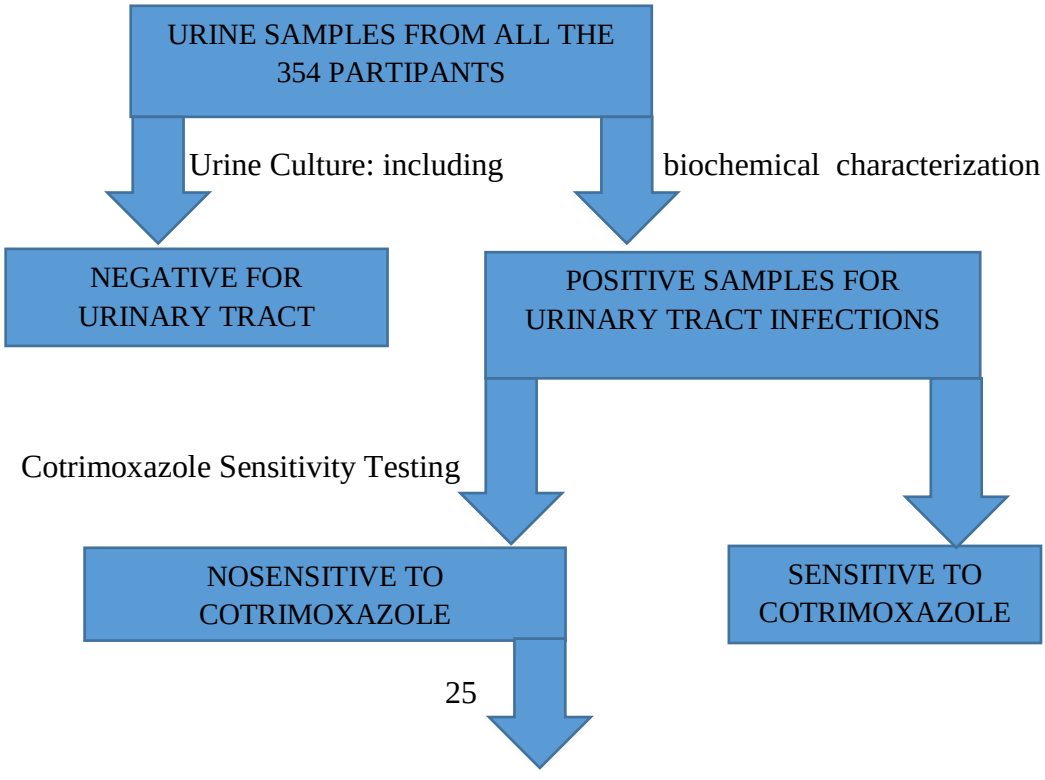
800mg sulfamethoxazole and 160mg trimethoprim (Forte 960mg) from (Aspen Bad Oldesloe GmbHA-Germany) were aseptically grounded in a mortar. Concentrated stalk solution of (960mg/1ml) was prepared by dissolving the entire powder in 2ml of sterile distilled water in tube (C) and well mixed. 1ml was transferred to tube (1) and again well mixed. Starting from tube (2), 1ml was similarly transferred to the next tube with pipette changing between tubes down to tube (7) from which 1ml was discarded leaving tube (8) with only Muller- Hinton broth. To tube (1), a loop full (0.02ml) of distilled water was added while to tubes (C) and 2 to 8, a loop full of test organism colony suspended in 1ml of normal saline and adjusted to 0.5 McFarland turbidity was added. All the tubes were incubated at 35^{o}C for 18hrs and examined for visible signs of bacterial growth as determined by turbidity against tubes (1) negative control (as it contained distilled water instead of the test organism) and tube (8) positive control as it did not contain any cotrimoxazole thus allowing growth unhindered. The highest dilution showing no growth was considered minimal inhibitory concentration (MIC). For interpretation of resistance, MICs for each isolated organism were compared to standard control organisms *Escherichia coli* (ATCC 25922) for gram negatives and *Staphylococcus aureus* (ATCC 25923) for gram positive bacteria in accordance with Clinical and Laboratory Standards Institute-CLSI document M23 (Baquer *at el.,* 2021).

3.11.4 Susceptibility Testing

Cotrimoxazole resistant uropathogens were subjected to antimicrobial susceptibility testing by way of disk diffusion technique in accordance with CLSI Subcommittee on Antimicrobial Susceptibility Testing (2018). Standard inoculum adjusted to 0.5 McFarland was swabbed onto Muller-Hinton agar and allowed acclimatization of 5 minutes at 37^{0}C. for each inoculated plate, a compound sensitivity testing disc specific for urinary tract isolates (KGL ¼) comprising of Ampicillin (AMP10 µg), Trimethoprim-sulfamethoxazole (SX23.75µg), Nalidixic acid

(NA30µg), Streptomycin (S50µg), Ceftriaxone (CTR30µg), Nitrofurantoin (NIT300µg), Tetracycline (TET30µg) from HiMedia Laboratories Pvt. Ltd-India was aseptically dispensed. Other single discs for commonly used antibiotics according to hospital treatment and medicine dispensation records were added. These included gentamicin (GEN10µg), ciprofloxacin (CIP5µg), ofloxacin (OFX10µg), vancomycin (VAN30µg), cefuroxime (CXM20µg), Ceftazidime (CAZ30µg), cefepime (CPM30µg), and Doxyccline (DX30µg) were added and incubated at 37^{o} C for 18 hours. Zones of growth inhibition for each drug across all isolated bacteria were measured in millimeters by use of graduated ruler. These were used to determine susceptibility for each antibiotic with reference to standard sensitivity tables of *Escherichia coli* (coded ATCC 25922) for gram negatives and *Staphylococcus aureus* (coded ATCC 25923) for gram positives. Susceptibility average rates for all drugs were obtained (as shown in Table 3.5) and used in establishment of empirical antibiograms for cotrimoxazole resistant isolates as determined by their patterns of susceptibility (Figure 4.3).

3.11.5 Summary of methodology



Antibiotic Susceptibility Testing

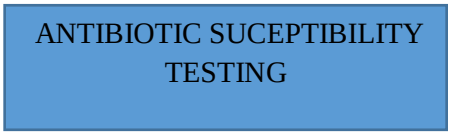


Figure 3.2:Flow chart for Urine culture processing. (UTI isolation and characterization, response to prophylaxis and antibiotic susceptibility testing).

3.12 Data Analysis

Descriptive statistics were used to analyze prevalence of bacterial uropathogens and patterns of antibiotic susceptibility while Chi-square evaluated at evaluated at 95 % confidence level was employed in determining of proportions of cotrimoxazole responding isolates per bacterial strain.

3.12.1 Prevalence of bacterial UTI

Table 3.1: Bacterial UTI Prevalence of Maseno Mission hospital HIV Seropositive Patients

No.	Bacterial Agent	Positive cases (P)	Percentage %
1	*Escherichia coli*	64 (54.7%)	54.7
2	*Klebsiella spp.*	21 (17.9%)	17.9
3	*Staphylococcus aureus*	14 (12.0%)	12.0
4	*Proteus mirabilis*	11 (09.4%)	09.4
5	*Staphylococcus epidermis*	04 (03.4%)	03.4
6	*Pseudomonas aureginosa*	03 (02.6%)	02.6
	Total =(N)	**Total (Ps)= TP (117)**	**100**

Percentage distribution for UTI causing bacteria amongst the (117) total positive cases from the Sample size of (354) X 100 = overall Prevalence of (33.05%)

Table 3.2: Age Based Bacterial UTI Prevalence of HIV Seropositive Patients.

No.	Age set	No. sampled (S) per age group	No. Positive (P) Per age Group	Overall positivity contribution. (P/117X 100)
1	< 17yr	77	20 (26.0%)	(17.1%)
2	18 – 40yr	184	67 (36.4%)	(57.3%)
3	41 – 60yr	86	27 (31.4%)	(23.0 %)
4	Over 60yr	07	03 (42.9%)	(02.6%)
		Total = 354	**P/S X 100**	**TOTAL = 100%**

Percentage UTI distribution amongst HIV seropositive patients according to age brackets

Table 3.3: Biochemical tests for Gram negative bacteria

No.	Bacteria	Catalase	Oxidase	Urease	Indole	Motility	TSI			
							Gas	Butt	Slant	H_2S
1	*E. coli* (64)	+	_	_	+	+	+	**Acid**	**Acid**	_
2	*K. pneumoniae* (21)	+	_	_	_	_	+	**Acid**	**Acid**	_
3	*P. aeruginosa* (3)	+	+	_	_	+	_	**(K)**	**(K)**	_
4	*P. mirabilis* (11)	+		+		+	+	**(K)**	**Acid**	+

Biochemical characterization results of gram negative bacteria.
KEY: K = Alkaline: (+) = Positive: (-) = Negative

Table 3.4: Biochemical tests for Gram positive bacteria

No	Bacteria	Catalase	Coagulase	Urease	M/Red	VP	Oxidase	Motility	Indole
1	***S. aureus* (14)**	+	+	+	+	-	-	-	-
2	***S. epidermidis* (4)**	+	-	+	-	+	-	-	-

Biochemical characterization results for gram positive bacteria.
KEY: (+) = Positive: (-) = Negative:

3.12.2 Cotrimoxazole response

Table 3.5: Bacterial Responses to Cotrimoxazole Prophylaxis

No	**Bacterial Isolates**	Number Isolated		Responsive (R)		**Non Responsive (NR)**			
		NO.	%	NO.	**%= R/12x100**	NO.	%		
							NR/117x 100	**NR/105 100**	**x**
1	***S. aureus***	**14**	**12**	**4**	**3.4**	**10**	**8.5**	9.52	
2	***P. mirabilis***	**11**	**9.4**	**4**	**3.4**	**7**	**6.0**	6.66	
3	***E. coli***	**64**	**54.7**	**2**	**1.7**	**62**	**53.0**	59.05	
4	***K.pneumoniae***	**21**	**17.9**	**1**	**0.6**	**20**	**17.0**	19.05	
5	***S. epidermis***	**4**	**3.4**	**1**	**0.6**	**3**	**2.6**	2.86	
6	***P. aeruginosa***	**3**	**2.6**	**0**	**0.0**	**3**	**2.6**	2.86	
	Total	117	100	12	**10.3**	1O5	**89.7**	**100**	

Prevalence of overall cotrimoxazole responsive (10.3%) and non-responsive (89.7%) UTI bacteria from HIV seropositive patients KEY: R = Responsive: NR = Non-Responsive:

Table 3.6: Intra species responses to prophylactic Cotrimoxazole.

No	Bacterial isolates	UTI Positive		Response to CTX			
				Sensitive (S)		Non sensitive (NS)	
		No	(%)	No	(%)	No	(%)
1	*Staphylococcus aureus*	14	12.0	4	33.3	10	9.5
2	*Proteaus mirabilis*	11	9.4	4	33.3	7	6.7
3	*Escherichia coli*	64	54.7	2	16.7	62	59.0
4	*Klebsiella pneumoniae*	21	17.9	1	8.33	20	19.0
5	*Staphylococcus epidermis*	4	3.4	1	8.33	3	2.9
6	*Pseudomonas aeruginosa*	3	2.6	0	0.0	3	2.9
	Total	**117**	**100**	**S= 12**	**100**	**NS= 105**	**100**

Both responsiveness and non-responsiveness of Individual UTI bacterial species prevalence to cotrimoxazole prophylaxis

3.12.3 Drug susceptibility Patterns

Table 3.7: Antibiotic Susceptibility Across isolated uropathogens.

NO.	Drugs	SUSCEPTIBILITY RATING												
		***E.coli* (60)**		***K. pneumoniae* (20)**		***S. aureus* (10)**		***P. mirabilis* (7)**		***S. epidermidis* (3)**		***P. aeruginosa* (3)**		**Mean**
		S.NO	**%**	**S**	**%**	**S**	**%**	**S**	**%**	**S**	**%**	**S**	**%**	**%**
1	GEN	51	**82**	18	**90**	9	**90**	6	**86**	2	**67**	2	**67**	**80.3**
2	CIP	55	**89**	18	**90**	8	**80**	6	**86**	2	**67**	2	**67**	**79.8**
3	S	55	**89**	17	**85**	8	**80**	6	**86**	2	**67**	2	**67**	**79.0**
4	NIT	51	**82**	13	**65**	8	**80**	6	**86**	2	**67**	2	**67**	**74.8**
5	TET	50	**81**	15	**75**	7	**70**	6	**86**	2	**67**	2	**67**	**74.3**
6	CXM	36	**58**	13	**65**	6	**60**	4	**57**	1	**33**	1	**33**	**51.0**
7	NA	35	**56**	8	**40**	5	**50**	4	**57**	2	**67**	1	**33**	**50.5**
8	CTR	25	**40**	10	**50**	6	**60**	3	**42**	1	**33**	1	**33**	**43.0**
9	DX	25	**40**	3	**15**	6	**60**	4	**57**	1	**33**	1	**33**	**39.7**
10	VAN	30	**48**	6	**30**	3	**30**	3	**42**	1	**33**	1	**33**	**36.0**

11	OFX	17	**27**	6	**30**	3	**30**	3	**42**	1	**33**	1	**33**	**32.5**
12	AMP	16	**26**	5	**25**	3	**30**	3	**42**	1	**33**	1	**33**	**31.5**
13	SX	6	**10**	3	**15**	1	**10**	1	**14**	1	**33**	0	**0**	**13.7**
14	CAZ	5	**8**	3	**15**	1	**10**		**10**	1	**33**	0	**0**	**12.7**
15	CPM	1	**2**	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**	**0.3**
Mean			**49.2**		**46.0**		**49.3**		**52.9**		**44.4**		**37.7**	**46.6**

Mean susceptibility rates across all tested antimicrobials for isolated bacterial UTI pathogens.
(GEN10µg) =Gentamicin, (CIP5µg) =Ciprofloxacin, (S50µg) =Streptomycin, (NIT300µg) =Nitrofurantoin, (TET30µg) = Tetracycline, (CXM20µg) = Cefuroxime, (NA30µg) = Nalidixic Acid, (CTR30µg) =Ceftriaxone, (DX30µg) =Doxycycline, (VAN30µg) =Vancomycin, (OFX10 µg) =Ofloxacin, (AMP10 µg) =Ampicillin, (SX23.7µg) =Trimethoprim-sulfamethoxazole, (CAZ30µg) =Ceftazidime, (CPM30µg) =Cefepime.

CHAPTER FOUR

RESEARCH FINDINGS AND OBSERVATIONS

4.1 Participants' Demographic Characteristics

Table 4.1: Demographic Characteristics of Study participants

STATUS		**Female**	**Male**	**Total**
Marital	Single	79	72	151 (42.7%)
	Married	101	58	159 (44.9%)
	Widowed	17	14	31 (8.8%)
	Divorced	09	4	13 (3.7%)
Total		**206**	**148**	**354 (100%)**
Age	≤17yrs.	42	35	77 (21.8%)
	18-40yrs.	102	82	184 (51.9%)
	41-60yrs.	57	29	86 (24.3%)
	> 60yrs.	5	2	07 (2.0%)
Total		**206**	**148**	**354 (100%)**
Level of Education	$< 1^0$	83	56	139 (39.3%)
	2^o	84	51	135 (38.1%)
	≥ Collage	39	41	80 (22.6%)
Total		**206**	**148**	**354 (100%)**
When diagnosed	≤ 1 yr.	102	77	179 (50.6%)
	2-5 yrs.	96	68	164 (46.3%)
	6- 10yrs.	05	02	07 (2.0%)
	>10yrs.	03	01	04 (1.1%)
Total		**206**	**148**	**354 (100%)**

Demographic characteristics indicating age, marital status, level of education for both male and female participants

4.2 Urinary tract infection prevalence in HIV seropositive participants at Maseno mission Hospital

Upon performance of urinalysis, five participants were found to have been infected with Candidiasis and one with Trichomoniasis. The six were treated and replaced from the study.

Out of 354 participants investigated, 117 (33.1%) were positive for bacterial urinary tract infections with various organisms being encountered as captured in figure 4.1 below.

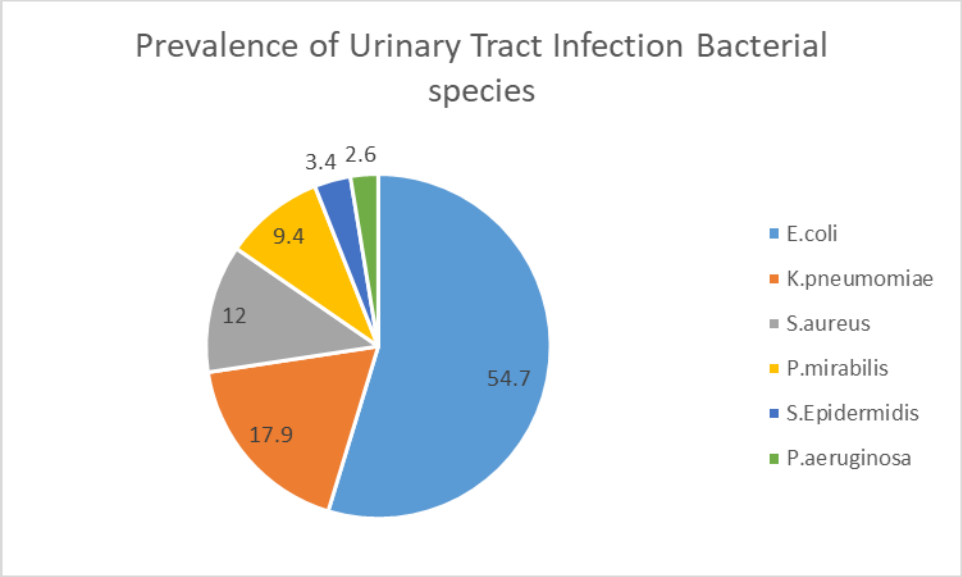


Figure 4.1: Individual bacterial species contribution towards the overall UTI Prevalence of 33.1% (117/354) amongst HIV Seropositive Patients from Maseno mission Hospital

4.2.1 Prevalence according to age groups

Most of the infections were realized in age group 18- 40yrs 67 (57.3%) while those over 60yrs had only 3 positive cases (2.6%) as shown in the table 4.3. Comparing the number of positive cases in relation to the actual number of participants sampled per age group, the elderly patients are more at risk than the rest as shown in figure 4.2 below.

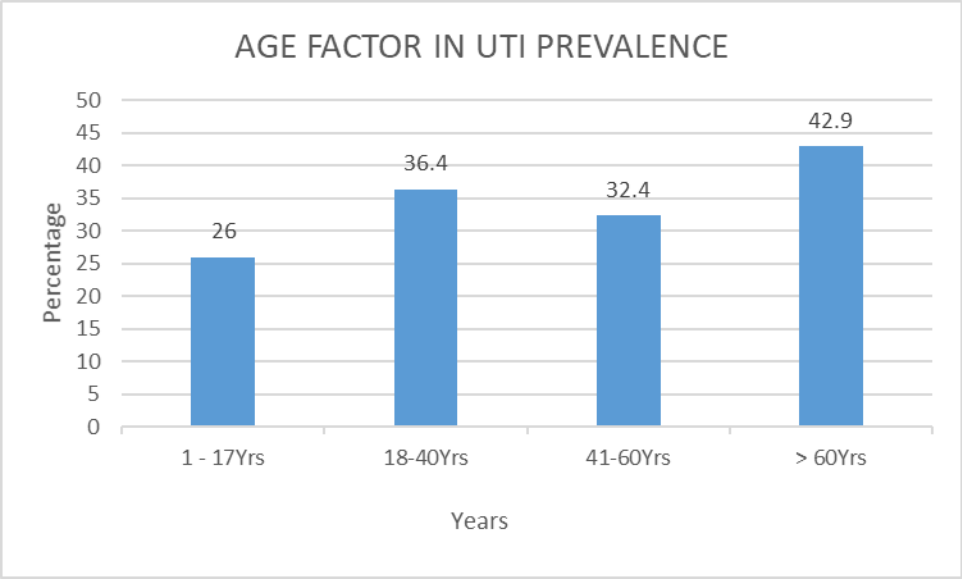


Figure 4.2: Graph of UTI prevalence as expressed in percentages of number sampled per age group

4.2.2 Gender and prevalence

According to gender distribution, most of the isolated cases were from women participants who were also the majority of the patients attending the comprehensive care clinic. Out of the 117 positive samples females contributed 87(74.4%) and males 30(25.6%). At least 206 females were sampled giving a positivity rate of 42% compared to males' total sample size of 148 yielding a positivity of 20.3%.

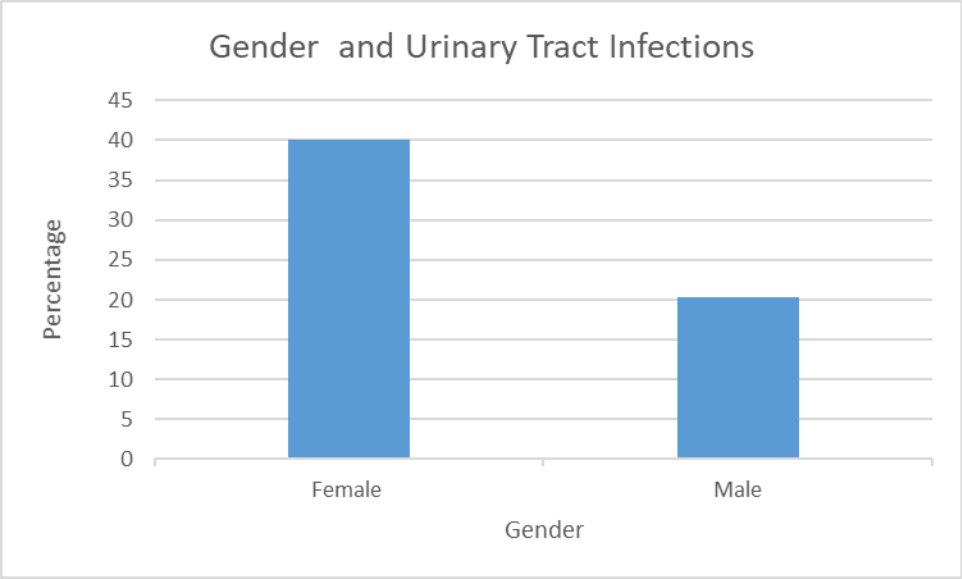


Figure 4.3: Bar graph showing prevalence of females against males in relation to positive cases against number sampled per group

4.3 Bacterial Response to Cotrimoxazole

Of all the 117 UTI positive samples that were examined for cotrimoxazole sensitivity, only 12 (10.3%) samples responded positively. There were however varied responses based on different bacterial strains isolated varying between E.coli (59.0%) and *Pseudomonas aureginosa (2.9%)*. Chi square analysis of bacterial responses to cotrimoxazole evaluated at 95 % confidence level was statistically significant at $P< 0.05$ $(x)^2 (5) = 14.3$, $p< 0.005$

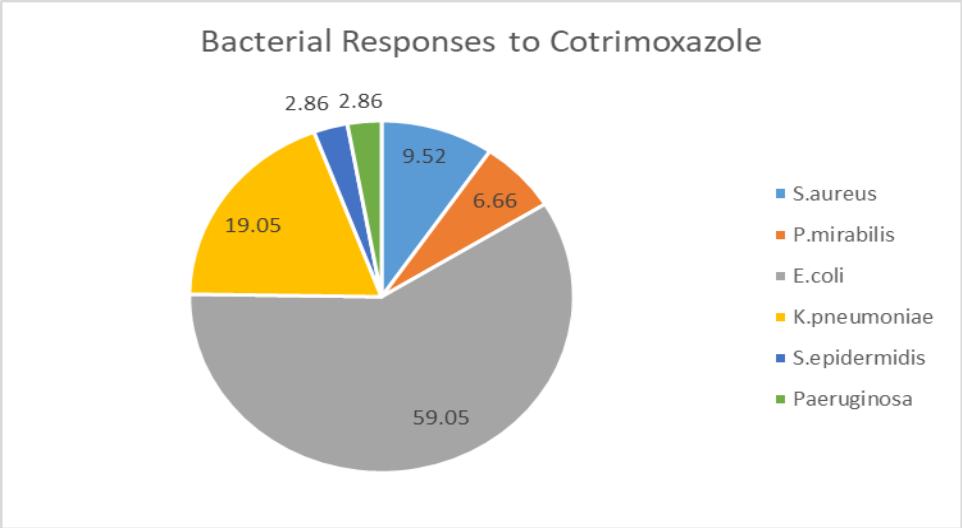


Figure 4.4: Pie chart representation of individual bacterial pathogenic species contribution to overall 89.7% (105/117) cotrimoxazole non-response

Table 4.2: Overall Response to Prophylactic Cotrimoxazole

No. Responding to Cotrimoxazole.	Conc.	Cotrimoxazole performance per dilution level 1:1	1:2	1:4	1:8	1:16	1:32	1:64
105	**R**	R	R	R	R	R	R	R
4	S	**S**	R	R	R	R	R	R
3	S	S	**S**	R	R	R	R	R
2	S	S	S	**S**	R	R	R	R
1	S	S	S	S	**S**	R	R	R
1	S	S	S	S	S	**S**	R	R
1	S	S	S	S	S	S	**S**	R
0	S	S	S	S	S	S	S	**S**
Total = 117			**(S)Sensitive = 12**			**(R) Resistant =105**		

Distribution of prophylactic resistant and sensitive bacteria at various cotrimoxazole dilution levels

4.4 Antibiotic Susceptibility Patterns

Several patterns of antibiotic susceptibility were obtained from the 105 (89.7%) non cotrimoxazole responding cases subjected to antibiotic testing in attempt to find remedy as captured in (Figure 4.5: Drugs Susceptibility patterns). On average, the most effective drug was gentamicin (80.3%) cepime being least active. Preferred antibiograms for isolated bacteria were CIP & S (89%) for *E.coli*, GEN & CIP (90%) for *Klebsiella pneumoniae*, GEN (90%) for *S.*

aureus, GEN,CIP,S,NIT&TET (86%) for *Proteus mirabilis*, GEN,CIP,S,NIT,TET,NA (67%) for *S. epidermidis* and finally GEN,CIP,NIT&TET (67%) for *P. aeruginosa*.

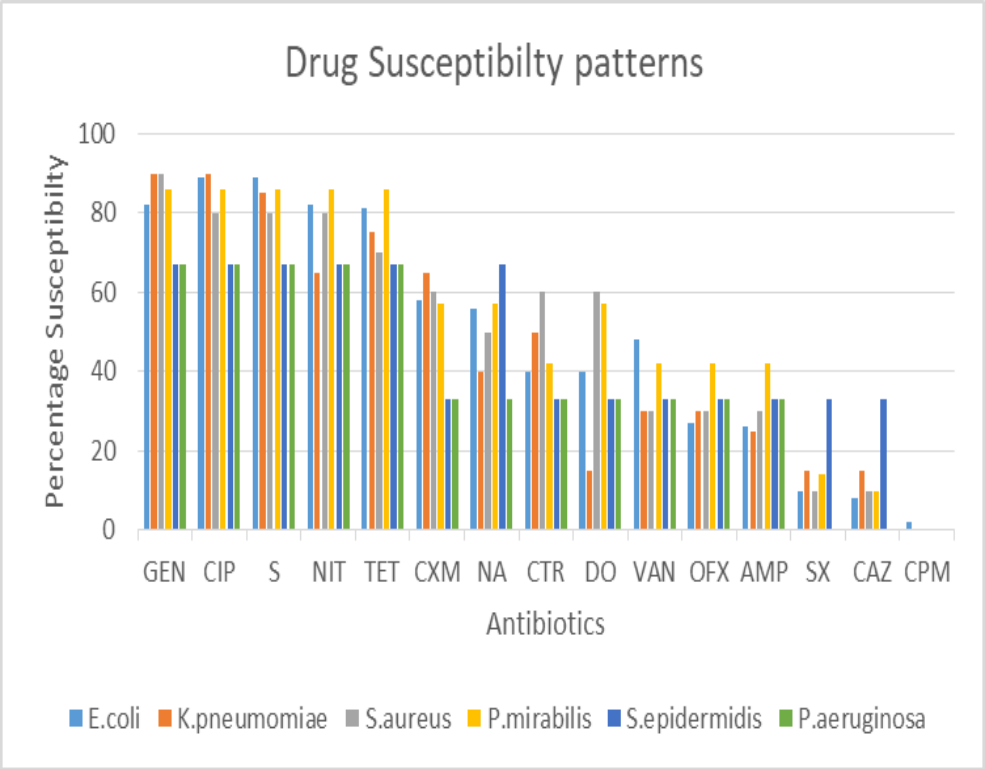


Figure 4.5: Graph of antibiotic susceptibility pattern showing variations on average for different isolated bacterial uropathogen ranging from *P. mirabilis* (52.9%), *S. aureus* (49.3%), *E. coli* (49.2%), *S. epidermidis* (44.4%) *K. pneumoniae* (46%), *P. aeruginosa* (37.7%)

CHAPTER FIVE
DISCUSSION

5.1 Demographic characteristics of study participants

Study participants who were selected from sero positive patients attending comprehensive care clinic cut across varied demographic characteristics (table 4.1). The fact that participants were across all age groups is proof that HIV is a non-selective virus that can infect any one exposing them to numerous opportunistic infections including urinary tract manifestations.

Several reasons can be attributed to the fact that more women were captured in the study compared to men. In line with the suburban nature of the region, it is positive that most men have moved to bigger cities and towns in search of employment as is the norm in developing countries leaving women and children behind. In addition, women are generally known to be more caring about their health and are likely to take relevant medical actions compared to men who only present at healthcare facilities when they can't avoid it any more.

The fact that the number of patients captured in the study tended to degrease with increasing educational levels may indicate that knowledge plays a big role in terms of creating awareness of risky behaviour like casual unprotected sex that can lead to contraction of the virus.

5.2 Prevalence of Urinary Tract Infections

Urinary tract infection prevalence of 33% compared to previous study from Kenya by Masika *et al.,* (2017) whose overall prevalence of UTI was 11.9% is higher. This could be attributed to differing immune status between these studies. Whereas participants in the initial study were randomly picked with no consideration to immunity as opposed to the later in which inclusion criteria was HIV seropositivity attending comprehensive care clinic. A fact that predisposes them to opportunistic infectious diseases. However, when compared with studies on patients with similar characteristics (immuno compromised participants), the results seem to agree in. In fact

previous studies reported higher prevalence for instance, working with adolescent/young adult Nigerians with acquired human immunodeficiency disease in Benin City (Michael *et al.,* 2006) reported prevalence of 41%. The drop in the current study compared to the earlier one can be attributed to improved strategies in management of opportunistic infections including easier access to ART/prophylaxis following efforts by donor communities and local governments.

Previous studies reported more gram-negative bacteria than gram positives, for example Megan et al., 2015) in his study on prevention of recurrent infections in women isolated (75%) gram negatives and (24.4%) gram positive bacterial with *Escherichia coli* (54.7%) and *Klebsiella pneumoniae* (17.9%) being predominant. Another study from India by Lekha *et al.,* (2016) recorded similar results where *Escherichia coli* (49.5%) was predominant followed by *Klebsiella spp*. (24.7%). A unique structure possessed by gram negative bacteria which assists in attachment to uro-epithelium prevents these pathogens from being easily washed away in urine as in gram positives. The same is used in tissue invasion and cellular growth thus partly accounting for their high prevalence. This explains the predominance of the troublesome infectious, invasive and pyelonephritis. *Escherichia coli* bacterium in many studies. Anal Cross infection from gastro intestinal tract (GIT) where *E. coli* is normal flora in adults is another reason for its predominance. The fact that *Staphylococcus aureus* (12.0%) was most prevalent gram positive and third overall can also be attributed to cross infection from the skin where the organism is a commensal owing to lowered immunity.

The highest rate of infections was realized in age groups (18-40yrs) where 67(57%) of the cases were positive. This could be attributed to the fact that this age bracket comprises members who are generally more active sexually which is a common lifestyle risk factor for UTIs, particularly for women. It’s thought that sexual intercourse may transport bacteria from the anus to the

genitals and urethra causing infection. This further explains high female prevalence compared to males in this age which agrees with study results from Uganda where highest prevalence of 28/86 (32.6%) was from the age group 20–29 linking it to sexual activities (Odoki *et al.,* 2019). Those aged 60 years and above seem to have contributed the least 3(2.6%) but considering that very few patients in this age bracket were captured in the study (7) and that 3 of them were positive, it actually emerged as the most vulnerable group at (42%) i.e. (3 out of sampled 7). These findings are in line with Girija *et al.,* (*202l*) who reported up to 49% UTI positivity rate among the Pakistan elderly patients. This is reasonable considering age related slowing down immunity which also agrees with Akhtar *et al*., (2021) whose results from an Iranian study of elderly patients recorded uropathogenes in high prevalence from various parts of the urinary systems as follows Cystitis (37.6%), bacteriuria (31.9%), urosepsis (10.2%), and prostatitis (6.4%) attributing it to age-related immune drop. Another reason for high UTI among the elderly can be attributed to the fact that with aging, bladder muscles and pelvic floor tend to weaken. This causes urine retention and/or incontinence which increase the potential for bacteria to thrive. Since elderly patients are likely to have been exposed to more antibiotics and other antimicrobial agents in the course of their long lives compared to their younger counterparts, it is possible that these drug/immunity interactions may have led to resistance exposing the elderly a more risky group of patients for urinary tract infections in HIV.

The occurrence of more infections amongst female patients (74.4%) compared to males (25.6%) is in agreement with Megan *et al.* (2015) whose findings suggested a 50% to 60% life time risk of urinary tract disease in women with a recurrent possibility of over 25%. A similar report by Kanu *et al., (2016),* showed female majority of 51%. Generally, the high urinary tract infections prevalence in women can be linked to the female urogenital anatomy and its anal proximity and

also to some birth control methods like diaphragms or spermicides which change the p^H making it favourable for bacterial pathogens to thrive.

5.3 Uropathogenic Response to Cotrimoxazole

Out of 117 isolated uropathogens subjected to cotrimoxazole sensitivity testing 105(89.7%) were non-responsive. This agrees with a Nigerian study from as far back as 2009 where bacterial resistance to cotrimoxazole stood at 83.9% by bacteria Nwadioha *et al.,* (2010). Continued daily low dose drug administration for a long time is likely to turn into development of resistance. Non strict adherence to prophylaxis dosage regimen is another contributory fact in the increasing loss of effectiveness of cotrimoxazole in protecting the seropositive patients as expected. As noted on the local scene, the lack follow up measures of ensuring patients compliance once they have received medicines to take home may have contributed to this non-responses as they end up not taking their medication on time or not sticking to prescript directions.

In general, gram negative bacteria were more resistant to cotrimoxazole than gram positives i.e. *Pseudomonas aureginosa* (100%), *Escherichia coli* (96.9%), *Klebsiella pneumoniae* (95.2%) and compared to *Staphylococcus aureus* (71.4%) and *Staphylococcus epidermidis* (75%). This could be attributed to the characteristic cell envelopes they possess. The envelope which is made up of a special layer of peptidoglycan wall between cytoplasmic inner and outer membranes makes it harder for antibiotics including cotrimoxazole to penetrate into the cells. Mostafa *et al.,* (2018) paints an almost similar picture from an Iranian study where high cotrimoxazole resistance prevalence was reported across most gram negative isolates i.e. *Escherichia coli 62%, Klebsiella* 54%, *Staphylococcus* 55%, and *Enterobacter* 52%. Even though results from the two studies agree for *Escherichia coli* the slide difference for *Klebsiella pneumoniae* and Staphylococcus family may be attributed to regional variations in drug sensitivity.

Chi-square analysis of resistance averages by various bacteria uropathogens was $x^2(5) = 14.3$, $p < 0.005$ shows statistical significance. None of the organisms had frequencies less than 5. With an expected minimum frequency of 83.7, the results show a significant statistical differences in the resistance patterns with *pseudomonas aureginosa* being the most resistant organism at 100%**.**

5.4 Antibiotic Susceptibility Patterns

Maseno region's average susceptibility of 46.6% (Table 3.7) can be attributed to the lack of involvement of the laboratory as a tool in management of opportunistic infections in seropositive patients. Instead patients are blindly treated on the basis of chance and history using outdated empirical antibiograms originated elsewhere. The lack of scientifically determined antibiotic guidance from the laboratory compromised bacterial management standards thus raising the level of bacterial resistance to commonly used antimicrobial agents. Cases of antibiotic misuse in the area where cases of under-dosages, skipping and/or delayed taking of drugs could be another reason for the high resistance reported for the study. Other reasons include over the counter drug prescriptions as a result of the numerous commercial chemists/pharmacies in the area including neighboring Luanda trading centre. Being an agricultural area overuse of antibiotics in livestock and fish farming may have contributed to this resistance development.

Even though no drug had 100% susceptibility, most bacteria were antibiotically affected by all the drugs. Generally, the emerging pattern had, GEN 80.3%, CIP 79.8%, S 79.0%, NIT 74.8% and TET 74.3% as the most effective drugs. Gentamicin being an injectable drug and therefore requiring some technical know-how to be administered makes it less likely to be misused. Being aminoglycoside exhibiting concentration-dependent killing, gentamicin's higher concentrations correlated with greater antimicrobial killing. Additionally, research shows that gentamicin has synergistic effects on gram-positive bacteria. This results compare favorably with those of another study within the same region. In Gucha sub-county hospital investigating pregnant

women for urinary tract infections, Maniga *et al.,* (2015) though with a smaller susceptibility rate (21%) recommended gentamicin as the empiric drug of choice for the region after. The same study results indicated ciprofloxacin (17%) and nitrofurantoin (13%) as next preferable drugs. This tend to agree with results of the current study where ciprofloxacin (79.8%) second best followed by streptomycin (79%).

Drugs showing mixed performance against various uropathogens included CXM 51.0%, NA 50.5%, CTR 43.3%, DOX 39.7% while least effective antimicrobials included VAN 36.0%, OFX3 2.5%, AMP 31.5%, SX 13.7%, CAZ 12.7%, CPM 0.3%. Ampicillin (31.5%) was ranked among the poorest performing drugs only better than Sulphamethoxazole, vancomycin Ceftazidime and Cefepime. This again agrees with the results of a related Tehran (Iranian) study whose results showed most of the isolated pathogens being resistant to ampicillin in very high rates i.e. *E. coli* (85.9%), *Proteus spp.* (88.3%) and *Klebsiella spp*. (94.5%) (Kashef *et al.,* 2010). This was an important observation because ampicillin is generally among the most used drugs world widely including in management of urinary tract infections. Being cheap and easily available as an over the counter drug, chances of being abused are high making it more resistant to common uropathogens as witnessed in this study. Despite being relative newer drug, cefepime (0.3%) ranking last is worrying. Possible reasons for this could include over reliance on the latest drugs and rapid over exposure while overlooking the traditional medicines. Like all other cephalosporins, previous researchers have linked cefepime resistance has to previous treatment exposures which either reduces affinity of existing target components, or causes acquisition of bacterial produced chromosomal or plasmid DNA supplementary beta-lactam-insensitive targets.

On average, study findings found *Proteus mirabilis* (53.1%) as most susceptible bacterium. This followed high susceptibility scores with most antibiotics i.e. 86% for ciprofloxacin, Gentamicin,

nitrofurantoin, streptomycin and tetracycline. There are slide difference compared with findings of a Pakistan by Kidwai *et al.,* (2017) who reported *E. coli* (58.6%) most susceptible compared to this study's (49.2%). The drop in *E.coli* susceptibility in the two studies can be explained by the fact that being among the most implicated uropathogen globally, its continuous subjection to various antibiotics is likely to have led to horizontal transfer and accumulation of resistance genes. However, despite the drop between then and now, *E.coli* showed susceptibility to all the drugs including sulphamethoxazole 10% ceftazidime 8% and even cefepime 2%. This shows the bacterium still retains its nature of being intrinsically susceptible to almost all clinically relevant antimicrobial.

Gram-negative bacteria exhibited more resistance across all antimicrobials compared gram-positive bacteria because of their thicker layer of peptidoglycan which easily absorbs antibiotics. These gram positive susceptibility pattern has some resemblance with an Ethiopian study which also had most Gram positive bacteria resisting majority of the test antibiotics including Ampicillin (90%), amoxicillin (Agersew *et al.,* 2013).The only notably difference between the two studies is the complete resistance reported for tetracycline in Ethiopia which directly contradicts high (74.8%) susceptibility reported in this current one. The resurgence noticed in tetracycline susceptibility may be attributed to initial hesitancy by most clinicians in prescribing the drug following massive development of resistance by most bacterial. This coincided with brief withdrawal from the counters, a strategy that seem to have worked well for it because upon reintroduction, it acted like a new drug with the strains that had developed resistance for it having been wiped out by more effective antimicrobial agents.

Most gram negative bacteria generally were susceptible to ciprofloxacin and gentamicin. For instance, for *E. coli*, rates were *82% & 89, Klebsiella spp*. 90% & 90% and *pseudomonas spp.*

were 67% & 67% respectively which is in agreement with a Nigerian study (Nwadioha *et al.*, 2010). Some uropathogens showed some resistance to both commonly used antibiotics and the latest drug interventions. *Pseudomonas aeruginosa* which was the most resistant uropathogen bacterium was completely resistant to cefepime, Ceftazidime and sulphamethoxazole. This phenomenon is not unique as previously identical patterns had been reported by Rajan & Prabavathy (2012). In fact, some worse situations where organisms showed resistance to almost all the antimicrobial agents have been reported (Ngowi *et al.,* 2021). Such situations are mostly hospital transmissions. Multi Drug Resistant (MDR) bacteria were mostly noted from samples of the elderly patients where 2 out of the 3 cases were resistant to at least three of the drugs in the panel. Bacteria in this category were *P. aeruginosa* and *S. epidermidis*. These are likely to have been indiscriminately exposed to many antibiotics throughout their longer lifespans leading to development of resistance (Gashaw *et al.*,2018). Study of emerging trends of susceptibility patterns obtained from this study point to the likelihood of multi-drug resistant uropathogens being domiciled likely in HIV patients making this group of patients a significant source to the rest of the communities.

5.5 Limitations of the Study

i. The study scope was limited to bacterial urinary tract pathogens leaving out viral, parasitic, fungal and other organisms associated urinary tract infections in HIV patients.
ii. Uneven patient presentation patterns for age and sex translated into skewed selection of participants in favour of women against males and adults against minors.
iii. The lack of follow up programs that would ascertain whether the patients stick to dosage regimen at home was a major limitation in establishing if cotrimoxazole unresponsiveness was due to drug misuse or genetic nature of the isolates and/or affected patients.
iv. Unavailability of CD4 Data was a limitation in linking UTI prevalence to HIV stage

CHA PTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary of Findings

1. The study documents a Urinary Tract Infections prevalence (33%) 117/354 with Gram-negative bacteria as the most dominant uropathogens and *Escherichia coli (54.7%)* being implicated most. Others were, *Klebsiella pneumoniae* (17.9%), *Staphylococcus aureus* (12.0%), *Proteus mirabilis* (9.4%), *Staphylococcus epidermidis* (3.4%) and *Pseudomonas aeruginosa* (2.6%).

2. Most isolates (89.7%) 105/117 failed to respond to prophylactic drug cotrimoxazole with individual bacterial species resistance contributions being *P. aeruginosa* (59.0%), *K. pneumoniae* (19.0%), *S. aureus* (9.5%), *P. mirabilis* (6.7%), *E. coli* (2.9%) and *S. epidermidis* 2.9%).

3. Despite an average susceptibility of 46.6%, encountered bacterial pathogen strains exhibited varying patterns to both commonly used antimicrobial agents and newer drugs with means ranging from the most susceptible *P. mirabilis* (52.9%), *S. aureus* (49.2%), *E. coli* (49.2%), *K. pneumoniae* (46.0%) and *P. aeruginosa* (37.7%) as the lowest.

6.2 Conclusions

i. One in every three seropositive patients is likely to be having a urinary tract infection probably caused by *Escherichia coli*.

ii. High prevalence (89.7%) of prophylaxis non-responsiveness exhibited by most uropathogens among HIV seropositive patients can be attributed to daily low dose administration of cotrimoxazole in addition to its routine use in management of non-complicated infections.

iii. Despite good susceptibility scores from a good number of antimicrobials, the average rate of insusceptibility to commonly used drugs was averaged at (53.4%) with gentamicin (80.3%) being the most effective drug and cefepime (0.3%) the least performing.

6.3 Recommendations from the study

i. Findings and conclusions of this study highlight the need for continuous monitoring of urinary tract infections and profiling of bacterial aetiologies amongst HIV seropositive patients by encouraging hospitals and CCCs to invest in diagnostic testing in order to establish traditional culprits emerging infection trends.

ii. Community health workers should be mobilized to ensure that patients stick to dosage regimen for prophylactic drug as well as prescribed antimicrobials. Periodic surveillance of cotrimoxazole effectiveness and continued updating of prophylaxis program should be adopted.

iii. There is need for CCCs to base UTI treatment on scientifically determined data as obtained from periodic studies of susceptibility patterns. This will aide effective therapy and limit development of antimicrobial resistance through maximal utilization of resources instead of prescription of outdated drugs. This study recommended gentamicin as the empirical drug of choice for Maseno region.

6.4 Recommendations for further studies

i. Since long term administration of anti-retro viral drugs has some negative effects on functionality of the nephron, studies that would investigate prevalence of urinary tract infections against duration of these drug use are recommended.

ii. Cotrimoxazole studies aimed at investigating whether high rate of bacteria not responding to prophylaxis is as a result of drug failure due to long term application of

daily doses combined with routine antimicrobial therapeutic use, bacterial gene mutations or genetic makeup of affected patients.

iii. Following the high rates of drug insusceptibility across commonly isolated uropathogens, antimicrobial susceptibility studies are recommended

REFERENCES

Agata S., Bartłomiej M., Agnieszka B., Marcin P., Ewa F., Andrzej H., Justyna D. Kowalska. (2018) "Factors associated with urinary tract infections among HIV-1 infected patients" PLoS One. ; 13(1): e0190564. 11. doi: 10.1371/journal.pone.0190564, PMCID: PMC5764262

Agersew A. 1, Mulat D.2, Meseret A.3 and Mucheye, (2013) "Uropathogenic Bacterial Isolates and their Antimicrobial Susceptibility Patterns among HIV/AIDS Patients Attending Gondar University Specialized Hospital Gondar, Northwest Ethiopia." *Journal of Microbiology Research and Reviews*, Vol. 1(4): 42-51, ISSN: 2350-1510

Alhumai S.1, Abbas A. M.2, 3, Zainab A.A. 4, Ahmad J. A.5, Mansou T.6, Ahmed M. A.1, Ibrahim B.S1, Issa A.H.1, Naif A.7, Mossa A.8, Ahmed H. A.9, Fadhil A.10, Haifa A.S.11, Ali A. R.12 and Awad A.O. (2021) "Antimicrobial susceptibility of gram-positive and gram-negative bacteria: a 5-year retrospective analysis at a multi-hospital healthcare system in Saudi Arabia" Annals of Clinical Microbiology and Antimicrobials. (2021) 20:43 https://doi.org/10.1186/s12941-021-00450-x.

Akhtar A., Azmi M., Hassali A., Zainal H., Irfhan A. and Amer H.K. (2021). "A Cross-Sectional Assessment of Urinary Tract Infections Among Geriatric Patients: Prevalence, Medication Regimen Complexity, and Factors Associated With Treatment Outcomes". BRIEF RESEARCH REPORT article Front. Public Health, Sec. Aging and Public Health https://doi.org/10.3389/fpubh.2021.657199

Ayoyi A.O., Kikuvi G., Bii C.,Kariuki S. (2017) "Prevalence, aetiology and antibiotic sensitivity profile of asymptomatic bacteriuria isolates from pregnant women in selected antenatal clinic from Nairobi, Kenya". Pan Afr Med J. 2017; 26: 41. 2017 Jan 30. doi: 10.11604/pamj.2017.26.41.10975. PMCID: PMC5398259. PMID: 28451019

Baquer, F.;Sawan A., Auzou A, Grillon M, Jaulhac A, Join-Lambert B., Boyer O., P.H. (2021) "Broth Microdilution and Gradient Diffusion Strips vs. Reference Agar Dilution Method" First Evaluation for Clostridiales Species Antimicrobial Susceptibility Testing. Antibiotics 2021, 10, 975. https://doi.org/10.3390/antibiotics10080975

Behzadi, P. (2019). “Introductory Chapter: An Overview on Urinary Tract Infections, Pathogens, and Risk Factors. From the Edited Volume of Microbiology of Urinary Tract Infections”. Intech Open. doi: 10.5772/intechopen.82230

Bryce A, Alastair D H, Lane I. L, Hannah V.T., Mandy W. Costelloe C.. (2016) “Global prevalence of antibiotic resistance in pediatric urinary tract infections caused by Escherichia coli and association with routine use of antibiotics in primary care: systematic review and meta-analysis”. BMJ2016;352:i939http://dx.doi.org/10.1136/bmj.i939:

CLSI Subcommittee on Antimicrobial Susceptibility Testing (2018) Janet A Hindler, MCLS MT (ASCP) F (AAM), Editor Audrey Schuetz, MD, MPH, FCAP, Editor. ” CLSI AST News Update. Volume 3, Issue 1 winter 2018.

Clinical and Laboratory Standards Institute (CLSI), (2015) “M100S-S25 Performance Standards for Antimicrobial Susceptibility Testing;” Twenty-Fifth Informational Supplement, Clinical and Laboratory Standards Institute (CLSI), Vol. 35, Clinical and Laboratory Standards Institute (CLSI), Pittsburgh, PA, USA,

Consolidated guidelines (2013) “on the use of antiretroviral drugs for treating and preventing HIV infection. Supplementary section to the world health organization guidelines”.

Dadi M. et al (2019) “Prevalence, Antimicrobial Susceptibility Pattern of Bacterial Isolates, and Associated Factors of Urinary Tract Infections among HIV-Positive Patients at Hiwot Fana Specialized University Hospital, Eastern Ethiopia.” *The Canadian journal of infectious diseases & medical microbiology = Journal canadien des maladies infectieuses et de la microbiologie medicale* vol. 6780354. 6 Feb. 2019, doi:10.1155/2019/6780354

Dat V.Q., Tran T.D, Vu H.V, Kim B G, Satoko O. (2022) ‘Antibiotic use for therapy in critical units in primary and secondary hospitals in Vietnam: A multicenter cross-sectional study”. The lancet regional health, western pacific, Volume 18, 100306 January 01, 2022.

Fifth International Conference (2019), “on Antimicrobials and Antibiotics Resistance”. Conference Series LLC Ltd, March 22-23, at Chicago, USA

Fourth International Conference (2018) “on Antimicrobials, Multiple Drug Resistance & Antibiotics Resistance” Conference Series LLC Ltd. April 20-21, 2018 Las Vegas, USA

Flores-Mireles, A., Walker, J., Caparon, M. *et al.* (2015). "Urinary tract infections: epidemiology, mechanisms of infection and treatment options." *Nat Rev Microbiol* 13, 269–284 https://doi.org/10.1038/nrmicro3432.

Gashaw M, Berhane M, Bekele S, Kibru G, Teshager L, Yilma Y, Ahmed Y, Fentahun N, Assefa H, Wieser A, Gudina EK, Ali S. (2018) "Emergence of high drug resistant

Girija S. AS, Vijayashree P. J & Paramasivam A (2021) Prevalence of *Acb* and *non-Acb* complex in elderly population with urinary tract infection (UTI), Acta Clinica Belgica, 76:2, 106-112, DOI: 10.1080/17843286.2019.1669274

Grimwade K, Swingler GH. (2003) "Cotrimoxazole prophylaxis for opportunistic infections in adults with HIV:" *Cochrane Database of Systematic Reviews*, Issue 3. Art. No.: CD003108.DOI: 10.1002/14651858.CD003108.im

Joanna L. (2020) "Treatment options for urinary tract infections | Understanding UTIs, Part 5". May 14, 2020 Scope. Standard Medicine

Joint United Nations Programme on HIV/AIDS/World Health Organization/ United Nations Children's Fund towards universal access: (2009) "scaling up priority HIV/AIDS Interventions in the health sector." Progress report 2009. Geneva, UNAIDS, WHO, UNICEF.

Kanu A.M, MgbajiakaN., Abadom N. (2016) "Prevalence of urinary tract infection among HIV patients in Aba, Nigeria"17th International Congress on Infectious Diseases / International Journal of Infectious Diseases 45S (2016) 1–477

Kashef N.1, Djavid G.E.2, Sahba S. (2010) 'Antimicrobial susceptibility patterns of community-acquired uropathogens' in Tehran, Iran'. *J Infect Dev Ctries*; 4(4):202-206

Kidwai S.S., Nageen A., Ghaznavi S., Bashir F., & Ara J. (2017). Antibiotic susceptibility in commonly isolated pathogens from urinary tract infection in a cohort of subjects from low socioeconomic strata. *Pakistan journal of medical sciences*, *33*(2), 254–259. https://doi.org/10.12669/pjms.332.11569

Kemajou T.S., Ajugwo A.O., Oshoma C.E., Enabulele O.I. (2016) "Antibiotic Resistance of Bacterial Isolates from HIV Positive Patients with Urinary Tract Infection (UTI) in Port Harcourt, Nigeria." J AIDS Clin Res 7: 594. doi:10.4172/2155-6113.1000594.

Kenya government census (20*19)* "Urban Poverty Estimates for Kenya's Provinces, Districts, Divisions and Locations"

Kenya HIV Estimates Report 2018; National Aids Control Council

Kimberly A. K. and Amanda L.L. (2016) "Microbiol Spectra. (2):10.1128 microbiolspec. UTI" - 0012-2012. doi: 10.1128/microbiolspec.UTI-0012-2012

Lekha T., Sujeet R ., Dilshad A., Deepak K. S. (2016) "Bacteriological profile and Antimicrobial Susceptibility pattern of isolates from Urinary Tract Infections in Eastern Uttar Pradesh, India." *Int.J.Curr. Microbiol .App.Sci.* 5(3): 428-435. doi:http://dx.doi.org/10.20546/ijcmas..503.049

Mageto V.M., Gatwiri, M.S., and 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*.

Maniga N.J., Mogaka G., Nyambane L. and Eilu E. (*2015*) "Prevalence and susceptibility pattern of bacterial Urinary Tract Infections among pregnant HIV positive women in Gucha sub county, Kenya." *Special Bacterial Pathogens Journal (SBPJ); Vol 1, No 1: p 00010-0015*

Marimuthu R., R., and Narasingam A. (2018) "Drug-Resistant Bacterial Infections in HIV Patients". DOI: 10.5772/intechopen.78657.

Marwa K.J., [1] Martha F. Mushi, [2] Konje E., [3] Alele P.E. [4] Kidola J.[5] and MiramboM.M. (2015) "Resistance to Cotrimoxazole and Other Antimicrobials among Isolates from HIV/AIDS and Non-HIV/AIDS Patients at Bugando Medical Centre, Mwanza, Tanzania" Hindawi / Publishing partnerships

Masika WG, O'Meara WP, Holland TL, Armstrong J. Contribution of urinary tract infection to the burden of febrile illnesses in young children in rural Kenya. PLoS One. 2017 Mar 21; 12(3):e0174199. doi: 10.1371/journal.pone.0174199. PMID: 28323886; PMCID: PMC5360311

Megan R.MS, Krista G., Susan E, and Debra V. (2015). " Prevention of recurrent infections in women"

Michael O., Abel O., and Ukoh G, (2006) "Urinary tract infection in adolescent/young adult Nigerians with acquired human immunodeficiency disease in Benin City," Journal *of Medicine and Biomedical Research,* vol. 5, no. 2, pp. 55–60.

Miftahussurur,M., Fauzia K.A., Nusi, I.A. *et al. (2020)* 'E-test versus agar dilution for antibiotic susceptibility testing of *Helicobacter pylori'*: a comparison study. *BMC Res Notes* **13,** 22 (2020). https://doi.org/10.1186/s13104-019-4877-9

Mwang'nde B.J., Mchami J.I (2022) "The aetiological and prevalence of urinary tract infections in Sub-Saharan Africa: a systemic review". J. Health Biol Sci.20222; 10(1):1-7. doi: 10.12662/2317-3206jhbs.v10i1.4501.p1-7.2022

National Institute for Health and Clinical Excellence-NICE (2012) "Urinary tract infection in children: diagnosis, treatment and long-term management". NICE clinical guideline. www.nice.org.uk/cg054.

National Kidney Foundation Inc. (2021)" HIV and Chronic Kidney Disease ". 30 East 33rd Street, New York, NY 10016

Nelson JM and Good E. *(2015)* "Urinary tract infections and asymptomatic bacteriuria in older adults. *"Nurse Pract. Aug 15; 40(8):43-8*

Ngowi, B. N., Sunguya, B., Herman, A., Chacha, A., Maro, E., Rugarabamu, L. F., Bartlett, J., Balandya, E., Mteta, K. A., & Mmbaga, B. T. (2021). Prevalence of Multidrug Resistant UTI among People Living with HIV in Northern Tanzania. *Infection and drug resistance*, *14*, 1623–1633. https://doi.org/10.2147/IDR.S299776

Nwadioha SI., Nwokedi EO., Ikeh I., Egesie J and Kashibu E (2010) "Antibiotic susceptibility pattern of uropathogenic bacterial isolates from Aids patients in a Nigerian tertiary hospital." *Journal of Medicine and Medical Sciences* 1(Suppl 11): 530-534

Odoki M., Adamu A., Aliero, Tibyangye J.,ManigaJ.,N., Wampande E., Kato C., D., Agwu E. and Bazira J. (2019), Prevalence of Bacterial Urinary Tract Infections and Associated Factors among Patients Attending Hospitals in Bushenyi District, Uganda: International Journal of Microbiology. Volume 2019, Article ID 4246780, 8 pages; https://doi.org/10.1155/2019/4246780,

Olowe O.A, Ojo-Johnson B.B., Makanjuola O.B., Olowe R.A, Mabayoje V.O., (*2015)* "Detection of bacteriuria among human immunodeficiency virus seropositive individuals in Osogbo, south-western Nigeria." *Eur J Microbiol Immunol (Bp). 2015 Mar; 5(1):126-30*

Opiyo W.O, Ng'wena A.G.M. & OfullaA.G. O. (*2010)* "Urine markers of kidney disorders and their risk associations in HIV-infected patients attending Nyanza provincial general hospital in Kisumu, western Kenya" *East Africa Medical Journal Vol. 87 No.*

Park, J. J., Seo, Y. B., & Lee, J. (2017). Antimicrobial Susceptibilities of Enterobacteriaceae in Community-Acquired Urinary Tract Infections during a 5-year Period: A Single Hospital

Study in Korea. Infection & chemotherapy, 49(3), 184–193. https://doi.org/10.3947/ic.2017.49.3.184

Paul R. (2018). State of the Globe: Rising Antimicrobial Resistance of Pathogens in Urinary Tract Infection. Journal of global infectious diseases, 10(3), 117–118. https://doi.org/10.4103/jgid.jgid_104_17

Prasada S. [1 2], Archana B. [1 2], Sevitha B. [1 2], Shalini. M. [1 2], Sanyuktha T. (2019) "Changing antibiotic susceptibility pattern in uropathogenic *Escherichia coli* over a period of 5 years in a tertiary care center" May 29;12:1439-1443. doi: 10.2147/IDR.S201849.e-Collection 201

Rajan S and Prabavathy J. (2012) "Antibiotic sensitivity and phenotypic detection of ESBL producing *E. coli* strains causing urinary tract infection in a community hospital, Chennai, Tamil Nadu, India" *Webmed Central Pharm Sci;* 3(11): WMC003840

Rashmi K.S, Ravi K. KL, Jagadeesh, Nimitha, Bhagyashree HN (2013) "Asymptomatic bacteriuria in HIV/AIDS Patients: Occurrence and risk associated with low CD4 counts." *JEMDS;* 2(19):3358-3366.

Sinawe H, Casadesus D. 'Urine Culture.' [Updated 2022 May 8]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557569/

Talha H. I. "Overview of Urinary Tract Infections (UTIs)"**,** MD, University of Riverside School of Medicine Reviewed/Revised Dec 2022.

Truong, W. R., Hidayat, L., Bolaris, M. A., Nguyen, L., & Yamaki, J. (2021). The antibiogram: key considerations for its development and utilization. *JAC-antimicrobial resistance*, *3*(2), dlab060. https://doi.org/10.1093/jacamr/dlab060

Vijayan V.K.K, Karthigeyan K.P., Tripathi S.P., & Hanna, L. E. (2017). Pathophysiology of CD4+ T-Cell Depletion in HIV-1 and HIV-2 Infections. Frontiers in immunology, 8, 580. https://doi.org/10.3389/fimmu.2017.00580

Wayne PA CLSI, (2013). "Performance standard for antimicrobial susceptibility testing, twenty-third informational supplement M100- S 23", *Clinical and laboratory standard institute;*

WHO guidelines of (2014). "On cotrimoxazole prophylaxis in resource limited settings".

Yang, N. J., & Chiu, I. M. (2017). Bacterial Signaling to the Nervous System through Toxins and Metabolites. *Journal of molecular biology*, *429*(5), 587–605. https://doi.org/10.1016/j.jmb.2016.12.023

ZeyaullahM.D.1 & Kaul V. 2 (2015), Prevalence of urinary tract infection and antibiotic resistance pattern in Saudi Arabia population. G.J.B.A.H.S., Vol.4 (1):206-214 (January-March, 2015) ISSN: 2319 – 5584

APPENDICES

Appendix I: SGS Letter of Approval

MASENO UNIVERSITY
SCHOOL OF GRADUATE STUDIES

Office of the Dean

Our Ref: MSc/PH/00072/2015

Private Bag, MASENO, KENYA
Tel:(057)351 22/351008/351011
FAX: 254-057-351153/351221
Email: sgs@maseno.ac.ke

Date: 15th MARCH, 2018

TO WHOM IT MAY CONCERN

RE: PROPOSAL APPROVAL FOR PHILIP SAKWA MUSASIA — MSc/PH/00072/2015

The above named is registered in the programme of Master of Science in Medical Microbiology in the School of Public Health and Community Development, Maseno University. This is to confirm that his research proposal titled "**Prevalence and antibiotic susceptibility patterns of contrimoxazole resistant uropathogens in HIV seropositive patients attending Maseno mission hospital.**" has been approved for conduct of research subject to obtaining all other permissions/clearances that may be required beforehand.

Prof. J.O. Agure
DEAN, SCHOOL OF GRADUATE STUDIES

14 MAR 2018

Maseno University *ISO 9001:2008 Certified*

Appendix II: Ethical approval

MASENO UNIVERSITY ETHICS REVIEW COMMITTEE

Tel: +254 057 351 622 Ext: 3050
Fax: +254 057 351 221

Private Bag – 40105, Maseno, Kenya
Email: muerc-secretariate@maseno.ac.ke

FROM: Secretary - MUERC

DATE: 7th August, 2018

TO: Philip Sakwa Musasia
PG/MSc/PH/00072/2015
Department of Biomedical Science and Technology
School of Public Health and Community Deveiopment
Maseno University
P.O. Box Private Bag, Maseno

REF: MSU/DRPI/MUERC/00557/18

RE: Prevalence and Antibiotic Susceptibility Patterns of Cotrimoxazole Resistant Bacterial Uropathogens from HIV Positive Patients Attending Maseno Mission Hospital. Proposal Reference Number MSU/DRPI/MUERC/00557/18

This is to inform you that the Maseno University Ethics Review Committee (MUERC) determined that the ethics issues raised at the initial review were adequately addressed in the revised proposal. Consequently, the study is granted approval for implementation effective this 7th day of August, 2018 for a period of one (1) year.

Please note that authorization to conduct this study will automatically expire on 6th August, 2019. If you plan to continue with the study beyond this date, please submit an application for continuation approval to the MUERC Secretariat by 15th July, 2019.

Approval for continuation of the study will be subject to successful submission of an annual progress report that is to reach the MUERC Secretariat by 15th July, 2019.

Please note that any unanticipated problems resulting from the conduct of this study must be reported to MUERC. You are required to submit any proposed changes to this study to MUERC for review and approval prior to initiation. Please advice MUERC when the study is completed or discontinued.

Thank you.

MASENO UNIVERSITY SECRETARY 07 AUG 2018 ETHICS REVIEW COMMITTEE

Dr. Bonuke Anyona.
Secretary,
Maseno University Ethics Review Committee.

Cc: Chairman,
Maseno University Ethics Review Committee.

MASENO UNIVERSITY IS ISO 9001:2008 CERTIFIED

Appendix III: Permission for study

Philip Sakwa Musasia
P. O Box 333,
Maseno
Email: spmusasia@gmail.com
Tel: 0722219622

17th Sep, 2018.

To

The Coordinator Comprehensive Care Centre (CCC).

Maseno Mission Hospital.

Dear Sir/Madam,

RE: REQUEST FOR PERMISSION TO CONDUCT RESEARCH AT MASENO MISSION HOSPITAL'S COMPREHENSIVE CARE CENTRE (CCC) PATEINTS.

Being a master of science student in medical microbiology at the School of Public Health and Community Development, in the Department of Biomedical Sciences of Maseno university and following the approval of my research proposal titled " **Prevalence and Antibiotic Susceptibility Patterns of Cotrimoxazole Resistant Bacterial Uropathogens in HIV Seropositive Patients Attending Maseno Mission Hospital**" at the school of graduate studies and clearance by Maseno University Ethical Review Committee, I hereby apply for permission to collect urine samples from patients in the Hospital's Comprehensive Care Centre (CCC) program .

Attached find the approval letters from School of Graduate Studies and Maseno University Ethical Review Committee.

Looking forward to your positive response

Thank You.

MASENO HOSPITAL TEL: 057-351006

Yours faithfully,

Philip Sakwa Musasia.
MSC/PH/00072/2015

Appendix IV: letter of consent

Participation Consent

Study Title: Prevalence of bacterial urinary tract infection and antibiotic susceptibility patterns of cotrimoxazole resistant uropathogens from HIV sero positive patients on cotrimoxazole attending Maseno mission hospital, western Kenya.

Dear participant,

You are hereby invited to participate in this study because you are affected. This form is meant to make you aware why this research is being carried out. Please read through and make decision if you want to take part or not. The principal investigator in this study is a master's student at Maseno University.

The purpose of this study is **to determine prevance of urinary tract infections, investigate prophylaxis sensitivity and establish antibiotic susceptibility patterns of cotrimoxazole resistant uropathogens from seropositive patients attending Maseno mission hospital in western Kenya**. Urine specimens will be collected for urinary tract infection screening a process that will not cause any pains.

You are free to decide whether to take part or not. It is of importance to note that by volunteering to participate there is no financial benefit just as there will be no cost implications. However, participating in this study is important as the findings of the may be helpful in your own personal management and for decision making in improving public health that will go a long way in prevention of urinary tract infection among people living with HIV in Kenya. Your results will be relayed personally to you through your comprehensive care centre (CCC) service provider at Maseno mission hospitals

Questions posed to the participants regarding their socio-demographic characteristics may cause discomfort. The researcher wishes to assure the participants that their information will be treated with high level of confidentiality but not guaranteed**.**

……

Read and Signed

N/B: In case of any queries and /or complaints arising from this study you can reach us on

1. Principal Investigator (Philip Sakwa Musasia – reg. MSc/PH/00072/2015)
   Maseno University,
   School of Public health and Community Development
   Department of Biomedical Sciences.
   P.O. Box 333 Maseno.
   Tel no: 0722219622.
   Email:spmusasia@gmail.com
1. Secretariat Maseno University Ethics Review Committee
   School of Graduate Studies (SGS).
   P.O. box 333 Maseno

Appendix V: Questionnaire (Questions answered by patient)

1. Age in Years

   18- 30 ☐ 31 – 44 ☐ 45- 60 ☐ Over 60 ☐

2. Gender: Male☐ Female☐

3. Marital Status

   Single ☐ Married☐ Widowed ☐ Divorced/separated☐

4. Level of education

   None ☐ Primary☐ Secondary ☐ College/University ☐

5. For how long have you been known to be sero positive?

   1-6 Months☐ 7- 12 Months ☐ Over 1 Year ☐

6. How long have you been on medication?

   Less than 6months☐ 5-12months ☐ 13-24months☐ 25-36months☐ over 3years

7. What type of medication?

   ARVs ☐ Antibiotics ☐ Others ☐

8. Are you on cotrimoxazole?
   Yes ☐ No ☐

9. If yes for [8] above, how long have you been on cotrimoxazole?

   1-6 Months☐ 7- 12 Months ☐ Over 1 Year ☐

Appendix VI: Laboratory data capture form (Questions answered by Laboratory Staff)

1. Was uropathogen(s) isolated from the HIV sero positive patient?

   Yes ☐ No ☐

2. If yes for (1) above, which uropathogen?

   E. coli ☐ *Klebsiella spp.* ☐ *Pseudomonas spp* ☐ *Proteaus spp* ☐

   Staphylococcus aureus ☐ Other bacteria ☐ Others /non bacteria ☐

3. Was the uropathogen(s) isolated sensitive to Cotrimoxazole?

   Yes ☐ No ☐

4. If no, for (3) above was the cotrimoxazole resistant uropathogen(s) susceptible to other antibiotics?

   Yes ☐ No ☐

5. If yes for (4) above, which other antibiotics were the cotrimoxazole resistant uropathogens susceptible to?

AMP	CXM	GEN
CIP	NA	SX
S	CTR	CAZ
NIT	DO	CPM
OFX	VA	TET