

ANTIBIOTIC SUSCEPTIBILITY AND GENOTYPES OF *ESCHERICHIA COLI* ISOLATED FROM HIV SERO-POSITIVE ADULTS AT MBAGATHI DISTRICT HOSPITAL, NAIROBI.

BY

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DECLARATION

I, Jacinta Emacar, hereby declare that this thesis is my original work and has not been presented for the award of a degree in any other university or any other a ward.

Signature.....Date.....

I/We confirm that the work reported in this thesis was carried out by the student under my/our supervision.

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DEDICATION

I would like to dedicate this thesis to my mother Angela Alungat and beloved daughter Favour Vanaya for enabling me to achieve all that I have.

A CKNOWLEDGEMENT

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ABBREVIATIONS AND ACRONYMS

AIDS – Acquired Immunodeficiency Syndrome

AMR- Antimicrobial resistance

AMP – Ampicillin

Amp^R – Resistant to ampicillin

Amp^S – Sensitive to ampicillin

ART – Antiretroviral treatment

ARVs- Antiretrovirals

AUG – Amoxicillin/clavulanic acid (augmentin)

Aug^R – Resistant to amoxicillin/clavulanic acid.

Aug^S – Sensitive to amoxicillin/clavulanic acid

Caz – Ceftazidime

Caz^R – Resistant to ceftazidime

Caz^S – Sensitive to ceftazidime

CCC- Comprehensive Care Clinic

CDC – Centre for Disease Control

Chl – Chloramphenical

Chl^R – Resistant to chloramphenical

Chl^S – Sensitive to chloramphenical

Cip – Ciprofloxacin

Cip^R – Resistant to ciprofloxacin

Cip^S – Sensitive to ciprofloxacin

Cxm – Cefuroxime

Cxm^R – Resistant to cefuroxime

Cxm^S – Sensitive to cefuroxime

DCA-Deoxycholate Citrate Agar

DHHS –Kenya Department of Health and Human Services

DHFR - Dihydrofolate reductase

DHPS - Dihydropteroic acid synthesis

DNA – Deoxyribonucleic Acid.

EC – *Escherichia coli*

Error bars – Standard deviation

Gm - Gentamicin

Gm^R – Resistant to gentamicin

Gm^S – Sensitive to gentamicin

GPA - Global AIDS Programme

HIV – Human Immunodeficiency Virus.

HIV⁺A⁻ - Human Immunodeficiency virus positive and not taking antibiotics

HIV⁺A^{+0.5} – Human Immunodeficiency virus positive and taking antibiotics for six months

HIV⁺A^{+1.0} - Human Immunodeficiency virus positive and taking antibiotics for one year

HIV⁺A^{+1.5} - Human Immunodeficiency virus positive and taking antibiotics for one and a half yrs

HIV⁺A^{+2.0} - Human Immunodeficiency virus positive and taking antibiotics for two years

HUS- Haemolytic Uremic Syndrome.

IDRS-Integrated Disease Surveillance and Response

KAIS- Kenya AIDS indicator survey

KDM – Kenya Department of Medicine

KCN – Potassium cyanide broth

KFDA –Kenya food and Drug administration

MAI - *Mycobacterium avium intracellulare*

MAC - *Mycobacterium avium* complex

MBCs – Minimum Bactericidal Concentration.

McCA – MacConkey Agar

MDH-Mbagathi District Hospital

MDR- Multidrug- resistance

MIC – Minimum Inhibitory Concentrations.

MW – Molecular weight

MOH – Ministry of Health

MSF- Medecin's San Frontiers

Na – Nalidixic acid

Na^R - Resistant to nalidixic acid

Na^S –Sensitive to nalidixic acid

NACC - National Aids Control Council

NCCLS- National Committee for Clinical Laboratory Standards.

NARMS- National Antimicrobial Resistance Monitoring System

NACP - National AIDS Control Programme

NASCOP – National AIDS/STI control program

Nit – Nitrofurantoin

Nit^R – Resistant to nitrofurantoin

Nit^S – Sensitive to nitrofurantoin

Nor –Norfloxacin

Nor^R – Resistant to norfloxacin

Nor^S – Sensitive to norfloxacin

OIs – Opportunistic Infections.

PBP - Penicillin binding protein

PCP- *Pneumocystis carinii* pneumonia

PLWHA – Persons living with HIV and AIDS

PMCTC – Prevention of mother to child transmission

PEGE – Pulse gel electrophoresis

Sxt - Trimethoprim–Sulfamethoxazole (Cotrimoxazole)

Sxt^R –Resistant to trimethoprim-sulphamethazole

Sxt^S –Sensitive to trimethoprim-sulphamethazole

STI's – Sexually transmitted diseases

Tet – Tetracycline

Tet^R –Resistant to tetracycline

Tet^S – Sensitive to tetracycline

UNAIDS – United Nations programme on HIV/AIDS

USPHS - United States Public Health Services

VCT – Voluntary counseling and testing

VRE –Vancomycin Resistant Enterococcus

ABSTRACT

Persons living with HIV may succumb to acquired immunodeficiency syndrome (HIV/AIDS), which renders them susceptible to opportunistic infections. Several antibiotics are recommended for prophylaxis and treatment of the infections. These usage of antibiotics has led to the development of multi drug resistant strains of *Escherichia coli* isolated from the gut of persons living with HIV/AIDS (PLWHA). Such resistance is of great concern as the organisms may become infectious and also serve as an important reservoir for resistant genes which can be transferred to potential pathogens. Available antibiotics would be rendered ineffective thus plugging the country into a possible post antibiotic resistance era. This study was carried out to determine the antibiotic susceptibility and genotypes among fecal *E. coli* isolates from PLWHA who were reporting at Mbagathi District hospital. *E. coli* were obtained by culturing stool samples from the study population on MacConkey agar and the isolates biochemically identified. The isolates were tested for antimicrobial susceptibility to commonly used antimicrobials using Kirby Bauer disk diffusion method and the E-test. Out of the 216 (81.81%) isolates from PLWHA and taking antibiotics (HIV⁺A⁺), 209 (96.76%), 198 (91.67%) and 188 (87.04%) isolates were resistant to trimethoprim-sulphamethazole, tetracycline and ampicillin respectively. The results also indicated that isolates from this group were highly resistant to cefuroxime and augmentin (51.39% and 58.33%). Thirty nine (83.33%) isolates of the 48 (18%) from PLWHA not taking antibiotics (HIV⁺A⁻) were resistant to trimethoprim-sulphamethazole. All the isolates from PLWHA not on antibiotics were sensitive to fluoroquinolones and nalidixic acid but less than 50% isolates were resistant to tetracycline, ampicillin and gentamicin, augmentin, cefuroxime, cefazidime and nitrofurantoin. Resistance trends increased with time in isolates from HIV⁺A⁻ to HIV⁺A^{+2.0} for nearly all the antibiotics with the most prevalent resistant pattern of Sxt^R Tet^R Amp^S Amc^S Gm^S Cxm^S Caz^S Na^S Chl^S Nit^S Nor^S Cip^S ($P \geq 0.05$). The minimum inhibitory concentration (MIC) of the antibiotics tested from HIV⁺A⁺ was much higher at $P \geq 0.05$ than for isolates from HIV⁺A⁻ for all the antibiotics except for ampicillin and augmentin that had the same MIC mode of 32 and 16 μ g/ml in the two groups. Plasmid analysis showed that Multi drug resistant (MDR) strains carried plasmids ranging in size from >4MDa to 98MDa and were transferred to *E. coli* strain K12 F. Prophylactic use of antibiotics improves quality of life for PLWHA, but regular monitoring of resistance developed to these antibiotics must be enforced in order to curb increased MDR strains and reduce their spread. This concern should receive equal attention if not more than what is given to other components of HIV/AIDS awareness campaign. Increasing resistant trends to antibiotics in the HIV/AIDS era could result to a public health problem if left to persist.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Human immunodeficiency virus (HIV) is the causative agent of Acquired Immunodeficiency Syndrome (AIDS), an acute and often fatal disease of human beings, which is pandemic and has reached almost all parts of the world (UNAIDS, 2005). Among its most devastating effects is that it compromises the immune system hence exposing the infected people to opportunistic infections (OIs) which in turn make the infected to be on prolonged antibiotic use. Prolonged usage of antibiotics has been thought to increase resistance levels of *Escherichia coli* in the gut of persons living with HIV/AIDS (PLWHA). The pandemic has led to increased morbidity and mortality and created widespread resistance of bacteria to antibiotics among populations.

AIDS was first reported in the United States in 1981 (CDC, 2005) and since then it has become the most devastating disease facing mankind (CDC, 2005). The AIDS epidemic began during the same period in North America, Western Europe and Sub-Saharan Africa (Bongaarts, 2003). Currently, HIV the causative agent of AIDS has spread to all corners of the world and the epidemic shows no sign of abating. By the end of the year 2007, it was estimated that 33.2 million people globally were infected with HIV (Table 1). One third of the infected persons were between the ages of 15-24 years. Ninety five percent of new infections occur in the developing world and almost 50% are women (UNAIDS, 2007). The current HIV/AIDS global overview report still gives alarming figures on the pandemic, Sub-Saharan Africa accounts for 68% of the cases. Death related to AIDS globally was reported to be 2.1 million in 2007, 1.7 million adults and 330,000 children less than 18 years (NASCO, 2007).

Table 1: Global estimates of HIV From 2001-2007

Global Estimates (in millions)							
	2001	2002	2003	2004	2005	2006	2007
People living with HIV	29.0	30.0	30.9	31.6	32.1	32.7	33.2
New infections	3.2	3.1	3.0	2.9	2.8	2.7	2.5
Deaths	1.7	1.9	2.0	2.1	2.2	2.1	2.1

(Source: UNAIDS, 2007)

The first HIV/AIDS case in Kenya occurred in 1978 but it was officially reported six years later by the Kenyan Ministry of Health (Obel *et al.*, 1984). The government was quick in response by establishing the National AIDS Control Council (NACC) in 1985 to coordinate all Aids programmes in Kenya with the support of the Global AIDS Programme (GAP) (NASCO, 2005). However it was not until 1990 that HIV/AIDS surveillance became functional in Kenya. In November 25th 1999, HIV/AIDS was declared a national disaster by the then president of the republic of Kenya, Hon. Daniel Toroitich Arap Moi. Since then several systems have been adapted for surveillance, which include: HIV sentinel surveillance in patients seeking treatment for sexually transmitted infections (STI), monthly reporting of AIDS cases, monthly reporting of HIV positive results of donated blood, Voluntary counseling and testing (VCT), Prevention of mother to child transmission (PMTCT), Behavioral surveillance survey, Reporting of new cases through the integrated Disease Surveillance and Response (IDSR).

The first sentinel survey report in 1990 showed that the HIV infection prevalence in Kenya was 5.1% which increased steadily from 1991 (6.3%), 1992 (7.4%), 1993 (8.5%), 1994 (9.5%), 1995 (10.4%), 1996 (11.2%), 1997 (11.9%), 1998 (12.5%), 1999 (13.0%) to 13.4% in the year 2000 (MOH Kenya, 2005; NACC Kenya, 2007). The National HIV prevalence showed a notable decline to 10.4%, 6.7% and 5.1% in 2002, 2003 and 2007 respectively (NASCO, 2005). This was attributed partly to significant behavioral change and increased access to antiretroviral treatment (ART). However, this

was difficult to quantify, as several factors needed to be considered including AIDS deaths, migrations or actual decline in new infections. The prevalence rates of HIV further shot to 7.4% in June 2008 (KAIS, 2008). This was attributed to PLWHA taking antiretrovirals (ARVs), regaining their health and having more sexual partners than before (WHO, 2008). Currently, the estimated prevalence of HIV infection in adults aged 15 to 49 years in Kenya per province is; Nairobi 175,000 (9.0%), Central 240,000 (3.8%), Coast 135,000 (7.9%), Eastern 380,000 (4.7%), North Eastern 15,000(1.0%), Nyanza 480,000 (15.3%), Rift valley 390,000 (7%) and Western 210,000 (5.1%) (KAIS, 2008).

Kenya is therefore home to over 2 million persons living with HIV infections. These persons may succumb to AIDS which renders them susceptible to opportunistic infections. Since 2000, over 1.5 million Kenyans have died of AIDS related diseases and 233 people die daily from HIV related complications (UNAIDS, 2007). Several antibiotics are recommended for prophylaxis and treatment of these infections but the increased usage of these antibiotics has lead to the development of multi drug resistant strains of normal flora for example *E. coli* in HIV sero-positive persons. The current study investigated the antibiotic resistance trends and patterns to commonly used antibiotics by PLWHA and attending Mbagathi District Hospital.

1.2 Problem Statement and justification

Several studies have shown that use of antimicrobial treatments produces selective pressure for resistance. This is particularly common in people who are on prolonged antibiotic therapy. Few studies in sub- Saharan Africa have assessed the impact of prolonged antibiotics exposure in HIV infected persons and even attempted to genotype resistant strains of *E.coli* isolated from HIV positive patients. Most available data are specific to certain pathogenic organisms and antibiotic resistance trends as well as patterns over time in these regions are rarely followed. However, prolonged antibiotic therapy

in HIV infection has caused widespread emergence of drug resistant strains of *E. coli* (Cornaglia *et al.*, 2003) hence exacerbated the problem of controlling microbes in PLWHA and resurgence of bacterial diseases worldwide.

There is little data on antibiotic resistance patterns and trends of *E. coli* isolated from HIV positive patients in Kenya. The current study sought to fill this gap in knowledge which would impact the care and management of enteric infections in PLWHA.

1.3 Research Hypothesis

Persons living with HIV/AIDS and on prolonged antibiotic therapy harbor strains of *Escherichia coli* that are significantly more resistant to antibiotics.

1.4 General objective

To determine the patterns and trends in resistance to antibiotic and the presence of transferable plasmids in strains of *E. coli* isolated from stool samples of persons living with HIV/AIDS (PLWHA).

1.4.1 Specific objectives

1. To isolate and identify strains of *E. coli* from persons living with HIV/AIDS (PLWHA) and attending Mbagathi's District Hospital (MDH) Comprehensive Care Centre (CCC)
2. To carry out *in-vitro* antimicrobial susceptibility tests on *E. coli* isolates
3. To determine the presence of self transferable resistance (R) - plasmids in antibiotic resistant *E. coli*.

1.4 Significance

The findings of this study will have major implications on public health policy guidelines on antimicrobial resistance in persons living with HIV/AIDS (PLWHA) and be valuable in antimicrobial resistance (AMR) detection modules and intervention schemes in order to slow down the emergence and reduce the spread of antimicrobial resistant *E. coli* species in PLWHA. It can also help in planning of regular monitoring of antibiotic resistance trends, patterns and development of resistance (R) plasmids to provide the basis for developing rational prescription guidelines, making policy decisions and assessing the effectiveness of both.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Acquired Immunodeficiency Syndrome.

Human Immunodeficiency Virus (HIV) is the causative agent of AIDS. It is often a fatal disease of human beings that results from the proliferation of the virus in infected host lymphocytes that invade lymphoid and non – lymphoid tissues (Gilks, 2005). The Virus destroys the immune system hence exposing the infected to opportunistic infections.

There are various ways by which HIV is transmitted but the most common identified ways of transmission in Kenya are heterosexual contact, perinatal transmission and blood transfusion (NASCO, 2005). 86% of HIV cases in Kenya occur as a result of heterosexual transmission (Kreiss *et al.*, 2006). Prevention addressing sexual behaviour has been widely advocated. This includes abstinence, non promiscuity and use of condoms. Children born of infected mothers have a higher chance of being infected perinatally. Over 100,000 children under the age of five years are infected with HIV (NACC Kenya, 2007). Although anti-retroviral drugs have been shown to protect babies from perinatal infection (Petra study team, 2002), the drug is not available to the larger population of infected expectant mothers.

Transmission via blood transfusion is rare since almost 100% of blood available for transfusion is screened for HIV. Potential risks of infection through contaminated blood and body fluids are usually limited to some groups such as health workers, traditional birth attendants and traditional circumcisers. Universal precautions of wearing gloves and use of sterile piercing equipment are also advocated (NASCO, 2007).

2.2 Opportunistic infections associated with AIDS

The HIV destroys the immune system hence exposing the infected to opportunistic infections (OIs). These are illnesses that occur because of a weakened immune system. They are clearly the most important complications of HIV infection and they cause considerable morbidity and mortality (Kaplan *et al.*, 2003) in PLWHA. These infections occur with greater frequency and severity in HIV-infected persons because of immunosuppression (Kaplan *et al.*, 2003). Following the rapid spread of HIV and AIDS, a plethora of opportunistic infections have been reported from various regions of the world. More than 100 pathogens including viruses, bacteria, fungi, protozoa and helminthes have been implicated as opportunistic infections in HIV – infected persons. In 1996, the United States Public Health Services (USPH) and Infectious Disease Society of America (IDSA) published guidelines for prevention of opportunistic infections in PLWHA. These infections were *Pneumocystis carinii* pneumonia, toxoplasmic encephalitis, cryptosporidiosis, tuberculosis, microsporidiosis, bacterial respiratory infections, bacterial enteric infections, Bartonella infection, candidiasis, cryptococcosis, histoplasmosis, coccidiomycosis, cytomegalovirus infection, herpes simplex virus infection, varicella – zoster infection and human papilloma virus infection (USPHS/IDSA, 2001). The infections selected are important in terms of incidence, morbidity, mortality and the guidance can be effectively used in North America and other industrialized regions of the world. However, applicability of such guidelines in developing countries remains unclear because of changed patterns of opportunistic infections (Corbett *et al.*, 2003)

Like other developing countries, especially those in Africa, Kenya has its burden of opportunistic infections to reckon with. Although hampered by overstretched diagnostic services, which are lacking in many parts of the country, a number of studies have been carried out to elucidate the relevant etiological agents (Kaplan *et al.*, 2003). Tuberculosis is now recognized as one of the most common

and devastating opportunistic infection in PLWHA (Johnson *et al.*, 2003; Kivihya *et al.*, 2004). In Kenya, Kivihya *et al.* (2004) showed that there was an increase in pulmonary tuberculosis infection as a result of HIV infection. The study demonstrated that tuberculosis infection increased with a decrease in CD4 lymphocyte count. During the same period, multiple drug resistant strains of *Mycobacterium tuberculosis* were detected by Githui *et al.* (2005). Disseminated *Mycobacterium avium intracellulare* (MAI) infection has also been reported in Kenya (Gilks *et al.*, 2002), although it was not detected in Ugandan patients with AIDS (Okello *et al.*, 2005).

Chronic diarrhoea is also a common clinical feature in African AIDS patients (Dallabeta and Miotti, 2002). Aetiology of HIV – associated diarrhoea has been investigated in previous studies in Kenya (Mwachari *et al.*, 1998; Batchelor *et al.*, 2003; David *et al.*, 2006). The spectrum of enteropathogenic bacteria isolated from PLWHA reporting with diarrhoea was similar to that detected from HIV negative persons with diarrhoea. Mwachari *et al.* (1998) and Batchelor *et al.* (2003) also reported on the spectrum of diarrhoeagenic agents in PLWHA. These were pathogenic *Escherichia*, *Salmonella*, *Shigella*, and *Campylobacter*. *S. typhimurium*, *E. coli* and *S. flexneri* were the most commonly isolated species. Newly recognized parasitic pathogens were also isolated, the most significant being *Cryptosporidium parvum* (Batchelor *et al.*, 2003). Pneumococcal disease is also an important feature of opportunistic infections and *Streptococcus pneumoniae* has been grossly implicated in these infections (Gilks *et al.*, 2003). Microbiology of bacteremia in PLWHA presenting with fever has also been reported (Petit *et al.*, 2001; Gilks *et al.*, 2003). Enterobacteriaceae featured significantly in causing life threatening infections. The most prevalent species isolated being *E. coli*, *Salmonella*, *Klebsiella* and *Enterobacter*.

Other Gram negative organisms include *Pseudomonas aeruginosa*, *Bacteriodes fragilis* and *Neisseria meningitidis*. Gram positive organisms isolated were *Staphylococcus aureus* and *Streptococcus*

species. Fungi isolated were *Candida* and *Cryptococcus neoformans* (Batchelor *et al.*, 2003; Catry *et al.*, 2003; Lori *et al.*, 2003). The HIV pandemic has therefore changed the pattern of medical needs, care and response as dictated by opportunistic infections. Control of these infections improves the quality of life of Persons living with HIV/AIDS.

2.3 Management of opportunistic infections

Opportunistic infections reduce the quality and length of life for PLWHA. Globally, emphasis was initially focused on chemoprophylaxis against *Pneumocystis carinii* (USPHS/IDSA, 2001) followed by chemoprophylaxis against disseminated *Mycobacterium avium* complex (MAC) (Masur, 2003). Trimethoprim–Sulfamethoxazole (SXT) is the preferred drug for *Pneumocystis carinii* pneumonia (PCP), toxoplasmosis and other bacterial infections (USPHS/IDSA, 2003). The risk to contract active tuberculosis can be stalled by preventive therapy (Quigley *et al.*, 2001). This is recommended in areas where diagnosis of latent TB is possible, for instance by the use of the tuberculin skin test. Isoniazid therapy with pyridoxine supplementation for nine months is administered when a positive case is detected by the tuberculin skin test. (Wilkinson *et al.*, 2000; Bucher *et al.*, 2003). With the recognition of opportunistic infection, the number of chemo prophylactic agents has also increased. The USPHS/IDSA (2003) has published disease – specific recommendations, which deal with seventeen opportunistic infections. These recommendations address prevention of exposure, disease and its recurrence.

The Kenya Ministry of Health has developed guidelines to be followed in the managing of opportunistic infections (NASCO, 2001). It is important to take note of the antibiotics included in the guidelines. The antibiotics recommended include benzyl penicillin, gentamicin, ceftazidime and cefuroxime for pneumonia, ciprofloxacin for *Salmonella* and *Shigella* infections and trimethoprim-

sulphamethazole, and azithromycin for most bacterial infections. Anti-retroviral therapy is the most effective means of reducing opportunistic infections (Palella *et al.*, 2002; Levy *et al.*, 2003). However antiretroviral drugs cannot be instant substitutes of antimicrobial prophylaxis because they only reduce the viral load but not bacterial infection. Antibiotics will not only eliminate target microbes, but will also have an impact on normal intestinal biota including *Escherichia coli*.

2.4 General characteristics of *Escherichia coli*

E. coli was first described by Theodore Esherich in 1885 and it was considered a non-harmful important member of the gut as its presence in the intestines is necessary for proper development and operation of the human body (Popoff *et al.*, 200; Nataro and Kaper, 2004). It is now thought to be an important opportunistic pathogen in some parts of the body with increasing antimicrobial resistance (Popoff *et al.*, 2001; Winokur *et al.*, 2001; Mitema *et al.*, 2004; Smith *et al.*, 2005). It belongs to the family enterobacteriaceae and it is the most significant species in the genus *Escherichia* (Smith *et al.*, 2005). It is a Gram negative bacillus that lives in the intestinal tract of humans and usually becomes an opportunistic pathogen when it gains access to extra intestinal organs and tissues such as urinary tract, bloodstream and wounds (Pratt and Taylor, 2003).

E. coli usually produces dry, pink (lactose positive) colonies with a surrounding pink area of precipitated bile salts on MacConkey agar and the colonies are occasionally mucoid. They are facultative anaerobes and grow on KCN. A large number of *E. coli* strains are motile, have sex pili and are able to grow on simple nitrogen and carbon compounds. They also possess capsules and fimbriae but not spores (Popoff *et al.*, 2001; Smith *et al.*, 2005). Biochemically, *E. coli* forms gas from glucose, ferment lactose, are indole positive, methyl red positive, Voges proskauer negative, oxidase negative, they do not utilize citrate, urease negative and they do not liquefy gelatin (Farmer *et*

al., 2004; Ewing, 2006). They have been shown to be prolific in the environment as a result of faecal contamination and survive for weeks in optimal conditions in the environment (Brenner, 2005). Intestinal strains of *E. coli* are the primary cause of urinary tract infections, septicemia, diarrhoea, neonatal meningitis and nosocomial infections. Individuals who are debilitated or have other predisposing factors are at much higher risk of infection than healthy persons (Levy, 2000). In developing countries *E. coli* have been reported to be the leading cause of diarrhoeal diseases in addition to pathogens such as *Salmonella*, *Shigella*, *Yersinia*, *Vibrio*, *Campylobacter* species, *Entamoeba histolytica*, and *Giardia lamblia* (Pratt and Taylor, 2003).

Although most strains are harmless, several others are known to produce toxins while others possess O and H antigens that can induce diarrhoeal diseases and other extra intestinal infections (Kelly *et al.*, 2002). Over the years strains of *E. coli* causing diarrhoea world wide, mostly in infants have been classified depending on their mechanisms of producing gastroenteritis. These groups are enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohaemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC) and enteroadherent *E. coli* (EAEC) or diffusely adhering *E. coli* (DAEC) (Patterson, 2002; Levin *et al.*, 2000; Berge *et al.*, 2003; Pratt, 2003).

The application of nucleic acid techniques such as DNA-DNA hybridization and 16S rRNA sequencing has resulted not only in the discovery of many new strains but proposed reclassification of older ones (Farmer *et al.*, 2004; Brenner, 2005).

2.5 Clinical Significance of *E. coli*

When Theodore Escherich first discovered *E. coli* in 1885, it was considered a normal flora. Since then it has accounted for 80% of significant isolates of enterobacteriaceae from stool specimens received in

clinical microbiology laboratories (Kelly *et al.*, 2002). There are six major categories of diarrhoeagenic *E. coli* diagnosed to cause gastroenteritis in human beings. These include: enterotoxigenic *E. coli* (ETEC) that produces a toxin resulting in travelers diarrhoea, enteroinvasive *E. coli* (EIEC) that penetrates the epithelial cells lining the intestinal mucosa hence diarrhoea, enteropathogenic *E. coli* (EPEC) that causes infantile diarrhoea, enterohaemorrhagic *E. coli* (EHEC) that causes diarrhoea as a result of haemolysin production and enteroaggregative *E. coli* (EAEC) that causes prolonged diarrhoea (Levin *et al.*, 2000; Patterson, 2002; Berge *et al.*, 2003). The enteropathogenic *E. coli* (EPEC) strain has been recognized to cause infantile diarrhoea since the 1940's. Particular O serogroups of EPEC were acknowledged in the late 60's and 70's as a cause of diarrhoea, but only particular groups of H types within each serogroup were associated with intestinal infections. Studies in 1998 now show that EPEC strains cause distinct diarrhoea (Neuhauser *et al.*, 2002). Diarrhoea outbreaks due to EPEC have occurred in hospital nursery's and day care centers but cases in adults are rare. Case characterization is established by the presence of low-grade fever, malaise, vomiting, and diarrhoea (Wiktor *et al.*, 2007). Contaminated stool contain large amounts of mucus, but blood is not usually present.

The significance of *E. coli* in diarrhoea has been reported in Kenya. Studies have been conducted on etiological agents of childhood diarrhoea by *E. coli* (Kariuki *et al.*, 1997, 1999; Mwachari *et al.*, 1998; Senerwa *et al.*, 2001; Sang *et al.*, 2003). In 1982, the O157:H7 strain of *E. coli* was first recognized during an outbreak of haemorrhagic diarrhoea and colitis in the United States (Nataro and Kaper, 2004). Strain serotype O157:H7 of the enterohaemorrhagic *E. coli* (EHEC) has since then been associated with haemorrhagic diarrhoea, colitis, and haemolytic uremic syndrome (HUS). HUS is characterized by low platelet count, hemolytic anemia, and kidney failure.

Extra intestinal infections caused by *E. coli* can at times supersede disease episodes by other micro-organisms. These infections include pneumonia, bacterimia, meningitis, septicaemia, abscesses, wound infection and urinary tract infections (Diekema *et al.*, 2000; Edmond *et al.*, 2000; Abbott, 2001). A surveillance report on nosocomial infections in the USA between 1986 to 1989 and 1990 to 1996 indicated that *E. coli* was the most isolated bacterium in urinary tract infections (Diekema *et al.*, 2000). Other enteric bacteria that featured in the infections were Enterobacter (6.2%), Klebsiella (5.4%), Proteus (2.3%), Serratia (1.5%), and Citrobacter (1.4%).

With the advent of HIV infection, *E. coli* has become the major cause of acquired bacterimia (Gilks *et al.*, 2002). Organisms in this group are a primary target for bombardment with antibiotics. Presence of antibiotics in the gut kills them or may induce development of antibiotic resistance.

2.6 *E. coli* and Drug resistance

Commensal bacteria which are naturally occurring host enteric flora constitute an enormous potential reservoir of resistant genes for pathogenic bacteria (Turnidge, 2004; Bartoli *et al.*, 2004). The prevalence of resistance in commensal bacteria in humans may reflect the selective pressure of antimicrobial usage and is a potential indicator of antibiotic resistance (Anderson *et al.*, 2003). *E. coli* has served as an indicator of AMR for Gram negative enteric bacteria and the emergence of strains showing multi-resistance in the public health sector (Guerra *et al.*, 2003). *E.coli* are used internationally as indicator bacteria for AMR because of their high prevalence in human faeces and also, because they harbour several resistance determinants (Witte, 2000; Catry *et al.*, 2003). The abundance of *E.coli* implicates them as likely candidates for the spread of resistance genes and vectors between the bacterial populations in humans; however, their abundance also makes such spread difficult to trace.

There has historically been a disagreement among scientists over the ability of some commensal strains of *E. coli* being pathogenic in HIV infected individuals (Caswel *et al.*, 2004). Recent research has indicated that all *E. coli* isolates from HIV positive patients are capable of causing an infection (Bogaard *et al.*, 2001). Studies employing molecular methodologies have found pathogenic *E. coli* strain differences in HIV positive and HIV negative isolates even when the two species are in close contact (Okoli *et al.*, 2002; Kariuki *et al.*, 2003). Within Gram negative bacteria, a substantial portion of resistance genes present on plasmids and transposons are incorporated into class 1 intergrons (Winokur *et al.*, 2001; Leverstein – van Hall *et al.*, 2002a).

2.7 Mechanisms of drug resistance

There are two forms of AMR in bacteria: intrinsic and acquired. Intrinsic or natural resistance is widespread in bacteria and results from evolutionary adaptation of bacteria to the environment (Heinemann, 2001). The inability of the antimicrobial to penetrate a bacterial cell and the lack of a target for the antimicrobial agent to act against, are all natural bacterial adaptations which result in AMR (Mitema *et al.*, 2004). Acquired resistance may be displayed in two forms: by the microorganisms withstanding relatively high levels of a specific antimicrobial agent. This could be as a result of either single or multiple step mutation in genes responsible for antibiotic uptake or binding sites, or may result from the lateral transfer of resistance traits between bacteria of the same species, of a different species, or acquired from the environment by a process known as transformation (McManus, 2000; Apley *et al.*, 2003). It may also be by acquisition of mobile extra chromosomal DNA elements such as plasmids, transposons and integrons rather than mutation (Heinemann, 2001).

A plasmid is a circular body of double stranded DNA which is separate from the chromosome and carries genes that encode various traits such as virulence and AMR (Kaye *et al.*, 2000). There are two types of plasmids: conjugative and non-conjugative. Conjugative plasmids transfer resistance via the

sex pili whereas non-conjugative plasmids must have direct contact for transfer to occur. In non-conjugative transfer, both the donor bacteria and the recipient bacteria have a copy of the transferred plasmid. Conjugative transfer is an important mechanism in AMR because transfer can occur in a wide range of bacterial species and can spread to unrelated organisms. Moreover, a single plasmid can contain multiple genes conferring resistance to multiple classes of antimicrobials (Akkina and Johnson, 2003).

Bacteria can also acquire DNA via transduction and transformation. Transduction occurs when DNA is transferred via bacteriophages, which are viruses that attack bacteria. Bacteriophages are very tightly packaged and do not have room to carry DNA. They also have a narrow host range, and as a result transduction is a less important mechanism for resistance gene transfer (Akkina and Johnson, 2003). Transformation occurs when bacteria pick up free DNA from the environment. While the presence of DNA is common after cell lysis, the compatibility between the free DNA and the intact recipient is narrow (McManus, 2000). Additionally, free DNA in the environment would be highly susceptible to digestion by nuclease. As a result, transduction and transformation are not thought to contribute significantly to the dissemination of AMR (Chopra *et al.*, 2001).

A transposon is a genetic element that contains an insertion sequence at each end. The insertion sequence allows the gene to jump to different locations on the chromosomal DNA, from plasmid to chromosome or from chromosome to plasmid (Wallis, 2000). Movement of a transposon is known as transposition and represents an important facet of AMR transfer because resistance genes can be moved from a non-conjugative plasmid or chromosome to a conjugative plasmid, and hence easily transferred to other bacteria (Emmanuel De *et al.*, 2001).

Integrations are genetic units that include the determinants of the components of a site-specific recombination system called gene cassettes (Hall and Collins, 2001). The essential components of an

integron are an *Int* gene which encodes a site-specific recombinase belonging to the integrase family, an adjacent site *attI*, which is recognized by the integrase and is the receptor site for the gene cassettes, and a promoter suitably oriented for the expression of the cassette-encoded genes (Hall and Collins, 2001). The integron is composed of a 5' conserved sequence (CS) (the integrase gene) and a 3' CS, which can vary for the four different classes of integrons; classes 1 through 4 (Roe *et al.*, 2003a). The majority of integrons described to date are class 1 integrons and the majority of those are associated with *su II*, a gene commonly found within the 3' conserved sequence (Fluit and Schmitz, 2000; Naas *et al.*, 2001). AMR gene cassettes are not always present in integrons, and the integrase gene (*Int*) can excise gene cassettes as covalently closed supercoiled circular molecules. Integron gene cassettes can be deleted, rearranged and duplicated within the integron (Wiktor *et al.*, 2007). Integron mediated AMR is a major mechanism for transfer of resistance traits within Gram-negative bacteria (Leverstein-van Hall *et al.*, 2002a; O'Brien, 2002; Roe *et al.*, 2003b; Mathai *et al.*, 2004). Integrons have been found to harbor the majority of resistance genes within the mobile resistance elements (transposons and plasmids), which allow for the transfer of resistance between bacteria (Hall and Collins, 2001; White *et al.*, 2001; Roe *et al.*, 2003a; Levesque *et al.*, 2003). Within the integron, more than 60 gene cassettes have been found that confer resistance to a variety of agents (White *et al.*, 2001). Of these, the most prevalent genes are those coding for aminoglycosides and trimethoprim resistance (Fluit and Schmitz, 2000; White *et al.*, 2001). Integrons also represent an important mechanism for transfer of resistance characteristics from commensal to pathogenic organisms and have been found to harbor multiple resistance genes (Goldstein *et al.*, 2001; Maguire *et al.*, 2001; Zhao *et al.*, 2001).

2.7.1 Molecular mechanisms of drug resistance

Antibiotics have targets, which are usually functional proteins such as enzymes and ribosomal proteins. The interaction between an antibiotic and target moiety is often quite specific. Alteration of target protein through mutations renders the bacterium resistant to drugs. A single amino acid change in the enzyme alters the sensitivity of the target for β -lactams, macrolides, and folate acid synthesis antagonists. Modification of penicillin binding protein (PBP) can affect the affinities of these molecules for β -lactam antibiotics. Mutations in *gyrA* gene leads to reduced affinity of the subsequent mutated DNA gyrase to quinolones. Mutations in genes coding for dihydropteroic acid synthesis (DHPS) and dihydrofolate reductase (DHFR) reduce affinity for sulfamethazole and trimethoprim respectively (Summers, 2002).

Burns *et al.* (2003) suggested that decreased sensitivity to chloramphenicol was due to low intracellular concentration of the drug. Resistance was observed even in the absence of chloramphenicol acetyltransferase. Decreased membrane permeability involves alteration of the outer membrane porins. Emmanuelle De (2001) demonstrated antibiotic resistance in *Enterobacter aerogenes* by inducing structural modification of the major porin. Decreased membrane permeability probably also enhances other mechanisms of antibiotic resistance (Nikaido, 2001; Pratt and Taylor, 2003).

Antibiotics can be inactivated by enzymatic cleavage or by chemical modification upon which the drug is rendered ineffective (Lancini, 2000; Pratt and Taylor, 2003). The degrading enzymes can be encoded on genes including transferable ones. Classical examples of these enzymes are the β -lactamases (Rice *et al.*, 2003), acetyltransferases and aminoglycosides modifying enzymes (Lancini, 2000).

Shifting to an alternative pathway is another way by which bacteria develop drug resistance. Organisms are able to utilize substrates from the immediate environment, bypassing the target enzyme. *Enterococcus* strains for example can resist activity of trimethoprim by using available folic acid (Bellaj *et al.*, 2003).

Bacteria may fail to metabolize the antibiotic into its active derivatives. This occurs when the drug needs to be converted by the bacterium into an active derivative. *Bacteriodes* are resistant to metranidazole as the bacteria cannot convert the drug to active metabolite (Fluit and Schmitz, 2000)

2.7.2 Plasmid mediated resistance

Plasmids are extrachromosomal DNA capable of autonomous replication (Mayer, 2000). The most important are the R-plasmids which code for antibiotic resistance and carry the fertility (F) factor. The F- factor is crucial in conjugation process, which eventually facilitates in the transfer of resistance genes/plasmids (Shears, 2003). Plasmids play an important role in the transfer and acquisition of antibiotic resistance (Mayer, 2000).

Plasmids coding for antibiotic resistance were first recognized in Japan in 1959 (Akiba *et al.*, 2005) following an outbreak of dysentery caused by multiple drug resistant (MDR) strains of *Shigella*. This was the foremost observation of multi-drug resistance among enterobactericiaeae (Akiba *et al.*, 2005). The use of plasmid profiles in tracing the spread of antibiotic resistance was later reviewed by Mayer. The R-factor was reported to be responsible for multidrug resistance (MDR) in *Klebsiella pneumoniae* isolates causing nosocomial infections in USA (Courtney *et al.*, 2003). Epidemics of dysentery in Burundi since 1979 were caused by multiple drug resistance *Shigella dysentery* type 1 (Ries *et al.*, 2000). Ezaki *et al.* (2002) identified an R-factor in *E. coli* which carried genes coding for lactose fermentation and drug resistance.

Several studies have since been carried out in Kenya on antimicrobial resistance of *E. coli* particularly entero-pathogenic strains (Githui *et al.*, 2005). Studies were done on *E.coli* to determine its antimicrobial activity and the type of resistance they have. The organisms were isolated from patients attending hospitals or in disease outbreak (Kariuki *et al.*, 2003; Oundo *et al.*, 2007). Drugs tested included: ampicillin, trimethoprim-sulfamethazole, chloramphenical, tetracycline, streptomycin, nalidixic acid and gentamicin. Except for the last two, prevalence was 100% for the other antibiotics. Molecular characterization of multidrug resistance strains confirmed that drug resistance of *E. coli* isolated from Kenyan children was plasmid mediated (Kariuki *et al.*, 2001). Therefore the importance of plasmid mediated resistance in such organisms and its potential transfer to other enteric bacteria cannot be overemphasized. In several decades, the spectrum and frequency of bacterial infections by antimicrobial resistance have increased in both the hospital and community (Cohen, 2002; Neu, 2002).

2.9 Antibiotic resistance following the HIV/AIDS pandemic

The use of antibiotics in the management of HIV/AIDS associated infections presents a potent opportunity for antibiotic resistance (Martin *et al.*, 1999). In the study carried out by Martin *et al.* (1999), it was reported that less than 5.5% drug resistance was observed before the AIDS era. This resistance increased to 25.4% after the pandemic. Increased multidrug resistance and selective colonization following prolonged antibiotic therapy was also reported in several other studies (DeMan *et al.*, 2000). Kenya has more than 1.4 million people living with HIV/AIDS (WHO, 2008). Although antiretroviral drugs which delay onset of AIDS have been shown to be effective (Lori *et al.*, 2005), the cost of such drugs is beyond the reach of the majority who are infected with HIV in the developing world (Richman, 2006). Treatment of opportunistic infections therefore remains the common practice. Kenyans with AIDS are exposed to various opportunistic infectious diseases including tuberculosis (Githui, 2000), diarrhoea (Batchelor *et al.*, 2003), bacteraemia and fever (Petit *et al.*, 2001; Gilks,

2005), bacteriuria (Ojoo *et al.*, 2006) among others. Guidelines prepared by the National AIDS'/STI control program (NAS COP, 2002) under the Ministry of Health should be followed for the management of opportunistic infections (Okeyo *et al.*, 2006). In spite of intensive laboratory investigations, Mwachari *et al.* (1998) were unable to detect any pathogen in almost half of the patients they investigated. Therefore the use of antimicrobials in persons with AIDS in most cases would be based on symptoms. This may encourage use of broad- spectrum antibiotics and could have an impact on the normal intestinal flora.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

The study was carried out at Mbagathi District Hospital (MDH) in Nairobi, which is an Infectious Disease Hospital (IDH) and has a Comprehensive Care Center (CCC) for HIV infected people and at the Kenya Medical Research Institute (KEMRI). MDH serves as a District Hospital for Nairobi residents. The largest population served by the study hospital are residents of Kibera and Dagoreti Divisions in Nairobi. The hospital attends to about 200 out-patients daily and has a bed capacity of 169. The average number of inpatients in a given day is 90-120. Over 50% of the inpatients have been reported to have HIV/AIDS (2007)

3.2 Study population

The study population was persons living with HIV/AIDS (PLWHA) who were attending MDH Comprehensive Care Center (CCC). Admission of PLWHA into the study was based on the following criteria; difficulty in breathing with elevated body temperatures, vomiting but without gastroenteritis or profuse diarrhoea. Other observations included persistent coughing, wasting and oral thrush. All cases had previously been confirmed to be HIV sero-positive.

3.3 Sample size determination

In a study on antibiotic resistance, Martin *et al.* (1999) reported less than 5.5% drug resistance in bacteria before the AIDS era and an increase to 25.4% after the pandemic started. This was thought to be due to increased and prolonged use of antibiotics. Assuming that the level of resistance is 5.5% in

healthy individuals, which can increase to 25.4% in persons with AIDS, the minimum sample size therefore was obtained using the following formulae: -

$$N = Z^2 \frac{1-\alpha/2 \{P1 (1 - P1) + P2 (1 - P2)\}}{P1 - P2} \quad (\text{Martin } et al., 1999)$$

Where:-

N = Sample size

P1 = Resistance in healthy subjects = 5.5%

P2 = Resistance in persons with AIDS = 25.4%

Z = Corresponding value to the 95% confidence interval

$1-\alpha/2$ = Level of significance (at 5%)

$$N = \frac{(95\%)^2 (5\%) \{5.5\% (1-5.5\%) + 25.4\% (1-25.4\%)\}}{5.5\% - 25.4\%}$$

In total 264 samples were collected and analyzed

3.4 Stool sample collection

In this study conducted from March to December 2007, 264 patients attending Mbagathi District Hospital (MDH) were recruited and their stool sampled randomly on Monday, Wednesdays and Fridays of March, April and May. Forty eight (48) samples were received from HIV sero-positive adults not taking antibiotics while two hundred and sixteen (216) from HIV sero-positive taking antibiotics. The samples were collected from the Comprehensive Care Centre (CCC) on Monday, Wednesday and Friday from 8:00 am to 12:00 am and at least 15 – 20 stool samples were received every day. The samples were collected in sterile wide mouth containers after obtaining informed consent from the patients. Demographic data on age, sex and antibiotics used in the preceding two years were also obtained for each individual from the interview and file records.

3.5 Isolation of *Escherichia coli*

Stool samples were transported to the laboratory in a cool box within 30 minutes of collection. The samples were then inoculated onto MacConkey agar and into Selenite-F broth using a sterile wire loop and the seeded plates/tubes incubated for 24h aerobically at 37°C (Pronadisa, Conda Laboratories, S.A). Cultures in Selenite- F broth were subcultured on MacConkey agar and incubated for 24h aerobically at 37°C.

3.6 Identification of *Escherichia coli*

Colonial morphology of the bacteria on the cultured plates was described and used to provide presumptive identification of *E. coli*. Gram staining of the colonies was done and recorded. Further identification of Gram negative lactose fermenting bacilli was done using biochemical tests. The bacteria were inoculated in triple sugar iron agar (Oxoid Ltd), Sulphur indole motility agar (SIM) (Oxoid Ltd), Simmons citrate agar (Oxoid Ltd), MR/VP broth and urea agar (Oxoid Ltd) and their reaction to oxidase reagent (1% dimethyl-p-phenylene-diamine dihydrochloride) noted. Confirmatory tests were done by the use of API 20E (bioMérieux, Basingstoke, United Kingdom) system in accordance with the manufacturer's instructions. API Lab^(R) software was used to interpret API-20E reactions. Organisms once identified were plated on nutrient agar and incubated for 24h at 37°C. After incubation, the bacteria were stored in 1 ml capacity cryotubes containing tryptic soy broth (Oxoid Ltd, Basingstoke, United Kingdom), containing 15% (V/V) glycerol at -70°C until further analysis.

3.7 Antibiotic susceptibility testing

3.7.1 Disk diffusion

Antibiotic susceptibility was performed on the isolates using Kirby – Bauer disk diffusion technique (Bauer *et al.*, 1996). Identified bacterial colonies were emulsified in sterile distilled water to conform

to 0.5 McFarland turbidity standard, which was then diluted ten times to give a density of approximately 10^6 CFU per ml for inoculation on Mueller Hinton agar (OXOID, UK). Using a sterile cotton swab, the suspension was uniformly swabbed on Muller Hinton agar (MH) (Pronadisa, Conda Laboratories, S.A). Antibiotics were aseptically applied on the seeded plates using a multidisk (Octodiscs; HiMedia Laboratories Ltd). The following antibiotic disks were used:- amoxicillin/clavulanic acid (25 μ g), sulphamethazole-trimethoprim (25 μ g), nalidixic acid (30 μ g), gentamicin (10 μ g), nitrofurantoin (30 μ g), norfloxacin (30 μ g), tetracycline (30 μ g), cefuroxime (30 μ g), ceftazidime (20 μ g), chloramphenicol (30 μ g), ampicillin (10 μ g) and ciprofloxacin (5.0 μ g). These antibiotics were chosen on the basis of their use in management of bacterial infections in PLWHA. The MH agar plates were then incubated aerobically at 37^oC for 18 – 24 h and diameter zones of inhibition measured and classified as sensitivity or resistance, all intermediate zone diameters were classified as sensitive. The results were interpreted according to the standard recommendations of the National Committee for Clinical Laboratory Standards (NCCLS, 2002). Media and antibiotic disks potency were quality controlled using commercially obtained *Escherichia coli* ATCC[®] 25922.

3.7.2 Minimum Inhibitory Concentration (MIC)

MIC was performed on MDR isolates using the E-test (AB Biodisk, Sweden). Identified bacterial colonies were emulsified in sterile distilled water to conform to 0.5 McFarland turbidity, which was then diluted ten times to give a density of approximately 10^6 CFU per ml for inoculation on Mueller Hinton agar (OXOID, UK). Using a sterile cotton swab, the suspension was uniformly swabbed on Muller Hinton agar (MH) (Pronadisa, Conda Laboratories, S.A) and the plates left to air dry on the bench. The E-test strips were aseptically placed in an equidistant radial fashion on the plates using a sterile forceps. The MH agar plates were then incubated aerobically at 37^oC for 18 – 24h.

The E-test MICs were read and interpreted directly from the test strip according to the instructions of the manufacturer, where the elliptical zone of inhibition intersected with the MIC scale on the strip when viewed from the upper agar surface with the plate lids removed.

3.8 Plasmid analysis

3.8.1 Harvesting and Extraction

Bacteria found to be Multi drug resistant (MDR) were selected for plasmid analysis using the protocol of Sambrook *et al.*, 1989. Bacteria stored at -70°C were subcultured on MH agar and incubated at 37°C for 24 h aerobically. Loopful growth of bacteria was transferred into 3ml of Luria – Bertani (LB) broth media in a loosely capped 15ml tube. The culture was incubated in a shaker at 37°C overnight. A volume of 1.5ml was transferred to Eppendorf tube and centrifuged at 13000g for 30 seconds at 4°C in a microcentrifuge (Tommy, Japan). The supernatant was removed by aspiration and the dry pellet resuspended in 100 μl of ice cold solution 1 (Appendix B) by gentle agitation. Bacterial cell wall was digested by addition of 200 μl of freshly prepared solution 11 (Appendix B) and on gentle shaking 150 μl of ice cold solution 111 was added to the preparation to precipitate the cell debris and protein material (Appendix B). The solution was centrifuged at 13000g for 5 minutes at 4°C in a microcentrifuge and the supernatant transferred to a fresh tube. The double stranded DNA was precipitated using 2 volumes of ethanol at room temperature, vortexed and allowed to stand for 2 minutes at room temperature (Birnboim and Doly, 1979). The mixture was centrifuged at 13000g for five minutes at 4°C in a microcentrifuge and the supernatant removed by gentle aspiration. The tubes were then placed in an inverted position and all the liquid allowed to drain. The pellet was rinsed using 1 ml of 70% ethanol at 4°C , the supernatant was removed and the pellet allowed to air dry for 10

minutes. Plasmid DNA was then redissolved in TE (pH 8.0) containing DNase free pancreatic RNase (20µg/ml).

3.8.2 Gel Electrophoresis

1% Agarose gel stained with 0.05% ethidium bromide was prepared with wells and mounted in an electrophoretic tank. Tris-borate (TBE) electrophoresis buffer (Appendix B) was added to cover the gel at a depth of about 1mm. The plasmid DNA was resuspended in Tris-phosphate – EDTA buffer (pH 8.0) and slowly 25 µl of the mixture loaded into the wells using disposable micropipettes. Plasmid DNA was subjected to electrophoresis at 100 volts DC for 3 hours. Plasmid DNA from standard strains of *E. coli* V517 and 39R861 (NCTC 50192) were used as molecular size markers.

Visualization of the bands was done using ultraviolet transilluminator (UVP, Upland USA) and pictures of the gel were taken using transmitted illumination camera fitted with a black and white Polaroid film.

3.8.4 Conjugation experiment

Conjugation experiment was performed as described in the manual of molecular cloning (Sambrook *et al.*, 1989). Resistant *E. coli* isolates (plasmid donors) were mated with standard strains of *Escherichia coli* K12 F- (plasmid recipients). The two strains were subcultured in 3 ml Brain heart infusion broth (Oxoid Ltd., Basingstoke, United Kingdom) and incubated at 37^oC for three hours in a rotating incubator. Brain heart infusion broth was freshly prepared and 4.5ml dispensed in universal bottles. Donor and recipient were diluted 1:10 by adding 0.5 ml of the three hour broth culture into 4.5 ml of freshly prepared broth. Duplicate mixtures of the donor and the recipient were made in equal proportions of 2ml each. One group of the mixture was incubated at room temperature and the other at

37°C aerobically for 24 h. The overnight culture were transferred into 1.5 ml tubes and centrifuged at 13,000xg for one minute in a microcentrifuge. The pellets were washed twice with sterile phosphate buffered saline by resuspending cells using a vortex mixer Using a sterile wire loop, each of the mixtures was inoculated onto:- (a) MacConkey agar containing 30µg/ml ampicillin and 30µg/ml nalidixic acid to select for transconjugants (b) MacConkey agar containing 30µg/ml ampicillin as control (only donor was expected to grow) and (c) MacConkey agar containing 30µg/ml of nalidixic acid as control (Only *E. coli* K12 was expected to grow Antibiotic susceptibility was repeated using the disk diffusion method and the presence of plasmids was used to confirm transferred resistance phenotype

CHAPTER FOUR

4.0 RESULTS

4.1 Data of the study population

In 2007, 264 patients attending Mbagathi District Hospital (MDH) were recruited and their stool samples examined after obtaining individual informed consent from them. All the 264 samples received during the study period were positive for *E. coli* growth (Table 2). 216 (82.0%) *E. coli* isolates were from HIV sero-positive adults taking antibiotics while 48 (18.0%) were not taking antibiotics. Age and sex of the population were recorded and the age category of 30 – 34 was the mean age of the population. Females had a percentage of 64.4% while males had 35.6%

Table 2: The age characteristics of the population.

Variable	Description
Age range	15 -50
Mean age	34.09
Standard deviation	7.184
Males	93 (35.2)
Females	171 (64.7)
Total	264

4.2 Physical appearance of stool samples

A total of 264 samples were collected, 216 (81.81%) from PLWHA taking antibiotics (HIV⁺A⁺) and 48 (18.18%) not taking antibiotics (HIV⁺A⁻) (Table 3). Presence of blood, mucus or both was noted. One hundred and thirty two (61.11%) of the stool samples from HIV⁺A⁺ and thirty (62.5%) from HIV⁺A⁻

were formed. Loose stool samples were collected from sixty four (29.63%) of HIV⁺A⁺ and fourteen (29.17%) from HIV⁺A⁻. Four (1.85%) loose/mucoid/blood stained and watery/mucoid were collected each from HIV⁺A⁺. Twelve (5.56%) of the watery samples were collected from HIV⁺A⁺ while four (8.33%) of them were collected from HIV⁺A⁻ (Table 3).

Table 3: Physical appearance of stool sample

Sample	Formed	Loose	LMB	Watery	WM	Total (N)
HIV ⁺ A ⁺	132 (61.11)	64(29.63)	4(1.85)	12(5.56)	4(1.85)	216(81.81)
HIV ⁺ A ⁻	30 (62.5)	14 (29.17)	0 (0.0)	4(8.33)	0(0.0)	48(18.18)

Legend:

HIV⁺A⁺ – Human immunodeficiency virus positive and taking antibiotics

HIV⁺A⁻ – Human immunodeficiency virus positive and not taking antibiotics

PLWHA – Persons living with HIV/AIDS

LMB – Loose, Mucoid and blood stained

WM – Watery and Mucoid

N – Number of stool samples

4.3 Distribution of *E. coli* into classes depending on time of antibiotic exposure

E. coli isolates from PLWHA were distributed into five classes depending on the length of time individuals patients had been exposed to antibiotics. The number of isolates tested in each category is as shown on table 4. All the isolates were resistant to either one or more antibiotics apart from six isolates from the HIV⁺A⁻ category that were sensitive to all the antibiotics tested. 87.5% of the HIV⁺A⁻

were resistant to either one or more antibiotics while all the isolates from the other classes were resistant to more than three antibiotics (Table 4)

Table 4: Number of isolates per the period of antibiotic use

Classes	HIV ⁺ A ⁻	HIV ⁺ A ^{+0.5}	HIV ⁺ A ^{+1.0}	HIV ⁺ A ^{+1.5}	HIV ⁺ A ^{+2.0}
Number tested (<i>n</i>)	48(18.18%)	40(15.15%)	60(22.73%)	52(19.70%)	64(24.24%)
No of isolates resistant	42	40	60	52	64

Legend: HI⁺A⁺ - Human Immunodeficiency Virus positive, HIV⁺A⁻ - and not taking antibiotics, HIV⁺A^{+0.5} -and taking antibiotics for six months, HIV⁺A^{+1.0} - and taking antibiotics for one yr, HIV⁺A^{+1.5} - and taking antibiotics for one and a half yrs, HIV⁺A^{+2.0} - and taking antibiotics for two yrs.

4.4 Antibiotic susceptibility testing by Kirby disk diffusion

4.4.1 Antimicrobial susceptibility of *E. coli* isolated from stool samples of PLWHA at MDH

The spectrum of antimicrobial resistance in the isolated organisms was not limited to a specific antibiotic. *E. coli* showed a percentage resistance difference of below 50% to nine antibiotics tested (Fig.1) but the isolates showed exceptionally high percentage resistance difference of 94.31%, 83.33%, and 78.78% respectively to trimethoprim-sulphamethazole, tetracycline and ampicillin. Susceptibility varied with antibiotics used whereby all the isolates from PLWHA showed a sensitivity profile ranging from 50 and 69% to cefuroxime, ceftazidime, chloramphenical, gentamicin and ampicillin. Isolates were highly sensitive to nalidixic acid (74.63%), ciprofloxacin (92.42%) and norfloxacin (93.56%) after incubating the seeded plates for 18 -24h at 37⁰C.

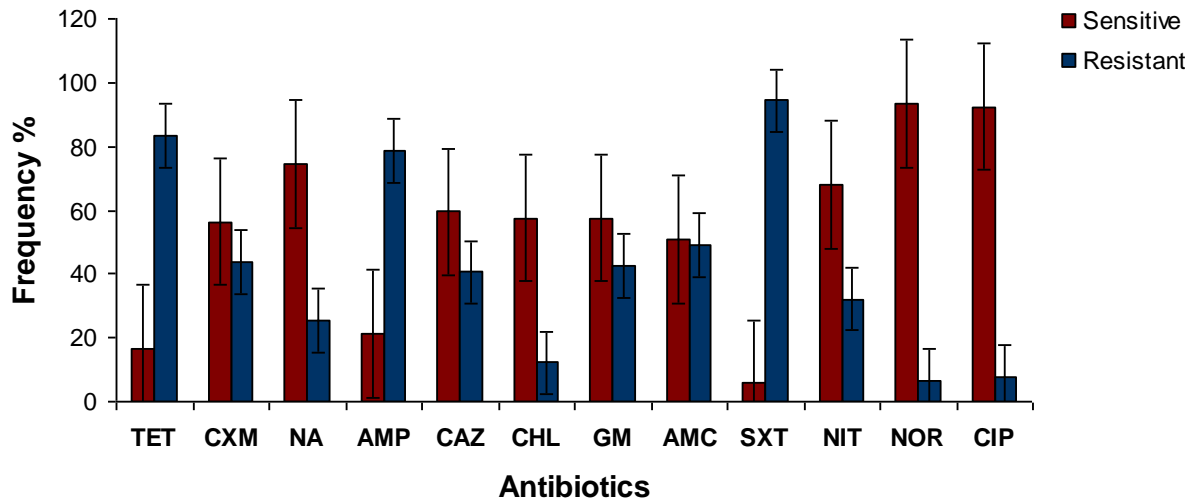


Figure 1: Antimicrobial susceptibility of *E. coli* from clinical samples of PLWHA at Mbagathi District Hospital (MDH) (N=264).

TET-Tetracycline, CXM-Cefuroxime, NA-Nalidixic acid, AMP – Ampicillin, CAZ – Ceftazidime, CHL-Chloramphenical, GM-Gentamicin, AMC-Augmentin, SXT-Cotrimoxazole, NIT-Nitrofurantoin, NOR-Norfloxacin, CIP-Ciprofloxacin

4.4.2 Comparison in prevalence of resistant *E. coli* isolated from PLWHA

The overall mean difference in percentage resistance of *E. coli* isolated from PLWHA is shown in figure 2. During the study period, ciprofloxacin, norfloxacin and trimethoprim-sulphamethazole showed the least significant difference in resistance between isolates from HIV⁺A⁺ and the HIV⁺A⁻ (9.25%, 7.85% and 16.67% respectively). Nitrofurantoin (29.17%), nalidixic acid (19%), ceftazidime (39.35%) and gentamicin (36.57%) showed a medium significant difference between the two groups while cefuroxime (43.09%), tetracycline (45.84%), ampicillin (45.47%) chloramphenical (45.67%) and amoxil/clavulanic acid (50.0%) showed the highest significant differences between isolates from PLWHA taking antibiotics and those not on antibiotics

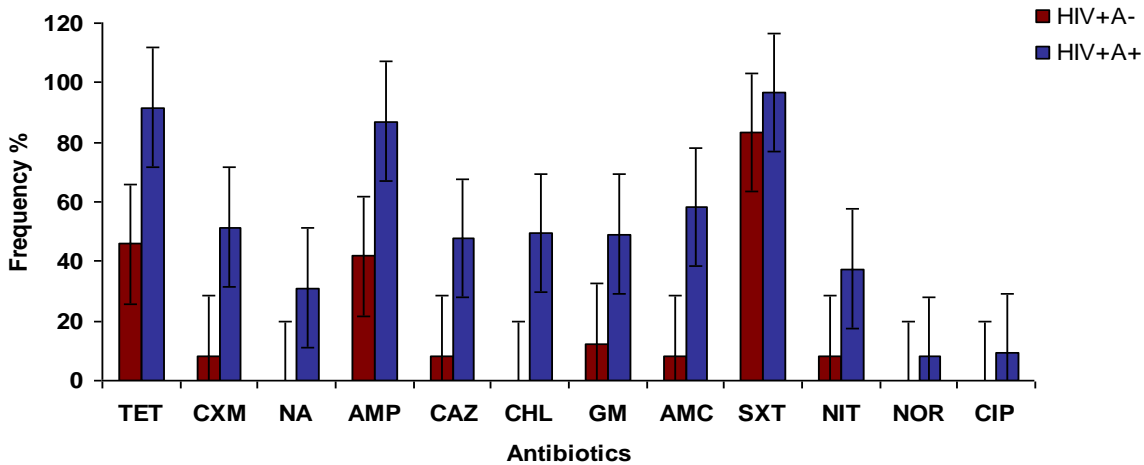


Figure 2: Comparison in prevalence of resistant *E. coli* strains isolated from PLWHA.

TET-Tetracycline, CXM-Cefuroxime, NA-Nalidixic acid, AMP – Ampicillin, CAZ – Ceftazidime, CHL-Chloramphenical, GM-Gentamicin, AMC-Augmentin, SXT-Trimethoprim-sulphamethazole, NIT-Nitrofurantoin, NOR-Norfloxacin, CIP-Ciprofloxacin

4.4.3 Antimicrobial resistance patterns (Antibiogram) of *E. coli* isolated from PLWHA stool specimen

Thirty three resistance patterns were developed in the five categories of *E. coli* with Sxt^R Tet^R Amp^S Amc^S Gm^S Cxm^S Caz^S Na^S Chl^S Nit^S Nor^S Cip^S (isolates resistant to at least two antibiotics) being the most prevalent multiresistance pattern. The antibiotics were clustered into patterns depending on the resistance frequency (Table 5). SXT^R TET^S AMP^R AMC^S GM^S CXM^S CAZ^R NA^S CHL^S NIT^S NOR^S CIP^S was the second most prevalent multiresistance pattern in *E. coli* isolated from HIV⁺A⁻ while Sxt^R Tet^R Amp^R Amc^S Gm^S Cxm^S Caz^R Na^S Chl^S Nit^S Nor^S Cip^S and SXT^R TER^R AMP^R AMC^R GM^R CXM^R CAZ^R NA^S CHL^S NIT^S NOR^S CIP^S turned out to be second most prevalent resistance pattern in isolates from HIV⁺A⁺ group (Table 5). Multi drug resistance was detected in 75% of all HIV⁺A⁻ *E. coli* strains, 90% of HIV⁺A^{+0.5} strains and 100% in isolates from HIV⁺A^{+1.0}, HIV⁺A^{+1.5} and HIV⁺A^{+2.0} persons.

Table 5: Antimicrobial resistance patterns of *E.coli* isolated from PLWHA at MDH

Resistance pattern	Frequency	Percentage Frequency
SXT	11	4.26
SXT, TET	11	4.26
SXT, AMP	8	3.10
SXT, TET, AMP	14	5.43
SXT,AMP, CAZ	8	3.10
SXT, TET, CXM	6	2.33
SXT, TET, AMP, GM	12	4.65
SXT,TET,AMC, CXM	11	4.26
SXT,TET, AMP, AMC	7	2.71
SXT, TET, AMP,AMC, GM	12	4.65
SXT, AMP, GM,CXM, CAZ	9	3.49
SXT, TET, AMP, CAZ, NIT	8	3.10
SXT, TET, AMP, AMC, GM	12	4.65
SXT, TET, AMP, AMC, GM, CHL	11	4.26
SXT, TET, AMP, AMC, CAZ, CXM	6	2.33
SXT, TET, AMP, GM, CXM, NIT	4	1.55
SXT, TET, AMP, AMC, GM, CXM, CAZ	14	5.43
SXT, TET, AMP, CXM, CAZ, NIT, NOR	8	3.10
SXT, TET, AMP, AMC, CXM, CAZ, NIT	8	3.10
SXT, TET, AMC, GM, CXM, CAZ, NA	8	3.10
SXT, TET, AMP, AMC, CXM, CAZ, CHL	8	3.10
SXT, TET, AMP, AMC, GM, CAZ, NIT, CHL	11	4.26
SXT, TET, AMP, AMC, GM, CXM, CAZ, CHL	4	1.55
SXT, TET, AMP, GM, CXM, CAZ, NIT, NA	6	2.33
SXT, TET, AMP, AMC GM, CXM, CAZ, CHL, NA	9	3.49
SXT, TET, AMP, AMC GM, CXM, CAZ, NIT, NA	6	2.33
SXT, TET, AMP, AMC GM, CXM, CAZ, CHL, NOR	3	1.16
SXT, TET, AMP, AMC GM, CXM, CAZ, CHL, NIT NOR	8	3.10
SXT, TET, AMP, AMC GM, CXM, CAZ, NA CHL, NIT	4	1.55
SXT, TET, AMP, AMC, CXM, CAZ, NA, CHL, NOR, CIP	3	1.16
SXT, TET, AMP, AMC, GM, CXM, CAZ, NA, CHL, NIT NOR,	5	1.94
SXT, TET, AMP, GM, CXM, CAZ, NA, CHL, NIT NOR, CIP	2	0.78
SXT, TET, AMP, AMC, GM CXM, CAZ, NA, CHL, NIT NOR, CIP	1	0.39

TET-Tetracycline, CXM- Cefuroxime, NA-Nalidixic acid, AMP – Ampicillin, CAZ – Ceftazidime, CHL- Chloramphenical, GM-Gentamicin, AMC- Augmentin, SXT- Trimethoprim-sulphamethazole, NIT- Nitrofurantoin, NOR- Norfloxacin, CIP-Ciprofloxacin

4.4.4 Summary of antimicrobial resistance profiles of *E. coli* from PLWHA

The results showed that about 76.65% of the *E. coli* were multidrug resistant that is, resistant to four or more antibiotics while 87.93% were resistant to three or more antibiotics (Table 6). The proportion of isolates resistant to three or more antibiotics increased steadily from HIV⁺A⁻ to HIV⁺A⁺ 2.0 confirming steady rise in multidrug resistance pattern.

Table 6: Resistance profiles of *E. coli* isolated from PLWHA.

Number of antibiotics showing resistance	Number of strains showing pattern (Frequency/percentage)
One	10 (3.90)
Two	21 (8.20)
Three	29 (11.32)
Four	31 (12.10)
Five	35 (13.67)
Six	26 (10.15)
Seven	42 (16.40)
Eight	23 (8.98)
Nine	18 (7.03)
Ten	14 (5.46)
Eleven	8 (3.13)

4.4.5 Antibiotic resistance in *E. coli* from PLWHA and not taking antibiotics (HIV⁺A⁻)

Strains of *E. coli* in this cluster did not show resistance to nalidixic acid, chloramphenicol, norfloxacin and ciprofloxacin but demonstrated resistance of 8.33% to cefuroxime, ceftazidime and nitrofurantoin with a resistance outline of SXT TET AMP AMC GM CXM CAZ NIT NA CHL NOR CIP. Isolates showed a slightly increased resistance of 12.5% to augmentin and gentamicin while isolates showed resistance of above 50% to trimethoprim-sulphamethazole, tetracycline and ampicillin (Fig 3).

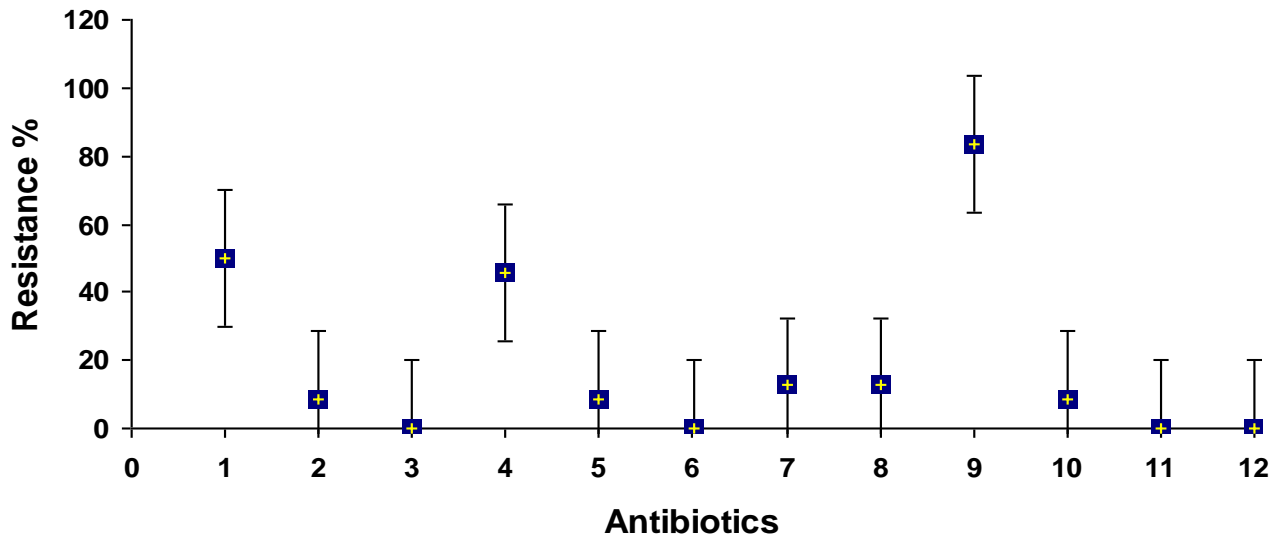


Figure 3: Frequency of resistant *E. coli* isolated from PLWHA not taking antibiotics (HIV⁺A⁻).

Legend: 1-Tetracycline, 2-Cefuroxime, 3-Nalidixic acid, 4-Ampicillin, 5-Ceftazidime, 6 – Chloramphenical, 7- Gentamicin, 8 – Augmentin, 9- Cotrimoxazole, 10- Nitrofurantoin, 11 – Norfloxacin, 12- Ciprofloxacin

4.4.6 Antibiotic resistance in *E. coli* from PLWHA and taking antibiotics for six months (HIV⁺A^{+0.5})

The antibiotic resistance pattern developed by *E. coli* in this group was SXT TET CXM AMP AMC CAZ NIT GM NA CHL NOR CIP (Fig. 4). Isolates did not show resistance to norfloxacin and ciprofloxacin but showed a slight resistance of 5% to chloramphenical and nalidixic acid. Isolates showed a resistance of less than 50% to augmentin, ampicillin, cefuroxime, ceftazidime, amoxicillin-clavulanic acid and nitrofurantoin while 87% and 60% resistance each to trimethoprim-sulphamethazole and tetracycline

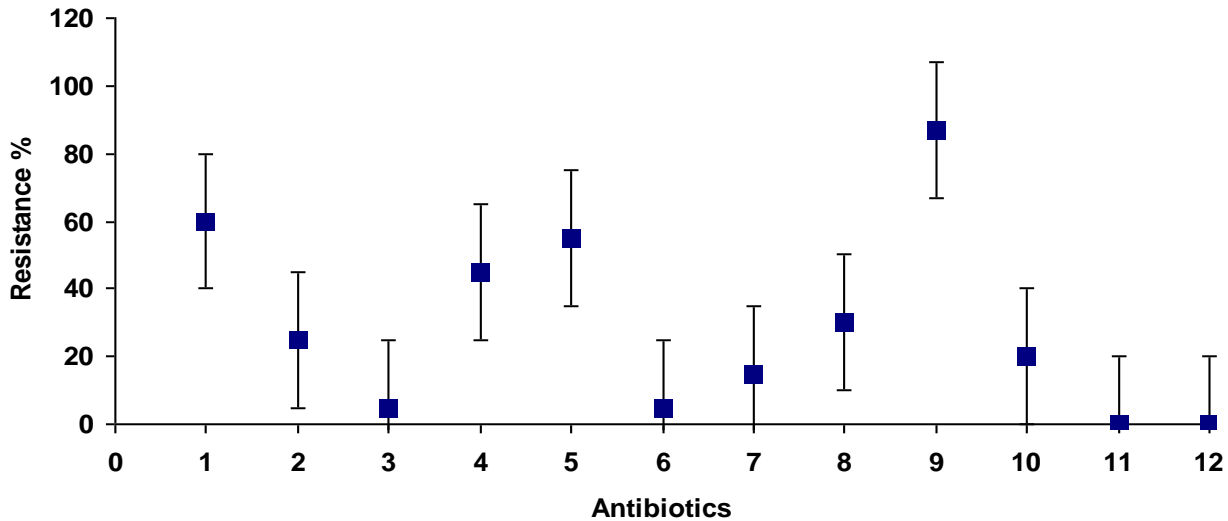


Figure 4: Frequency of resistant *E. coli* to antibiotics in six months. (HIV⁺A^{+0.5})

Legend: 1-Tetracycline, 2-Cefuroxime, 3-Nalidixic acid, 4-Ampicillin, 5-Ceftazidime, 6 – Chloramphenical, 7- Gentamicin, 8 – Augmentin, 9- Cotrimoxazole, 10- Nitrofurantoin, 11 – Norfloxacin, 12- Ciprofloxacin

4.4.7 Antibiotic resistance in *E. coli* from PLWHA and taking antibiotics for twelve months (HIV⁺A^{+1.0})

Out of the 216 samples isolated from HIV⁺A⁺, sixty (27.77%) were from persons living with HIV/AIDS and taking antibiotics for twelve months (HIV⁺A^{+1.0}). The percentage resistance of *E. coli* in this group varied from 3.33% for norfloxacin and ciprofloxacin to 90% in ampicillin. Isolates showed a resistance of 97.67% to both tetracycline and sulphamethazole-trimethoprim (Fig. 5) but demonstrated a resistance of less than 50% to nalidixic acid, cefuroxime, chloramphenical, augmentin, ceftazidime, gentamicin and nitrofurantoin with a resistance pattern of SXT TET AMP AMC CXM CHL CAZ NIT GM NA NOR CIP.

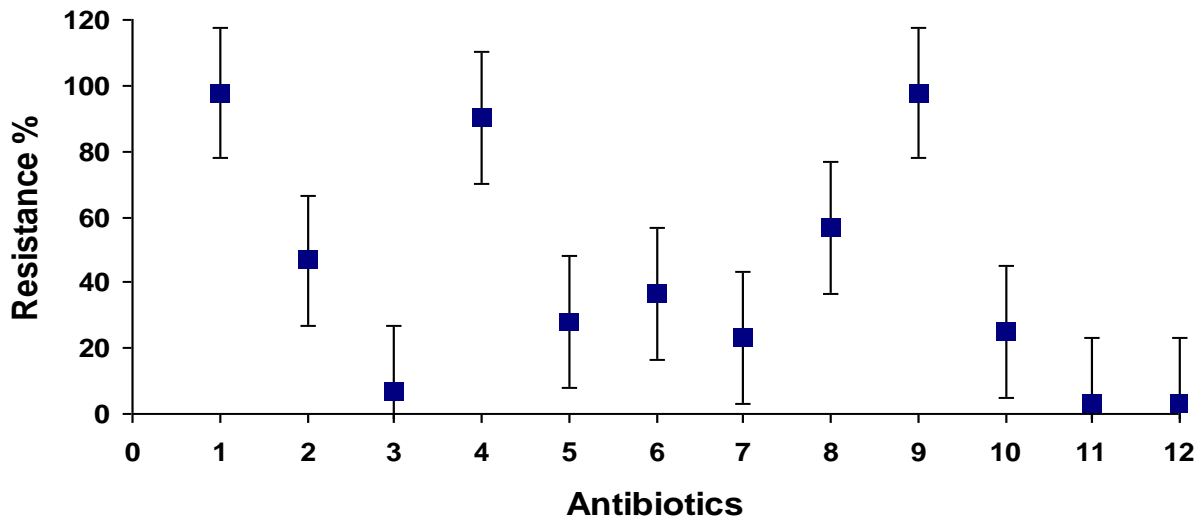


Figure 5: Frequency of resistant *E. coli* to antibiotics in twelve months (HIV⁺A^{+1.0}).

Legend: 1-Tetracycline, 2-Cefuroxime, 3-Nalidixic acid, 4-Ampicillin, 5-Ceftazidime, 6 – Chloramphenical, 7- Gentamicin, 8 – Augmentin, 9- Cotrimoxazole, 10- Nitrofurantoin, 11 – Norfloxacin, 12- Ciprofloxacin.

4.4.8. Antibiotic resistance in *E. coli* from PLWHA and taking antibiotics for eighteen months (HIV⁺A^{+1.5})

Resistance developed by bacteria isolated from PLWHA and taking antibiotics for eighteen months (HIV⁺A^{+1.5}) was 3.33% for norfloxacin and ciprofloxacin (Fig. 6). The resistance pattern for isolates in this group was SXT TET AMP GM CXM AMC NIT CHL CAZ NA NOR CIP. *E. coli* showed a resistance of 32.69% to nalidixic acid, 40% to ceftazidime and 50% to both chloramphenical and nitrofurantoin. Cefuroxime, gentamicin and augmentin demonstrated a slightly higher resistance of between 55% and 70% while all the isolates in this cluster were 100% resistant to tetracycline, ampicillin and trimethoprim-sulphamethazole.

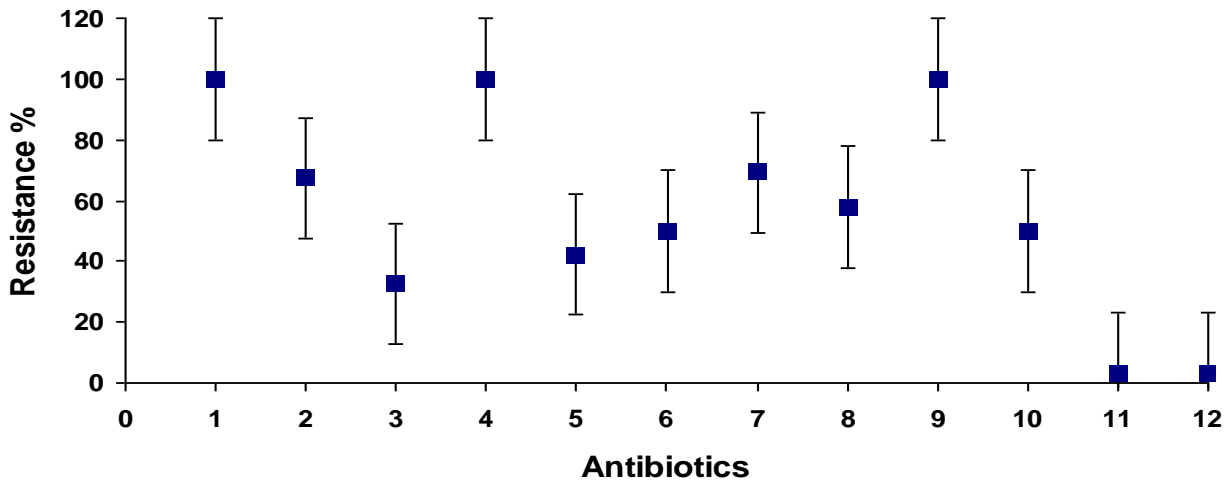


Figure 6: Frequency of resistant *E. coli* to antibiotics in eighteen months (HIV⁺A^{+1.5}).

Legend: 1-Tetracycline, 2-Cefuroxime, 3-Nalidixic acid, 4-Ampicillin, 5-Ceftazidime, 6 – Chloramphenical, 7- Gentamicin, 8 – Augmentin, 9- Cotrimoxazole, 10- Nitrofurantoin, 11 – Norfloxacin, 12- Ciprofloxacin

4.4.9. Antibiotic resistance in *E. coli* from PLWHA and taking antibiotics for twenty four months (HIV⁺A^{+2.0})

E. coli isolates from PLWHA and taking antibiotics for twenty four months showed a significant difference in resistance to all antibiotics (Fig. 7). The antibiotic resistance pattern was Sxt Amp Tet Chl Na Caz Cxm Gm Amc Nit Cip Nor. The isolates showed the least resistance to nitrofurantoin, norfloxacin and ciprofloxacin (Less than 50%) while to cefuroxime, nalidixic acid, gentamicin and amoxicillin-clavulanic acid, resistance was ranging from 50 to 69%. Resistance was 96.87% to both chloramphenical and tetracycline while all the isolates were resistant to sulphamethazole-trimethoprim and ampicillin.

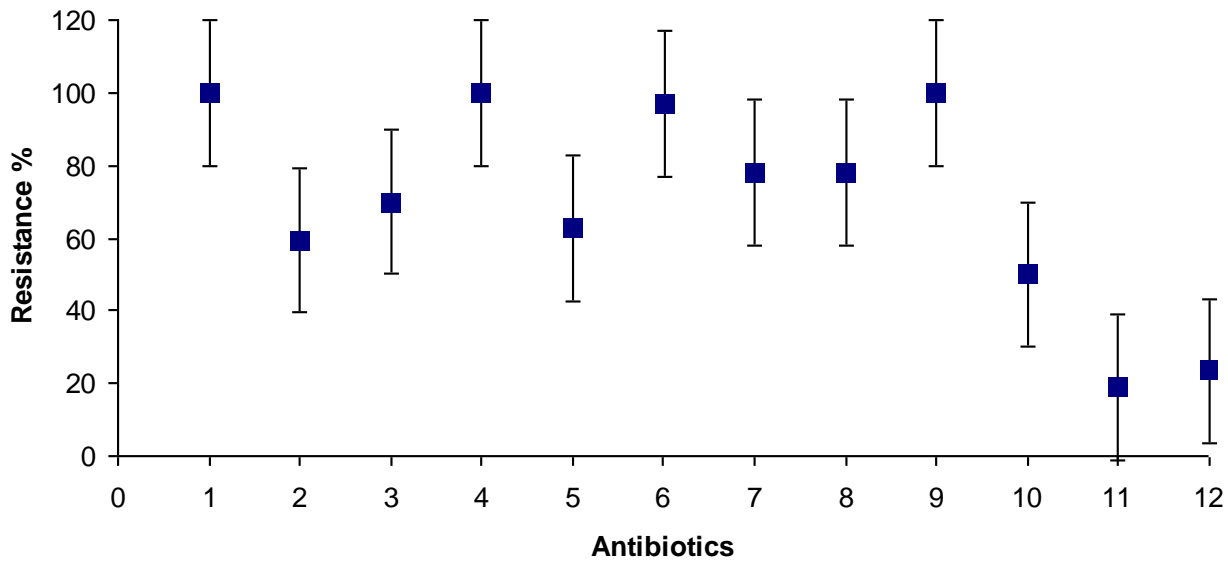


Figure 7: Frequency of resistant *E. coli* to antibiotics in twenty four months (HIV⁺A^{+2.0}).

Legend: 1-Tetracycline, 2-Cefuroxime, 3-Nalidixic acid, 4-Ampicillin, 5-Ceftazidime, 6 – Chloramphenical, 7- Gentamicin, 8 – Augmentin, 9- Cotrimoxazole, 10- Nitrofurantoin, 11 – Norfloxacin, 12- Ciprofloxacin

4.4.10 Antibiotic resistance trends of *E. coli* isolated from HIV sero-positive persons to specific antibiotics

There was a statistical percentage difference in resistance to tetracycline by species of *E. coli* isolated from HIV⁺A⁺ and HIV⁺A⁻ (45.84%) (Table 7). One hundred and ninety eight (91.67 %) of *E.coli* from HIV⁺A⁺ and 22 (45.83%) of the 48 from HIV⁺A⁻ were resistant to tetracycline. Increasing resistance trends in *E. coli* was observed and the proportion of resistance was high with the least resistance seen in isolates from HIV⁺A⁻ (45.83%) group. *E. coli* from HIV⁺A^{+0.5} group showed an increase in resistance (60%) which further shot to 96.67% in the HIV⁺A^{+1.0} cluster. Isolated from HIV⁺A^{+1.5} and HIV⁺A^{+2.0} groups demonstrated the highest form of resistance where all the strains were 100% resistant to this antibiotic.

Resistance of *E. coli* isolates to cefuroxime varied slightly from one category to another (Table 7). 51.39 % of the 216 *E. coli* isolates from HIV⁺A⁺ and 4 (8.3 %) of the 48 from HIV⁺A⁻ were resistant to cefuroxime with a significant difference of 53.09%. Frequency of resistant to this antibiotic was much higher in isolates from HIV⁺A^{+1.5} (67.31%) category compared to others groups. *E. coli* isolated from HIV⁺A^{+1.0} (46.67%) and HIV⁺A^{+2.0} (59.37%) showed a resistance frequency that was slightly higher than isolates from HIV⁺A^{+0.5} (25%) while isolates from HIV⁺A⁻ group illustrated the least resistance frequency of (8.33%).

Isolates from HIV⁺A⁻ cohort were all sensitive to nalidixic acid while 19 % of the 67(31.02%) isolates from HIV⁺A⁺ cluster were resistant to nalidixic acid (Table 7). Percentage resistance of the *E. coli* isolates varied in all the groups whereby isolates from HIV⁺A^{+0.5} and HIV⁺A^{+1.0} showed the least resistance of 5.0% and 6.67% each while those from HIV⁺A^{+1.5} showed a resistance of 32.69%. Isolates from HIV⁺A^{+2.0} cluster showed the highest resistance to this antibiotic of 68.75%.

Two hundred and nineteen (82.95%) of the 264 *E. coli* isolates from PLWHA were resistant to ampicillin, 188 (87.04%) from HIV⁺A⁺ group and twenty (41.67%) from HIV⁺A⁻ cluster (Table 7). Resistance trend increased slightly from 45.83% in HIV⁺A⁻ isolates to 47.74 in HIV⁺A^(0.5) isolates. The antibiotic resistance trend then rose steadily in isolates from HIV⁺A^(1.0) and HIV⁺A^(1.5) then reached 100% in the HIV⁺A^(2.0) group.

There was a slightly significant difference in resistance between the *E. coli* isolates from HIV⁺A⁻ and HIV⁺A⁺ of 39.35% (Table 7) to ceftazidime. Out of 48 isolates from HIV⁺A⁻ group, only 4(8.33%) were resistant to this antibiotic. 103 (47.68%) isolates from HIV⁺A⁺ were resistant to this antibiotic. Resistance increased from 8.33% in isolates from HIV⁺A⁻ to 55.0% in isolates from HIV⁺A^{+0.5}. Resistance further sharply declined to (28.33%) in isolates from HIV⁺A^{+1.0} group but increased to 42.31% in isolates from HIV⁺A^{+1.5} and to 62.63% in isolates from HIV⁺A^{+2.0} category.

All the 48 isolates from HIV⁺A⁻ were susceptible to chloramphenicol but 5% resistance was observed in isolates from HIV⁺A^{+0.5} (Table 7). The proportion of resistance increased steadily to 35% in isolates from HIV⁺A^{+1.0}, 50% in HIV⁺A^{+1.5} then to 96.88% in isolates from the HIV⁺A^{+2.0} with a resistance difference of 45.67% between isolates from HIV⁺A⁺ and HIV⁺A⁻.

Six (12.5%) of the isolates from HIV⁺A⁻ and 106 (49.07%) from HIV⁺A⁺ were resistant to gentamicin (Table 7). Resistance slightly rose to 18.5% in isolates from HIV⁺A^{+0.5} group then further increased to 24.30% in isolates from HIV⁺A^{+1.0}, 69.23% in HIV⁺A^{+1.5} and 78.13% in isolates from HIV⁺A^{+2.0}

Although susceptibility of *E. coli* from HIV⁺A⁺ and HIV⁺A⁻ to augmentin was high, there was statistically significant difference in resistance between isolates from the two groups (50%) (Table 7). Four (8.33%) of the 48 *E. coli* from HIV⁺A⁻ were resistant to augmentin compared to 58.33% of the 216 isolates from HIV⁺A⁺. Resistance increased from one category to another. *E. coli* isolated from HIV⁺A⁻ showed a percentage resistance of (8.33%) which increased to 30.0% in isolates from HIV⁺A^{+0.5} cluster. A slightly higher resistance to amoxicillin/clavulanic acid was detected in isolates from HIV⁺A^{+1.0} and HIV⁺A^{+1.5} categories (58.30 and 60%) each while isolates from HIV⁺A^{+2.0} group showed the highest resistance of 78.13%.

E. coli from all the five groups demonstrated a high resistance percentage to trimethoprim-sulphamethazole (Table 7). Isolates from HIV⁺A⁻ showed a resistance of 83.33%. This slightly increased to 87.0% in isolates from HIV⁺A^{+0.5} and further rose to 96.67 in HIV⁺A^{+1.0}. All the *E. coli* isolates from the HIV⁺A^{+1.5} and HIV⁺A^{+2.0} category had developed 100% resistant to trimethoprim-sulphamethazole.

E. coli isolates from PLWHA showed a moderate to a high resistance proportion to nitrofurantoin depending on the span of antibiotic exposure (Table 7). 4(8.33%) of the 48 isolates from HIV⁺A⁻ and 37.50 % of the 216 *E. coli* isolates from HIV⁺A⁺ were resistant to nitrofurantoin with a significant difference of 31.17%.

Frequency of resistance to nitrofurantoin was low in isolates from HIV⁺A⁻ (8.33%) category but gradually increased to 20.0% in isolates from HIV⁺A^{+0.5}. Slowly the trend rose to 25.0% in the HIV⁺A^{+1.0}. The highest proportion of resistance was 50% which was observed in isolates from HIV⁺A^{+1.5} and HIV⁺A^{+2.0} groups.

Resistance by *E. coli* isolated from PLWHA to this antibiotic was low. All the isolates from various classes had a percentage resistance of not more than 50% to norfloxacin (Table 7). Isolates from HIV⁺A⁻ were sensitive to norfloxacin while 17 (7.87%) of the 216 *E. coli* isolates from HIV⁺A⁺ were resistant to this antibiotic. Isolates from HIV⁺A^{+0.5} and HIV⁺A^{+1.0} showed a resistance of 3.33% and 5.0 % each while *E. coli* from HIV⁺A^{+1.5} and HIV⁺A^{+2.0} showed a resistance of 8.33% and 18.75% respectively.

E. coli showed the least antibiotic resistance percentages to ciprofloxacin for all the five classes of PLWHA in the study (Table 7). Among 216 *E. coli* isolates from HIV⁺A⁺ only 20 (9.25%) were resistant to ciprofloxacin. Isolates from the HIV⁺A⁻ and HIV⁺A^{+0.5} groups were all sensitive to ciprofloxacin unlike isolates from other groups. *E. coli* from HIV⁺A^{+1.0} category showed a resistance of 3.33% which increased slightly to 5.22% in isolates from HIV⁺A^{+1.5} and further shot to 23.44% in isolates from HIV⁺A^{+2.0}

Table 7: Antibiotic resistant trends of *E. coli*

Antibiotics	HIV ⁺ A ⁻ (%)	HIV ⁺ A ^{+0.5} (%)	HIV ⁺ A ^{+1.0} (%)	HIV ⁺ A ^{+1.5} (%)	HIV ⁺ A ^{+2.0} (%)
Tetracycline	45.83	60.00	96.60	100	100
Cefuroxime	8.33	25.00	46.67	67.31	59.37
Nalidixic acid	0.00	5.00	6.67	32.69	68.75
Ampicillin	41.67	45.00	90.00	100	100
Ceftazidime	8.33	55.00	28.33	42.31	62.63
Chloramphenical	0.00	5.00	36.67	50.00	96.88
Gentamicin	12.50	15.00	23.33	69.23	78.13
Amoxicillin/clavulanic acid	8.33	30.00	56.67	57.69	78.13
Trimethoprim-sulphamethazole	83.33	87.00	96.67	100	100
Nitrofurantoin	8.33	20.00	25.00	50.00	50.00
Norfloxacin	0.00	0.00	3.33	5.00	18.75
Ciprofloxacin	0.00	0.00	3.33	5.22	23.44

4.5 Minimum inhibitory concentration (MIC)

E. coli isolated from PLWHA and taking antibiotics (HIV⁺A⁺) demonstrated resistance of less than 50% to ciprofloxacin, norfloxacin, nitrofurantoin, gentamicin, chloramphenical, ceftazidime and nalidixic acid (Table 7) but revealed a high resistance to trimethoprim-sulphamethazole, tetracycline and ampicillin of 96.76%, 91.67%, 87.02% respectively (Table 7). On the other hand, resistance of *E. coli* isolated from PLWHA not taking antibiotics (HIV⁺A⁻) was less than 50% for all the antibiotics a part from trimethoprim-sulphamethazole that illustrated a resistance of 83.33%.

The frequency of MIC mode for isolates from the two groups varied for some antibiotics but some demonstrated the same mode. Isolates expressed the same MIC mode to ampicillin and augmentin (32µg/ml and 16µg/ml) each (Table 7). *E. coli* from PLWHA and taking antibiotics revealed a sensitive MIC to nalidixic acid, gentamicin, norfloxacin and ciprofloxacin but for isolates from PLWHA not on antibiotics, a sensitive MIC mode was demonstrated to nitrofurantoin and cefuroxime. All isolates tested on chloramphenical demonstrated a resistant MIC.

Table 8: Minimum inhibitory concentration (MIC) for twelve antimicrobials against *E. coli* isolated from PLWHA

Antibiotics	MIC range ($\mu\text{g/ml}$)	HIV ⁺ A ⁻ mode	HIV ⁺ A ⁺ mode	Percentage resistance	
				HIV ⁺ A ⁺ (216)	HIV ⁺ A ⁻ (48)
Tetracycline	1-128	64	64	198(91.67)	22 (45.83)
Cefuroxime	2-64	32	2	111(51.39)	4(8.33)
Nalidixic acid	1-64	16		67 (31.02)	0(0.0)
Ampicillin	8-32	32	32	188 (87.02)	20(41.67)
Ceftazidime	8-64	4	64	103 (47.68)	4(8.33)
Chloramphenical	0.5-64	16		107(49.54)	0(0.0)
Gentamicin	0.25-32	2		106(49.07)	6(12.5)
Augmentin	2-8	16	16	126(58.33)	4(8.33)
Cotrimoxazole	0.02-64	64	64	209(96.76)	40(83.33)
Nitrofurantoin	32-128	64	128	81(37.50)	4(8.33)
Norfloxacin	2-8	4		17 (7.78)	0(0.0)
Ciprofloxacin	0.0625-0.25	0.062		20(9.25)	0(0.0)

MIC-Minimum inhibitory concentration, N-Number of isolates tested

4.6 Plasmid analysis

Two hundred and forty six multi drug resistant (MDR) strains were selected for plasmid analysis. The strains were selected from organisms showing resistance to two and more antibiotics. Plasmids were detected in all MDR strains that were analyzed (Fig. 8, 9 and 10).

Most of the isolates from PLWHA and not taking antibiotics carried 20.8 MDa plasmid though some of them had the 60 MDa plasmid (Fig. 8). Isolates from HIV⁺A^{+0.5} and HIV⁺A^{+1.0} had almost the same molecular sized plasmids (Fig. 9). The most predominant plasmid in isolates from the HIV⁺A^{+0.5} group was 20.8 MDa (Fig. 9) while 98 and 20.8MDa plasmids featured most in the HIV⁺A^{+1.0} cluster. Isolates from HIV⁺A^{+1.5} and HIV⁺A^{+2.0} demonstrated a variety of plasmids ranging from the heaviest plasmid (98MDa) to the lightest (1.4MDa) (Fig 10). 3.7 and 4.6MD plasmids were observed in the isolates from HIV⁺A^{+1.5} while

3.4, 4.8 and >98 MDa plasmids featured most in the isolates from HIV⁺A^{+2.0}. The most prevalent plasmids detected in MDR isolates was 42, 24, 8, 4, <4 (Table 9).

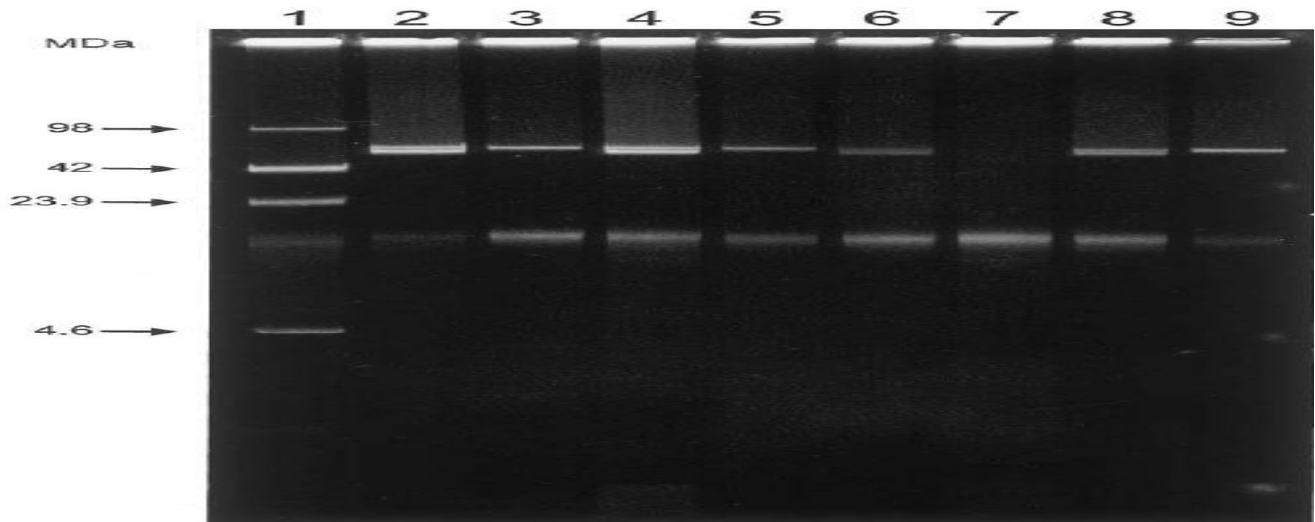


Figure 8: Analysis of plasmid DNA of *E. coli* isolates from PLWHA and not taking antibiotics.

Legend

Lane 1 *E. coli* 39R861 molecular size marker (98, 42, 24, 4.6, and 3 MDa)

Lane 2 to 9 *E. coli* isolates from PLWHA and not taking antibiotics (HIV⁺A⁻)

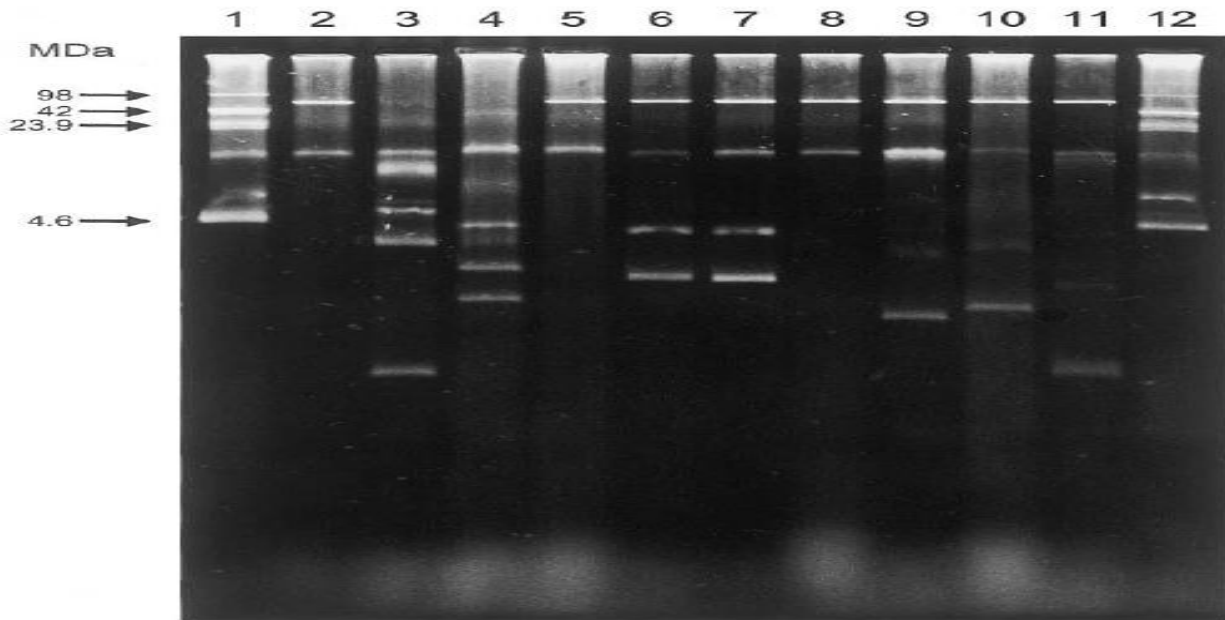


Figure 9: Analysis of plasmid DNA of *E. coli* isolated from PLWHA and taking antibiotics for six and twelve months

Legend

- Lane 1 *E. coli* 39R861 molecular size marker (98, 42, 23.9 and 4.6 MDa)
- Lane 2 to 6 *E. coli* isolates from PLWHA and taking antibiotics for six months (HIV⁺A^{+0.5})
- Lane 7 to 12 *E. coli* isolates from PLWHA and taking antibiotics for one year (HIV⁺A^{+1.0})

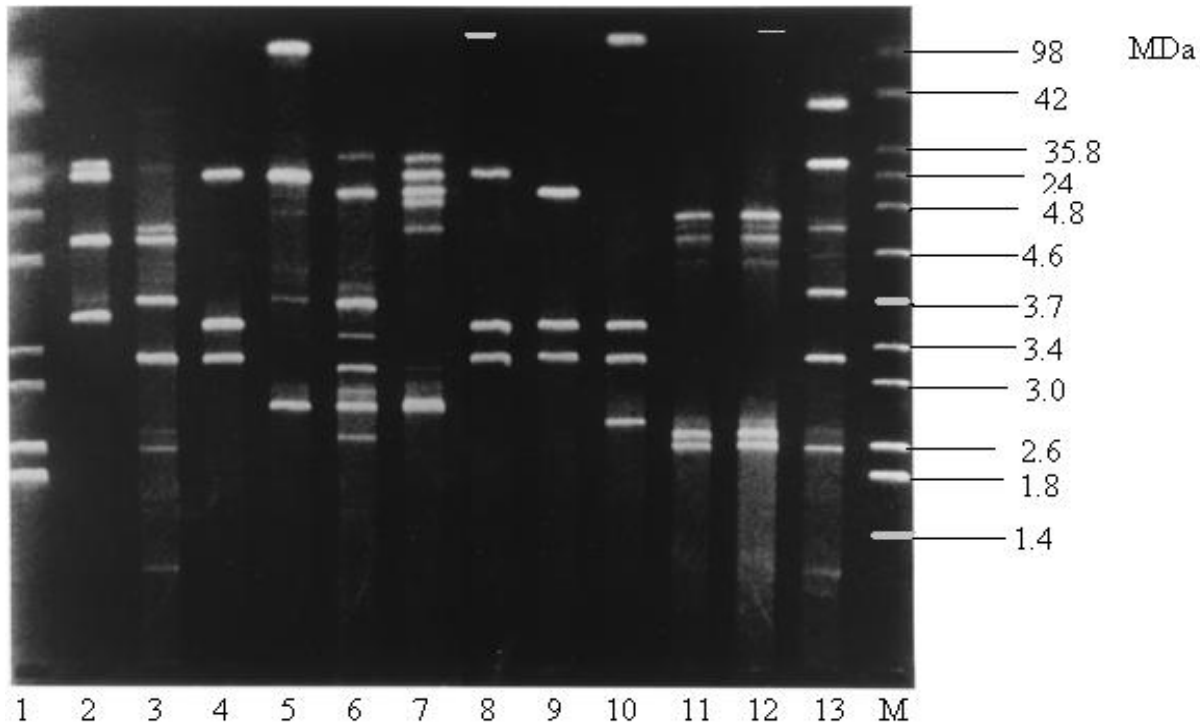


Figure 10: Analysis of plasmid DNA of *E. coli* isolates from PLWHA and taking antibiotics. Lane M is plasmid profile *E. coli* 39R861 and *E. coli* V517 that were used as plasmid size markers.

Legend

- Lane 1 *E. coli* 39R861 and *E. coli* V517 molecular size marker (98, 42, 24, 4.8, 4.6, 3.7, 3.4, 3.0, 2.6, 1.8 and 1.4 MDa).
- Lane 1-6 *E. coli* isolates from PLWHA and taking antibiotics for one and a half years (HIV⁺A^{+1.5})
- Lane 7 to 13 *E. coli* isolates from PLWHA and taking antibiotics for two years (HIV⁺A^{+2.0})

Table 9: Frequency of plasmids of different sizes detected in MDR *Escherichia coli*

Plasmid size in MDa	Frequency	Percentage
4.8	26	10.57
98, 4.8, 3.8	40	16.26
24, 8, 4.2	38	15.45
98, 4.8	31	12.60
4.2, 35	40	16.26
42, 24, 8, 4, <1.4	71	28.86

4.7 Conjugation results

Two hundred and eight strains of *E. coli* resistant to ampicillin were mated with *Escherichia coli* K12 F nalidixic acid resistant strain. The most common resistance phenotype transferred to *E. coli* K12 is shown in table 9. The success of resistance transfer was high for MDR to all the drugs tested. Transfer of resistance plasmid was evident following plasmid analysis of transconjugants. The 98 MDa plasmid were transferred to the recipient *E. coli* K12 alongside other plasmids (Table 10).

Table 10: Transferable plasmids detected in *Escherichia coli* K12 transconjugant

Plasmid size in MDa	Frequency	Percentage
98, 24, 4.8	59	23.98
98, 24	78	31.70
98, 4.8, <4.8	109	44.31

More than half (67%) of the transconjugants carried the large 98 MDa plasmid, 4.8MDa and several plasmids of less than 4.8MDa.

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 DISCUSSION

The broad objective of the present study was to determine patterns and trends in resistance with time and the presence of self transferable plasmids in strains of *E. coli* isolated from HIV sero-positive adults attending Mbagathi District Hospital. 264 strains of *E. coli* were isolated from PLWHA, 216 (81.81%) from PLWHA and taking antibiotic (HIV⁺A⁺) and 48 from PLWHA and not taking antibiotics (HIV⁺A⁻). The isolates were distributed into classes depending on the length of time the patient had been exposed to drugs. All the isolates were resistant to either one or more antibiotics a part from six isolates from HIV⁺A⁻. The isolates were highly sensitive to fluoroquinolones and nalidixic acid but quite resistant to first line antibiotics. This could have been because these antibiotics are rarely used partly because they are expensive.

5.1.1. Antibiotic resistance patterns

Thirty three different resistance patterns were developed from the isolated strains of *E. coli*. SXT^R TET^R AMP^S AMC^S GM^S CXM^S CAZ^S NA^S CHL^S NIT^S NOR^S CIP^S was the most prevalent pattern whereby most isolates were resistant to trimethoprim-sulphamethazole (cotrimoxazole) and ampicillin. The most prevalent resistance pattern in *E. coli* isolates from PLWHA not taking antibiotics was SXT^S TET^S AMP^R, AMC^S GM^S CXM^S CAZ^R NA^S CHL^S NIT^S NOR^S CIP^S while SXT^R TET^R AMP^R AMC^S GM^S CXM^S CAZ^R NA^S CHL^S NIT^S NOR^S CIP^S and SXT^R TET^R AMP^R AMC^R GM^R CXM^R CAZ^R NA^S CHL^S NIT^S NOR^S CIP^S were the most prevalent multiresistance patterns in isolates from PLWHA taking antibiotics (HIV⁺A⁺). Antibiotic resistance patterns differed in *E. coli* isolated from the five classes of isolates and this depended on the length of time the isolates had been exposed to the antibiotics. For isolates from HIV⁺A⁻ and HIV⁺A⁺ groups, the antibiotic resistance pattern was SXT TET CXM AMP AMC CAZ NIT GM NA CHL NOR

CIP whereby the isolates were highly resistant to trimethoprim-sulphamethazole and least resistant to ciprofloxacin. The pattern was the same in the two groups probably because the isolates had not been exposed to the antibiotics for a long time or possibly because analysis was done on two different populations. For isolates from HIV⁺A^{+1.0}, the antibiotic resistance pattern almost remained the same as for isolates from HIV⁺A^{+0.5} (SXT TET AMP AMC CXM CHL CAZ NIT GM NA NOR CIP) but ampicillin turned out to be highly resistant than Cefuroxime. Ampicillin being an inexpensive easily available antibiotic, its resistance rate could have increased as a result of increased usage. The antibiotic resistance pattern of *E. coli* isolated from HIV⁺A^{+1.5} was SXT TET AMP GM CXM AMC NIT CHL CAZ NA NOR CIP. It was almost similar to that of isolates from HIV⁺A^{+1.0} but gentamicin, nitrofurantoin and chloramphenicol were more resistance. The antibiotic resistance pattern for isolates from HIV⁺A^{+2.0} was SXT AMP TET CHL NA CAZ CXM GM AMC NIT CIP NOR whereby resistance was high in the first line antibiotics.

Antibiotic resistance patterns in *E. coli* to commonly used antibiotics have been previously reported (Laverstein-van Hall, 2002a; Brook *et al.*, 2003; Clearly *et al.*, 2005). Slightly different patterns were observed by Gniadkowski *et al.*, (2006). The investigators worked on isolates from units with PLWHA and wards admitting HIV negative patients in the same hospital. The most prevalent pattern of resistance was SXT^R TET^R AMP^S AMC^S GM^S CXM^S CAZ^S NA^S CHL^S NIT^S NOR^S CIP^S for *E. coli* isolated in wards with PLWHA. Several studies on the resistance patterns developed by strains of *E. coli* isolated from HIV sero-positive adults have been carried out. Madhi *et al.* (2002) have reported variations in patterns developed by strains of *E. coli* to antibiotics with time. Studies carried out in Cote d'ivoire have shown significant variation in antibiotic resistance patterns with time (Anglaret *et al.*, 2005; Wiktor *et al.*, 2007). Wininger and Fass (2004) observed that, faecal strains of *E. coli* isolated from PLWHA receiving prophylactic and chemoprophylactic administration of antibiotics commonly used by HIV sero-positive

adults developed 25 resistance patterns with SXT^R TET^R AMP^R AMC^S GM^S CXM^S CAZ^S NA^S CHL^S NIT^S NOR^S CIP^S being the most prevalent pattern. In Kenya, *E. coli* was isolated from PLWHA attending MDH, but the investigators did not report on variations in the antibiotic resistance pattern with time (Okeyo *et al.*, 2006) though the prevalent resistance pattern was SXT^R TET^R AMP^R AMC^S GM^S CXM^S CAZ^S NA^S CHL^S NIT^S NOR^S CIP^R which was almost same to the resistance pattern developed by *E. coli* isolated from PLWHA and taking antibiotics for one year. Prophylactic and chemoprophylactic use of antibiotics by PLWHA can improve persons lives but the impact of these antibiotics on development of antibiotic resistance strains in *E. coli* and circulating bacterial pathogens needs to be monitored.

5.1.2. Antibiotic resistant trends

The antibiotic resistance trends to most drugs tested on *E. coli* from PLWHA increased from HIV⁺A⁻ to HIV⁺A^{+2.0}. The general trend towards increasing prevalence of resistance was marked by recovery of an increasing proportion of strains from HIV⁺A⁻ to HIV⁺A^{+2.0} that were simultaneously resistant to several drugs. Most of the isolates had developed resistance to most antibiotics a part from the fluoroquinolones, augmentin and nalidixic acid before the study was initiated. *E. coli* isolates from PLWHA and not taking antibiotics had developed over 40% resistance to tetracycline, ampicillin, gentamicin and nitrofurantoin and over 80% to trimethoprim-sulphamethazole. This may explain how frequent these antibiotics are used even without prescription. These antibiotics are also inexpensive and easily acquired over the counter.

Antibiotic resistance trends to tetracycline, ampicillin, gentamicin and nitrofurantoin increased rapidly from PLWHA not on antibiotics to PLWHA taking antibiotics for two years. Tetracycline and ampicillin are one of the oldest and extensively used bacteriostatic antibiotics in sectors other than health and they are a first line treatment for most bacterial infections. Tetracycline was discovered in 1940s, it is inexpensive and extensively used in prophylaxis and therapy of human and animal infections (Chopra and Roberts, 2001).

Resistance to this antibiotic could have been due to decreased intracellular accumulation of the drug due to either impaired influx or increased efflux by an active transport protein pump, ribosomal protection due to production of proteins that interfere with tetracycline binding to the ribosomes and enzymatic inactivation of drug. In Kenya, it is the leading antibiotic used in food producing animals. Tetracycline contributes to approximately 55% of the total consumption (Mitema *et al.*, 2004) of the antibiotic in animals. O'Connor *et al.* (2002) isolated *E.coli* with an increasing resistance trend to tetracycline from cattle receiving chlortetracycline supplemented animal feeds.

Gentamicin is rarely abused as the mode of delivery is by intramuscular administration. It is possible then to deduce that the increasing trend of resistance to gentamicin observed among strains of *E.coli* was due to increased administration of the antibiotic. Gentamicin was introduced in 1969 as a broad spectrum aminoglycoside which was effective *in vitro* against a majority of Gram negative bacilli by irreversibly inhibiting protein synthesis (Siebert *et al.*, 2004). They observed in their laboratory increasing gentamicin resistance in indole negative *Proteus* (44%) Musoke and Revathi (2003) and Kariuki *et al.*(1999) also isolated gentamicin resistant *E. coli*. The results from this study agree with these findings as the patients had already been treated with gentamicin.

When this study began, fluoroquinolones and nalidixic acid resistance rates were <1% and 8.33% for amoxicillin/clavulanic (augmentin) acid in isolates from PLWHA not taking antibiotics. The prevalence of resistance in *E. coli* subsequently rose slowly and progressively in isolates from HIV⁺A^{+2.0}. These antibiotics are toxic, expensive and used only on prescription although over the years they are first antibiotic of choice in treatment of *Salmonella* and *Salmonella* like infections (Hart and Kariuki, 2003). However, MDR strains of *Salmonella typhi* which have emerged carry resistance to chloramphenicol. High level of susceptibility of *E. coli* isolated from HIV⁺A⁻ may be due to decreased use of the antibiotics because of their toxicity. Resistance to fluoroquinolones in *E. coli* is mainly associated with mutation in chromosomal genes

for DNA gyrase (*gyrA*) or topoisomers IV (topo IV), and these are usually targets of action by quinolone class (Hopper, 2000; Villa *et al.*, 2000). Chloramphenicol is a potent inhibitor of microbial protein synthesis, its resistance could have occurred as a result of mutations that are less permeable to the drug or production of chloramphenicol acetyltransferase plasmid encoded enzyme by the bacteria that inactivates the drug. Resistance to chloramphenicol featured in most of the MDR strains of *E.coli* isolated in Kenya by Sang *et al.* (2003). Bellaaj *et al.* (2002) isolated clinical strains of *E.coli* which exhibited high resistance to tetracycline and chloramphenicol. This resistance was associated with accumulation of the antibiotic in the bacterial cells and was not mediated by large conjugation plasmids. However, Kariuki *et al.* (2002) showed that chloramphenicol resistance in MDR strains of *E. coli* isolated in Kenyan children was transferable.

Ciprofloxacin is one of the most expensive antibiotic which is usually prescribed for typhoid treatment. It is administered as a 2nd line treatment for infection by Gram negative bacteria. However, it's recommended by the Ministry Of Health as one of the antibiotics to be used in treating opportunistic infections in PLWHA. Increasing resistance to ciprofloxacin among isolates from PLWHA is probably related to the increased use of the antibiotic. Garau *et al.* (2002) reported prevalence of quinolones resistant *E. coli* (QREC) in the faeces of healthy people in Spain (24% in adults and 26% in children) and a very high proportion of QREC in animal faeces (up to 90% of chicken harbored QREC). The investigator concluded that high prevalence of QREC in stool samples of healthy humans was associated with the use of the antibiotic. Nalidixic acid has effectively been used in the outbreaks of *Shigella dysenteriae* in Kenya (Lijima *et al.*, 2001). Increasing antibiotic resistance trends to the fluoroquinolones was most pronounced for *E. coli* (Karlowsky *et al.*, 2003). These reports are in agreement with our observation that implicates quinolones and fluoroquinolone resistance is as a result of widespread use of the antibiotic (Neuhauser *et al.*, 2002). Inappropriate use of quinolones therefore poses a threat not only to quinolones resistance but also prevalence of MDR by organisms.

Amoxicillin/clavulanic acid (augmentin) is not accessible because it is more expensive. Presence of β -lactamase inhibitor in clavulanic acid makes it more potent. However, *E. coli* isolates from HIV⁺A⁺ were more resistant than isolates from HIV⁺A⁻ to this antibiotic. This suggests that consumption of augmentin is considerable in HIV⁺A⁺ and may contribute to the development of an increasing antibiotic resistance trend, this compares well with previous reports. Okoli *et al.* (2004) reported increasing resistance trends to amoxicillin/clavulanic acid in *E. coli* isolated from free range chicken in Nigeria. Similar levels of susceptibility to a lactamase inhibitor antibiotic were reported by Karlowsky *et al.* (2003.) The investigators observed increasing trend in resistance among strains of *E.coli* isolated from intensive-care-unit (ICU) patients to ampicillin –sulbactam (45.5% and 57.2%) respectively. Cross resistance to beta lactam antibiotics in fecal *E. coli* and *Klebsiella* strains from neonates was implicated on the use of ampicillin and cefuroxime (Tullus *et al.*, 2000).

Trend in resistance to trimethoprim increased substantially from HIV⁺A⁻ (83.33%) to HIV⁺A^{+2.0} (100%). This drug is heavily used in health institutions and in the community in Kenya, generally in combination with sulfamethoxazole. The selective pressure generated by overuse explains the relatively high prevalence of resistance in *E. coli* isolates in HIV⁺A^{+2.0} cluster. However, it is not clear why the trend observed with other widely used drugs was not seen in this case. Trimethoprim-sulphamethazole (SXT) is a first line treatment antibiotic for upper respiratory infections. The antibiotic is relatively cheap and can illegally be acquired over the counter even without prescription. These factors can therefore contribute to a misuse of SXT and hence the high antibiotic resistance trends. SXT is the most commonly used antibiotic since the advent of HIV/AIDS (Maartens, 2002). UNAIDS recommended the use of SXT on a wide scale in Africa in 2000 among PLWHA (UNAIDS, 2007). This has already been started in Kenya by doctors (MSF Belgium), who are working from Mbagathi District Hospital and by the Center for Disease Control (CDC)

in western Kenya. SXT is currently the most used antibiotic as a prophylactic drug and for treatment of most bacterial infections. In Kenya SXT is retailed for as little as Kenya shillings 60.00 (USD 1) per dose and is even sold without prescription. Studies on SXT resistance trends in *E. coli* isolated from HIV sero-positive adults has been carried out (Anietie *et al.*, 2006; Obi *et al.*, 2007; Kaul *et al.*, 2007). In Kenya, *E. coli* was isolated from PLWHA and attending MDH and on continues use of cotrimoxazole (Oundo *et al.*, 2003). The results showed that the trend of trimethoprim-sulphamethazole resistance increased from 1999 to 2002.

Trend in antibiotic resistance to cefuroxime increased rapidly from *E. coli* isolated from PLWHA and not taking antibiotics (HIV⁺A⁻) to PLWHA and taking antibiotics for one and a half years (HIV⁺A^{+1.5}). The trend then dropped in PLWHA and taking antibiotics for two years (HIV⁺A^{+2.0}) while for ceftazidime, resistance increased from PLWHA not taking antibiotics (HIV⁺A⁻) to PLWHA and taking antibiotics for six months (HIV⁺A^{+0.5}). The antibiotic resistance then decreased in PLWHA and taking antibiotics for one year (HIV⁺A^{+1.0}) and further shot in PLWHA and taking antibiotics for one and a half years (HIV⁺A^{+1.5}). A rapid increase in resistance is probably due to increased use of the antibiotic as a second line treatment among the population. However, it is an inexpensive antibiotic and is probably administered when the first line of antibiotics fail to treat infection. A swift decrease in the antibiotic resistance in HIV⁺A^{+2.0} can be as a result of a back mutation of the bacteria, loss of the fertility factor to transfer resistant genes to other bacteria or as a result of using different populations in the study (Zemelman, 2001). Administration of cephalosporins is common in PLWHA especially in the management of upper respiratory tract infections, thereby exposing enteric bacteria to the antibiotics may lead to the emergence of resistance. Cefuroxime, a second generation cephalosporin represents a valid therapeutic option even in the presence of most beta-lactamase producing bacteria (Kallman, 2003). This has been due to production of extended-spectrum beta lactamase (ESBL) by these bacteria (Zemelman, 2001). Musoke and Revathi (2003) raised concern to increased resistance trend to cefuroxime in their study, which reported 27% resistance by clinical isolates of *E. coli*. Kariuki *et al.* (2001)

identified a novel extended spectrum beta-lactamase which they designated CTX-M -12 that coded for resistance to ceftriaxone, a 3rd generation cephalosporin. The ESBL was encoded on a large 160 kbp plasmid which was self transferable. These reports support our findings and calls for re-evaluation of cephalosporin's administration and monitoring of resistance to the same.

5.1.3. Minimum inhibitory concentrations

The minimum inhibitory concentration (MIC) for all tested antibiotics was higher in isolates from HIV⁺A⁺ compared to isolates from HIV⁺A⁻. Increased MIC has been reported for *E. coli* (Kariuki *et al.*, 2003). Kariuki *et al.* (2003) showed that MDR strains of *E. coli* isolated between 1997-1999 were resistant to tetracycline, ampicillin and Sulphamethazole-trimethoprim and all had an MIC above 32µg/ml. Reports on increased MIC among strains of *E. coli* isolated from HIV sero-positive adults has increased over the years. In this study, 42% of the isolates from PLWHA were multi-drug resistant (resistant to more than 7 antibiotics). These organisms cause life-threatening bacteraemias in PLWHA (; Petit *et al.*, 2001; Manfred *et al.*, 2001; Gilks *et al.*, 2002). Acquisition of such resistance by highly contagious pathogens such as *Yersinia pestis*, the plague bacillus (Galimaud *et al.*, 2003) will render such infections untreatable.

5.1.4. Plasmid analysis

Plasmids were detected in virtually all MRD strains. Transfer of resistant phenotype to *E. coli* K12 was also achieved. Sixty two percent of the strains transferred a resistant phenotype and a 98 MDa plasmid was implicated. Previous studies show the significance of plasmid of 40, 65 and 100 MDa in MDR *E. coli* in Kenya (Oundo *et al.*, 2000; Kariuki *et al.*, 2001). Plasmid mediated resistance has been clearly demonstrated in MDR isolates in this study. The findings are also supported by Lervestein-van Hall *et al.* (2002a). In their study, Lervestein and colleagues reported that complete resistance pattern was transferred by multi-antibiotic resistant *E. coli* at high frequency in conjugation experiment. The investigators also reported that, combined

resistance to SXT and ampicillin was a starting point for the development of additional resistance to beta-lactams, aminoglycosides, cephalosporins and ciprofloxacin, a role which can easily be achieved by intergron carrying plasmids (Lervestein-van Hall *et al.*, 2002b). *E. coli* may serve as a convenient pool of multi-antibiotic resistance genes which can be spread among species and genera. It is therefore important to study further the role of plasmid mediated resistance with a view to addressing ways to minimize rapid spread of antibiotic resistance among strains of *E. coli*

5.2 CONCLUSION

This study demonstrated that prolonged antibiotic therapy by PLWHA makes them harbour strains of *E. coli* that are more resistant to antibiotics with varying resistance patterns and increasing trends with time. The antibiotic resistance patterns generated using the antibiotic disc diffusion susceptibility test produced 33 varying resistance patterns with each pattern showing resistance to at least two antibiotics. Antibiotic resistance trends also differed depending on the antibiotic used. Trends increased rapidly to most of the older, less expensive antimicrobial drugs used in the management of opportunistic infections in Kenya but for fluoroquinolones, resistance trend increased slowly and progressively in isolates from PLWHA and taking antibiotic for two years.

All multidrug resistant strains of *E. coli* carried R- Plasmids but of varying molecular weights (ranging from 14 to 98MDa). Heavy weight plasmids i.e. >98MDa were detected in *E. coli* isolates from PLWHA and on prolonged antibiotic exposure although the isolates also had R-plasmids of lower MW. R- Plasmids of a particular MW were not restrictive to a specific group of the isolates, but were randomly placed among the isolates in all the classes. The R- plasmids were also successfully transferred to *E. coli* K12 though the 98, 4.8 and <4.8 plasmids were frequently transferred.

Antibiotic use whether as prophylactic agents or for treatment are important in improving quality of life for PLWHA but they will not only inhibit target bacteria but will also influence organisms that are part of the normal biota. These bacteria will acquire resistance genes and eventually a pool of resistance genes will be transferred to other bacteria. Transfer of resistance genes can even occur between different species and genera. Broad spectrum antibiotics enhance development of MDR strains as cross resistance to several antibiotics is conferred.

5.3 RECOMMENDATIONS

- Consider the option of food supplements to the current antibiotic therapy, which requires prolonged consumption with the possible risk of antibiotic resistance. This could help boost the immune system thus reducing the problem of opportunistic infections (OIs).
- Massive use of antibiotics should be augmented with relevant programs on knowledge, attitude, hygiene and practice to raise awareness on drug resistance.
- Change the route of inoculation or mode of administration of the antibiotics. It would be recommended to consider other routes of antibiotic inoculation like intramuscular or intradermal rather than oral administration.
- Extra work is needed on antibiotics whose trends were increasing in order to determine its consistency under constant variable. Close monitoring of antibiotic resistance should go hand in hand with expanded programs on chemoprophylaxis in Kenya .Susceptibility testing should be given more attention like other components of HIV in all hospitals and research institutes. WHO has recognized KEMRI as a reference institute for antibiotic susceptibility testing including monitoring MDR *Mycobacterium tuberculosis*. It is recommended that private hospitals and other stake holders network with KEMRI, so that drug resistance can be effectively addressed in Kenya.
- The government should enforce regulation on the usage of antibiotics
- Further studies should be conducted on antibiotic resistant trends of E. coli isolated from PLWHA but on the same study population.

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APPENDICES**APPENDIX A****ANTIBIOTIC PANELS USED**

TET – Tetracycline (30µg)

CAZ - Ceftazidime (20µg)

AMC – Amoxicillin/clavulanic acid (Augmentin) (20µg)

CXM – Cefuroxime (30µg)

CHL - Chloramphenical (30µg)

SXT – Cotrimoxazole (1.25µg)

NA – Nalidixic acid (30µg)

CIP – Ciprofloxacin (5µg)

NIT – Nitrofurantoin (300µg)

AMP – Ampicillin (10µg)

GM – Gentamicin (10µg)

NOR – Norfloxacin (30µg)

APPENDIX B**PLASMID EXTRACTION REAGENTS****Solution 1**

50 Mmol glucose

25 Mmol Tris Cl (Ph 8.0)

10 mMol EDTA (Ph 8.0)

Solution 1 can be prepared in batches of approximately 100 ml, autoclaved for 15min at 10lb/sq. in. on liquid cycle and stored at 4⁰C.

Solution 11

0.2 N NaoH (Freshly diluted from a 2 N stock)

1% SDS

Solution 111

5 M potassium acetate 60 ml

Glacial acetate 11.5 ml

H₂O 28.5 ml

The resulting solution is 3 M with respect to potassium and 5 M with respect to acetic cid

Electrophoresis buffer

Buffer	Working solution	Stock solution
Tris-borate EDTA (TBE)	0.5X:0.045M TBE 0.001M EDTA	5X:54g Tris base 27.5g boric acid 20ml 0.5M EDTA (PH 8.0)

APPENDIX C

INFORMED CONSENT STOOL COLLECTION

ANTIBIOTIC SUSCEPTIBILITY AND GENOTYPES OF *ESCHERICHIA COLI* ISOLATED FROM HIV SERO-POSITIVE ADULTS AT MBAGATHI DISTRICT HOSPITAL, NAIROBI

INTRODUCTION

Good morning/afternoon. My name is Jacinta Emacar, a postgraduate student at Kenyatta University. The aim of my study is to carry out microbiological analysis on stool samples from HIV positive patients on and not on antibiotics in order to determine the development of resistance in strains of *Escherichia coli*.

Why have you been chosen for the study?

You have been chosen to participate in this study because you are an adult client of the Comprehensive Care Centre (CCC) at Mbagathi District Hospital.

What do I have to do?

You will be required to give a stool sample, once on enrolment into the study.

Are there any risks

There are no risks in stool sample collection

What will I benefit?

In the event of any unanticipated discovery of disease, you will be notified, counseled and referred for further medical attention. The results of this study will be given to the hospital and pharmaceutical firms directly concerned with you to be able to make a sound decision in order to improve provision and management of antimicrobials

Who will have access to this information?

Any information given is confidential and the responses will only be used for the intended purpose, and will be accessible to members of this study only. It will be kept in a lockable cabinet and any information transferred to the computer will be password protected. Only the principal investigator and supervisors will have access to the information.

Must you participate?

Your participation in this study is voluntary. If you decide not to participate, you will not be denied any services in this facility, now or in the future.

Whom can I ask questions about the study?

You can contact me at Jacinta Emacar 0722563270

You can also contact Dr. K. M. Wasunna, secretary KEMRI/NERC at 254-20-272241

CONSENT AGREEMENT

I have been explained to all the risks and benefits of the study and all my questions answered to my satisfaction. I have also been assured that participation in the study is voluntary and I may refuse to participate, and that will not affect my service provision in the facility

I agree Signature.....date.....

I disagree Signature.....date.....

Study staff name _____ Sign _____ Date _____

APPENDIX D**MEDIA PREPARATION****MACCONKEY AGAR***Formulae*

Gelatin peptone	17.0g
Lactose	10.0g
Sodium chloride	5.0g
Bacteriological agar	13.5g
Bile salt No: 3	1.5g
Neutral red	0.03g
Peptone mixture	3.0g

Preparation

Suspend 50g of the powder media in 1 liter of distilled water. Boil to dissolve the media completely. Sterilize by autoclaving at 121⁰C for 15minutes. Cool to between 45 – 50⁰C in a water bath and then pour 15 – 20ml into Petri dishes.

Use: For selection of enteric bacteria

MULLER HINTOIN AGAR*Formulae*

Beef infusion	300.0g
Starch	1.5g
Acid hydrolysate casein	17.5g
Agar	17.0g

Preparation

Dissolve 42g of the powder media in 1 litre of distilled water. Boil to dissolve the media completely. Sterilize by autoclaving at 121⁰C for 15minutes. Cool to between 45 – 50⁰C in a water bath and then pour 15 – 20ml into Petri dishes.

Use: For sensitivity testing

PEPTONE WATER***Formulae***

Peptone	10.0g
Distilled water	1.0litres
Sodium chloride	10.0g

Preparation

Add the above ingredients to distilled water and adjust the pH to between 9.0 - 9.2 with concentrated sodium hydroxide solution. Distribute and autoclaving at 121⁰C for 15minutes. Store the alkaline medium in bottles with tightly screwed caps to prevent a drop in PH