EXPLORING THE TRENDS IN PREVALENCE OF HUMAN IMMUNODEFICIENCY VIRUS DRUG RESISTANCE IN SOUTH AFRICA OVER THE COURSE OF THE HIV EPIDEMIC

Denis Rutendo Chopera

Student Number: 3418765

A mini-thesis submitted in partial fulfilment of the requirements for the degree of Masters in Public Health, School of Public Health, Faculty of Community and Health Sciences, University of the Western Cape

Supervisor: Associate Professor Lucia Knight

October 2018

Abstract

Background: Antiretroviral therapy (ART) was rolled out in South Africa in the public sector in 2004 and the treatment coverage has increased over the years to 56% in 2016. The increased treatment coverage has the potential to increase the level of HIV drug resistance. Drug resistance presents a major challenge to the management of HIV infection through antiretroviral therapy at the population level. The aim of this study was to determine the impact of the public sector antiretroviral therapy rollout on the prevalence of HIV drug resistance in South Africa and the factors associated with drug resistance.

Methodology: A cross-sectional analytical study was used to determine the prevalence of drug resistance before and after ART rollout. The study population was HIV infected South Africans (infected between 1996 and 2011) who were not on antiretroviral therapy. The study sample was therapy naïve HIV infected South Africans who participated in published studies conducted between 1996 and 2011. HIV DNA sequences and associated data (participants' age, gender, geographic location and estimated year of HIV infection) were accessed through the Los Alamos HIV Database. The database contains all HIV DNA sequences and associated data from all published studies and the data was freely accessible. A descriptive analysis was carried out on the data to determine characteristics of the study sample. Drug resistance mutations were detected using Calibrated Population Resistance Program on the Stanford University HIV Drug Resistance database. The output from the Calibrated Population Resistance Program analysis were used to determine the prevalence of drug resistance mutations.

Results: There were 1701 DNA sequences obtained from the Los Alamos HIV Database for the three gene regions targeted by ART (reverse transcriptase, protease and integrase). Of these, 604 (35,5%) were for reverse transcriptase, 794 (46,7%) were for protease and 303 (17,8%) were for integrase. There was overrepresentation of DNA sequences from female participants (91%). There was no significant difference in the prevalence of drug resistance mutations between 1996-2004 (before ART rollout) and 2005-2011 (after ART rollout) in all the drug classes. There was also no association between drug resistance and age as well as gender.

Conclusion: The data from this study suggest that the public sector rollout of ART did not result in an increase in the prevalence of drug resistance mutations in therapy naïve HIV-infected South Africans. There is need for further studies, which have a wider coverage of the South African population.

DECLARATION

I declare that **Exploring the trends in prevalence of Human Immunodeficiency Virus drug resistance in South Africa over the course of the HIV epidemic** is my own work, that is has not been submitted for any degree or examination at any other university, and that all sources I have used or quoted have been indicated and acknowledged by complete references.

Denis Chopera

Acknowledgements

I would like to thank my supervisor, Professor Lucia Knight, for the support and guidance throughout this study. Without her kind persistence and expertise this work would not have been possible.

List of Acronyms

3TC - Lamivudine

ABC - Abacavir

AIDS - Acquired Immune Deficiency Syndrome

ART – Antiretroviral therapy

ARV - Antiretroviral

ATV - Atazanavir

AZT - Zidovudine

CCR5 - Cysteine-Cysteine Chemokine Receptor 5

CD4 - Cluster of differentiation 4

d4T – Stavudine

DDL - Didanosine

DLV - Delavirdine

DNA – Deoxyribonucleic acid

DRM – Drug resistance mutation

DTG - Dolutegravir

EFV - Efavirenz

ETR - Etravirine

EVG - Elvitegravir

FDC - Fixed-dose combination

FPV - Fosamprenavir

FTC - Emtricitabine

gp120 – Glycoprotein 120

gp41- Glycoprotein 41

HIV - Human Immunodeficiency Virus

IDV - lndinavir

InSTI – Integrase inhibitor

LPV - Lopinavir

MVC - Maraviroc

NFV - Nelfinavir

NNRTI – non-nucleoside reverse transcriptase inhibitor

NRTI - nucleoside reverse transcriptase inhibitor

NVP - Nevirapine

ORV - Darunavir

PEPFAR - US President's Emergency Plan for AIDS Relief

PI – Protease inhibitor

RAL - Raltegravir

RPV - Rilpivirine

RT – Reverse transcriptase

RTV - Ritonavir

SQV - Saquinavir

STI – Sexually transmitted infection

TDF - Tenofovir

TPV - Tipranavir

WHO – World Health Organisation

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Chapter 1: Introduction

1.1 Background

In South Africa, antiretroviral therapy (ART) was rolled out nationally in the public sector in 2004 and since then there has been considerable coverage of treatment as well as increasing drug combinations (Johnson, 2012). The public rollout was made possible through bilateral and multilateral human immunodeficiency virus (HIV) treatment financing such as the Global Fund and the US President's Emergency Plan for AIDS Relief (PEPFAR) (Bateman, 2002; Nattrass, 2006). Being the country with the largest population of HIV-infected people globally, the national response to HIV in South Africa has great implications on the global response to the epidemic (UNAIDS, 2016a). For example, if a large proportion of HIV-infected people in South Africa are receiving treatment, this translates to a large proportion of infected people globally who are receiving treatment (Muula, 2008). However, the increased treatment coverage comes with an increased risk of antiretroviral drug resistance due to increased chance of nonadherence. The extent of drug resistance accumulation since the introduction of ART in South Africa is unclear (Johnson, 2012). Drug resistance is the ability of a disease agent to resist the effects of a drug against it. Several studies have examined the prevalence of drug resistance mutations in South Africa (Nwobegahay et al., 2012, Parikh et al., 2013, Hunt et al., 2012, Parboosing et al., 2011) but their major limitation has been the fact that they looked at small samples, from specific regions and populations, and the results may not be generalizable to represent the national prevalence of HIV drug resistance.

While antiretroviral treatment is the best option for the management of HIV infection, the development of drug resistance presents a challenge to effective health care and prevention of transmission of the virus (Tanser et al., 2013). Antiretroviral drugs target specific steps in the HIV lifecycle and due to the virus' ability to change rapidly (high mutation rate), it can change to forms that are not recognised by the antiretroviral drugs (Roberts et al., 1988). To put this into perspective, an HIV infected individual typically produces over ten billion viruses per day and the key protein in the virus' replication is prone to making errors such that numerous viruses harbouring any given mutation can be produced per day (Roberts et al., 1988). As a result, viral variants that harbour drug resistant mutations can outgrow other variants. The net effect of this is that the drugs become less effective or completely ineffective (Clutter et al., 2016) .

In terms of resources, the South African government spent about R4.2 billion on antiretroviral drugs between 2014 and 2015 (Venter et al., 2016) which is a considerable burden to the healthcare system. Despite the vast amount of resources dedicated to combating HIV, the national prevalence among antenatal clients remains high (27% in 2011) (Massyn, 2013). In some parts of the country such as the KwaZulu-Natal province, the prevalence of HIV in pregnant women over 18 years was as high as 52% in 2011 (Abdool Karim et al., 2012). A recent study reported a population-weighted HIV prevalence of 36,6% in the province with a 66,6% prevalence in women aged 35-39 years (Kharsany et al., 2018). Recently, UNAIDS reported a national HIV prevalence of 12% for South Africa in 2015 (UNAIDS, 2016a). Although ART has been made available, the national coverage is still low with 40% of eligible patients receiving treatment in 2011 (Johnson, 2012) which increased to 56% in 2015 (UNAIDS, 2016a). While antiretroviral therapy has the advantage of preventing the development of AIDS in HIV infected individuals and reducing their chances of transmitting the virus, the emergence of drug resistance in circulating viral strains will pose an additional challenge to combating the HIV/AIDS pandemic in the country (Johnson, 2012). Individuals infected by drug resistant viruses fail treatment (have high viral loads and low CD4 counts). This will increase their chances of infecting other people with the drug resistant HIV strains, thereby offsetting the benefits of antiretroviral therapy (Clutter et al., 2016). In addition, to treatment failure, treatment of patients with resistant viruses will have an additional disadvantage to them, as they will suffer from the side effects of the drugs (Clutter et al., 2016).

In order to minimise the spread and negative impact of HIV drug resistance on the treatment and management of HIV, there is need for surveillance and resistance testing (WHO, 2015b) . The most effective way would be to conduct point of care testing for HIV drug resistance. Point of care refers to the simple testing that takes place at the bedside and the results are obtained rapidly as compared to the historical practice where testing is confined to the laboratory (Kost, 1995). The major limitation to point of care resistance testing in low-income countries is the cost factor. The low-income countries, such as those in sub-Saharan Africa, are already struggling to increase coverage of ART due to limited resources (e.g., South Africa has 40% of eligible patients on treatment) (Johnson, 2012). This underscores the need for the determination of population level drug resistance prevalence in order to assess the effectiveness of the currently used regimens, as well as guide possible future treatment regimens.

Drug resistance mutations are classified by the World Health Organization (WHO) into three categories based on their prevalence. These are; low prevalence (<5%), moderate prevalence

(5-15%) and high prevalence (>15%) (Jordan et al., 2012). The ART program is said to be optimally functioning when the national prevalence of drug resistance is below 5% (Jordan et al., 2012). When the prevalence is moderate (5-15%), the WHO recommends public health intervention such as increased prevention programs to reduce transmission of drug resistant viruses, examination of ART practices which include adherence, and increased support to treatment programs (Jordan et al., 2012). In the event of drug resistance prevalence of more than 15%, the WHO strongly advises that the first line regimens be changed (Jordan et al., 2012).

Concerns that large-scale antiretroviral therapy programs in Africa would result in the widespread emergence and circulation of drug resistant viruses were expressed very early in the development of such programs in Africa (Stevens et al., 2004, Popp and Fisher, 2002). These concerns arose from the assessment of challenges such as limited resources which would cause drug 'stockouts' as well as drug toxicity. It is important to investigate whether the prevalence of drug resistance has changed from the period prior to introduction of ART and whether factors such as age, geographical location and gender are associated with drug resistance in the South African population.

1.2 Problem Statement

South Africa has the largest ART programme globally, accounting for 20% of people on ART in the world (UNAIDS, 2016a). Drug resistance in HIV infected individuals presents a major challenge to the management of HIV infection through ART at the population level. In particular, individuals infected with resistant HIV strains do not respond to treatment and therefore are unable to control their viral loads and maintain high CD4 counts (Cohen et al., 2011). This, in turn, increases their chances of infecting other people, resulting in increased incidence and prevalence of HIV infections (Li et al., 2011). Drug resistance also threatens the effectiveness of pre-exposure prophylaxis (PrEP) where antiretroviral drugs are used to prevent infection in high risk individuals (Wood and Montaner, 2011, Cohen et al., 2011). PrEP has been shown to be very effective at reducing HIV infection both sexually and through injection drug use, reducing the risk of HIV infection by up to 90% (Cohen, 2018). At the population level, drug resistance will have the effect of reducing the benefits of ART, such as increasing the life expectancy of infected individuals and reducing HIV incidence and prevalence. In 2016, the World Health Organisation (WHO) announced new HIV treatment guidelines for universal testing and treatment whereby all individuals diagnosed with HIV infection have to be put on ART immediately (UNAIDS, 2016b). The overall goal is to end AIDS by 2030. This

is a welcome strategy in that it minimises the damage that untreated HIV cause to the immune system during the early phases of infection and significantly improves the quality of life of the infected individuals (UNAIDS, 2016b). Getting infected individuals onto treatment early and while they are still well reduces the likelihood of symptoms and illness developing. However, there is a possibility that it could result in increased drug resistance in circulating viruses. Due to the increased period of time that infected individuals have to be on treatment when they initiate treatment early, and lack of drug resistance monitoring capacity in low-income countries, there is a risk that early treatment could drive the emergence of resistant viruses in early infection which will increase the prevalence of drug resistance at the population level (Clutter et al., 2016). This will increase the cost of treatment due to the need for alternative drugs once resistance develops (Johnson et al., 2017).

Knowledge of the drug classes that drive resistance and those where resistance does not easily develop in the South African population is necessary for the effective management of the HIV epidemic. Antiretroviral drugs are classified according to the steps they target in the viral lifecycle. Due to the genetic diversity of HIV globally (Volberding and Deeks, 2010), there is need to determine which classes of drugs the viral strain (known as subtype) circulating in a specific region is able to easily develop resistance to and which classes are less prone to the development of resistance. Analysis of the HIV drug resistance patterns over a period of time (for example, more than a decade) will give an idea of which drugs and drug classes drive resistance in a given population. This knowledge will inform policy-makers on which drugs and drug classes to include in the treatment regimen for the country. The most effective management of HIV would be to include drugs where the circulating strains in the country cannot easily develop resistance to. That is, the viruses within the population cannot readily mutate to develop resistance.

1.3 Rationale

This study proposed to investigate the change in prevalence of drug resistance mutations in the South African population over a period of 16 years starting from the pre-ART era (1996-2004) well into the current age of combination therapy (2005-2011). The study assessed the distribution of drug resistance in South Africa by gender, age, and geographical location. An evaluation of the factors associated with accumulation of drug resistance provides useful information on what intervention strategies need to be put in place to combat the problem of drug resistance. It also provides information on the prevalence of drug resistance and the drugs or drug classes that are driving this resistance, and therefore guide treatment policy. For

example, if a specific drug or drug class predominantly drives HIV drug resistance in the South African population, it may be more effective to exclude it from the first line treatment regime in order to reduce development of resistance.

If the problem of drug resistance is not given enough attention and careful consideration, it could result in failure to manage the HIV epidemic through ART and worsen the already overwhelming situation in South Africa and the sub-Saharan region in general. Studies of the nature of this one are valuable to assess the true extent of the problem of drug resistance and the factors that are associated with it.

In addition, it will not be possible to conduct studies of this nature in future as the current treatment guidelines stipulate that HIV-infected individuals have to be put on treatment as soon as they test positive (test and treat). As a result, it will be very difficult to determine the level of transmitted drug resistance as everyone will be on ART (i.e., if resistance is detected in an infected individual who is on treatment, it is difficult to determine whether the resistance developed due to current treatment or it was transmitted). From a public health perspective, knowing the source of resistance would be important to enable the necessary interventions.

1.4 Aim and objectives

1.4.1Aim

The aim of this study was to describe the prevalence of HIV drug resistance in South Africa from 1996 to 2011 and determine the factors associated with drug resistance.

1.4.2 Objectives

a) To determine the prevalence of HIV drug resistance before ART rollout (1996-2004) and after ART rollout (2005-2011).

b) To determine the drug resistance by ARV class.

c) To determine if there is an association between drug resistance and selected risk factors over time.

1.5 Summary

In order to determine the effect of ART rollout on drug resistance prevalence, this study assessed the patterns of HIV drug resistance in South Africa to all the major drug classes over a period spanning the pre-ART era well into the era of combination antiretroviral therapy (1996-2011), retrospectively. The study participants were therapy-naïve HIV-infected South

Africans whose HIV sequence data as well as geographic (province), gender and age information was available through the Los Alamos HIV Database (www.hiv.lanl.gov). The results from this study provide useful information on which drugs and drug classes are driving HIV drug resistance in South Africa and will inform treatment strategies.

Chapter 2: Literature Review

2.1 Introduction

This chapter is a review of the literature relevant to this study. It starts with exploring the global HIV epidemic and the advances that have been made in the management of HIV through antiretroviral therapy. It goes further to describe the different classes of antiretroviral drugs and the mechanisms through which HIV develops drug resistance as well as the factors associated with drug resistance.

2.2 HIV epidemic

Since its identification as the cause of AIDS, HIV has emerged as a major global pandemic which had claimed more than 35 million lives by 2016 (UNAIDS, 2017). An estimated 36.7 million people were living with the virus in 2016, including 1.8 million children (UNAIDS, 2017). HIV has infected some 77.3 million people since the start of the epidemic and in 2017 alone, 940 000 people died from AIDS-related illnesses (UNAIDS, 2018). The sub-Saharan African region remains the worst affected with an estimated 25.5 million individuals who were living with HIV in 2016, constituting 67% of infected people globally (UNAIDS, 2017). South Africa had 7.1 million HIV infected people in 2016, making it the country with the highest number of infected individuals (UNAIDS, 2017).

Despite the dire situation, global statistics have shown some improvement (UNAIDS, 2018). For example, the number of new HIV infections per year has decreased from 3 million in 2001 to 1.8 million in 2017 (UNAIDS, 2018). This has been enabled by several strategies aimed at reducing HIV transmission which include, among others, the test and treat approach, increased testing and wider treatment coverage (UNAIDS, 2018). Recently, the World Health Organisation (WHO) announced new guidelines for ART which stipulate that treatment should be initiated when an individual tests positive for HIV (previously it was CD4 count below 500) (UNAIDS, 2016b). In 2017, approximately 75% of infected people knew their HIV status and of these, 79% were accessing treatment. Of those accessing treatment, 81% were virally suppressed suggesting that they had very little probability of transmitting the virus (UNAIDS, 2018). This is remarkable progress towards the ‘Fast-Track’ strategy of ending AIDS by 2030 (UNAIDS, 2018). The Fast-Track strategy targets that by 2020, 90% of infected people should know their HIV status and of these, 90% should be on treatment and of those on treatment, 90% should be virally suppressed (the so called 90-90-90 target). By 2030, 95% of infected

people should know their HIV status and of these, 95% should be on treatment and of those on treatment, 95% should be virally suppressed (UNAIDS, 2018).

The introduction of antiretroviral therapy (ART) in the mid-1990s resulted in dramatic and sustained declines in HIV related morbidity and mortality among those with access to treatment (Palella et al., 1998, Tanser et al., 2013). Since then, increasingly potent and effective drugs and drug classes continue to be developed (Palella et al., 1998). Although in 2009 and 2010 history was made with the first evidence of protective efficacy in an HIV vaccine trial (Rerks-Ngarm et al., 2009) and microbicide trial (Abdool Karim et al., 2010), respectively, an effective vaccine or cure is still not in sight. In South Africa, ART was only rolled out publicly in 2004 and the treatment coverage has increased markedly since then (Johnson, 2012). In 2015, 85% of HIV infected South Africans knew their HIV status (Johnson et al., 2017). However, on 56% of those who knew their HIV status were on ART and 78% of those on treatment were virally suppressed (Johnson et al., 2017).

2.3 HIV subtype distribution

The viral strains assessed in this study were all subtype C which is the predominant strain in the Southern Africa region (van Loggerenberg et al., 2008). HIV has three main groups; group M (major), group O (outlier), and group N (non-M/ non-O) (Robertson et al., 2000). Recently, a new group that is distinct from the other HIV-1 groups has been identified and named group P (Plantier et al., 2009). HIV-1 M infections account for over 95% of HIV infections worldwide (McCutchan, 2000) and Group M has been further sub-divided into 9 genetic subtypes named subtypes A, B, C, D, E, F, G, H, J and K. HIV-1 subtype C is the predominant HIV-1 lineage driving the global HIV epidemic, accounting for approximately 50% of all infections (Hemelaar et al., 2006). In South Africa, nearly all HIV infections result from subtype C with a few isolated cases of other subtypes (van Loggerenberg et al., 2008). This is quite different from Western countries such as the USA were subtype B is the predominant HIV strain.

2.4 HIV replication and antiretroviral drugs

HIV depends on human cells for its replication (Wyatt and Sodroski, 1998). The virus contains unique genes that encode various proteins crucial for the virus' structure and function. Antiretroviral drugs target these proteins and the associated steps in the lifecycle of the virus (Shafer, 2006). The most important viral targets are the virus' enzymes which catalyse steps in the cycle of the virus. The antiretroviral drugs act by inhibiting specific stages of the viral cycle while having minimal adverse effects on the functioning of the human cells.

Table 1: Classes of antiretroviral agents

Class	Abbreviation	Mechanism of action	Specific action
Nucleoside reverse transcriptase inhibitors	NRTIs	Reverse transcriptase inhibition	Nucleic acid analogues mimic the normal building blocks of DNA, preventing transcription of viral RNA to DNA
Non-nucleoside reverse transcriptase inhibitors	NNRTIs	Reverse transcriptase inhibition	Alter the conformation of the catalytic site of reverse transcriptase and directly inhibit its action
Protease inhibitors	PIs	Protease inhibition	Inhibit the final maturation stages of HIV replication, resulting in the formation of non-infective viral particles
Integrase inhibitors	InSTIs	Inhibition of viral integration	Prevent the transfer of proviral DNA strands into the host chromosomal DNA
Entry inhibitors	-	Entry inhibition	Bind to viral gp41 or gp120 or host cell CD4+ or chemokine (CCR5) receptors

[Source:(Meintjes et al., 2017)]

In order to put the mechanisms of action of antiretroviral drugs into perspective, here is a brief basic description of the HIV life cycle: The HIV envelope protein (viral coat) binds to the CD4 receptor on the surface of the human cells (Wyatt and Sodroski, 1998). This is followed by the binding of the HIV envelope to a second co-receptor called CCR5. Following binding of the virus to the receptors on the human cell, the virus fuses with the human cell allowing the contents of the virus to be released into the human cell (Arthos et al., 2008, Chan and Kim, 1998). One of the viral enzymes, reverse transcriptase, converts the virus' genetic material (RNA) to DNA which is then integrated into the human genome by another viral enzyme known as integrase. When the human cell synthesises its own protein using its genetic material, the virus' proteins are also synthesised as the viral DNA has now become part of the human cell's DNA (Wyatt and Sodroski, 1998). Another viral enzyme, protease, then processes the synthesised viral proteins which then assemble into the virus particles which bud off the cell and infect other cells and the cycle continues. A single infected cell can produce thousands of new viruses making the replication of HIV exponential (Wyatt and Sodroski, 1998). The three

HIV enzymes, reverse transcriptase, protease and integrase are the primary targets of antiretroviral drugs although there are drugs which target other steps in the viral cycle (Shafer, 2006).

There are several classes of antiretroviral drugs which are currently in use for treatment of HIV. These drugs are classified based on what phase in the viral cycle they target in order to inhibit the replication of the virus (Shafer, 2006). The classes are; nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), entry inhibitors and integrase inhibitors (InSTIs) (Shafer, 2006). The table below summarises the classes of antiretroviral drugs;

The different classes of HIV drugs are summarised in Table 2 below.

Table 2: Antiretroviral drugs and their classes

Class	**Drugs**
NRTIs	Abacavir (ABC) Didanosine (ddl) Emtricitabine (FTC) Lamivudine (3TC) Stavudine(d4T) Tenofovir (TDF) Zidovudine (AZT, ZDV)
NNRTIs	Delavirdine (DLV) Efavirenz (EFV) Etravirine (ETR) Nevirapine (NVP) Rilpivirine (RPV)
PIs	Atazanavir (ATV) Darunavir (ORV) Fosamprenavir (FPV) lndinavir (IDV Lopinavir (LPV) Nelfinavir (NFV) Ritonavir (RTV) Saquinavir (SQV) Tipranavir (TPV)
InSTIs	Raltegravir (RAL) Elvitegravir (EVG) Dolutegravir (DTG)
Entry inhibitors	Maraviroc (MVC)

[Source: (Shafer, 2006)]

2.5 HIV drug resistance

The high replication rate of HIV coupled with the fact that one of the key enzymes in the replication cycle of the virus, reverse transcriptase, makes a lot of errors, the virus is able to change rapidly to forms that are not recognized by antiretroviral drugs. Because HIV can easily become resistant to treatment with a single drug, this has necessitated the use of combinations of drugs for treatment in order to delay the appearance of resistance (WHO, 2015b). Typical drug combinations contain two NRTIs (backbone) and one NNRTI or PI or InSTI (base). Treatment failure occurs when an infected person on treatment is unable to control the virus. Once a patient begins to fail treatment with the first regimen, known as the first line, they are put on a second regimen (second line) and eventually on a third regimen (third line) upon failure of second line treatment (WHO, 2015b). Combination antiretroviral therapy is effective since it is able to suppress the HIV replication (Lange, 1995). The low replication significantly reduces the risk of resistance development due to the suppressed spontaneous generation of viruses that are resistant to all the three drugs.

2.6 Treatment guidelines in South Africa

In 2013, the South African government introduced new treatment guidelines (Health, 2015). These guidelines were in line with the international guidelines (WHO, 2015b) where the first line contains two NRTI drugs as the backbone and one NNRTI or PI or InSTI as the base. The drugs are given fixed-dose combination (FDC) pills (Health, 2015). These guidelines were revised in 2017 (Meintjes et al., 2017). The most commonly used FDC is made up of the regular three drugs used in the first line regimen (TDF, FTC and EFV) (Table 3). The FDC pills help to improve the adherence and therefore minimise the risk of drug resistance due to non-adherence. The regimens for South Africa as defined by the national Department of Health are shown in Table 3 below.

Table 3: South African ART regimens for adults and adolescents (Source: (Meintjes et al., 2017))

First Line
TDF + FTC (or 3TC) + EFV
TDF + (FTC or 3TC) + NVP
AZT + 3TC + EFV (or NVP) D4T + 3TC +EFV (or NVP)
ABC + 3TC + EFV (or NVP)
TDF + FTC (or 3TC) + EFV
Second Line
AZT + 3TC + LPV
TDF + 3TC (or FTC) + LPVr
TDF + 3TC (or FTC) + ATVr
Third Line
Raltagravir/Darunavir/Etravirine

2.7 Drug resistance prevalence in developed countries

The global prevalence of HIV drug resistance has been increasing due to the rollout of ART in low to middle-income countries (Gupta et al., 2012). In 2012, the global prevalence had increased to 9% from 6.9% in 2010 (Gupta et al., 2012). There are no clear differences in drug resistance prevalence between developed and developing countries with some developed countries having higher prevalences while others have lower than developing countries (WHO, 2017).

In Canada, the prevalence of drug resistance has been reported for various regions with the Alberta Province having a prevalence of 12% (Ragonnet-Cronin et al., 2013). Ragonnet-Cronin et al. (2013), conducted a surveillance study in Alberta Province, Canada where they genotyped remnant blood from newly diagnosed HIV infections from 2007 to 2010. This moderate prevalence was partly explained by the high coverage of HIV treatment in the country at the time. Canada introduced universal HIV treatment earlier than most countries and 70% of infected people were diagnosed and 80% of these were accessing treatment (Nosyk et al., 2014). A study conducted by Yerly and colleagues (2007) reported that Switzerland had a transmitted drug resistance prevalence of 7.7% which was generally stable for the 10-year period between 1996-2005.

2.8 Drug resistance trends in South Africa and other African countries

In Africa, drug resistance has been on the rise and is largely driven by resistance to NNRTIs (Gupta et al., 2012). This has significantly impacted the resistance levels globally (WHO, 2017).

A study carried out in Lusaka, Zambia, to assess the baseline drug resistance in patients who were initiating first-line therapy in 2007-2008 reported a 6% prevalence of drug resistance (Hamers et al., 2010). A recent study conducted in Uganda reported that the prevalence of drug

resistance in Kampala was 7% while it was 3% in Mbarara (another part of the country) (Lee et al., 2014).

In South Africa, there have been conflicting reports on the prevalence of HIV drug resistance mutations based on where the studies were carried out. For example, one study conducted on patients enrolling into antiretroviral therapy at a hospital in Limpopo Province reported a drug resistance prevalence of 9.3% in drug naïve patients (Nwobegahay et al., 2012) while a cross-sectional study of reproductive-aged women in KwaZulu-Natal reported a prevalence of 7.4% (Parikh et al., 2013). Only one study has assessed drug resistance over time through a literature review data mining study to determine temporal trends in transmitted drug resistance in South Africa (Manasa et al., 2012). The study reported on the annual prevalence of HIV drug resistance mutations in South Africa over a 10-year period between 2000-2010 (Manasa et al., 2012). The study reported that the drug resistance prevalence over those 10 years was consistently below 5% with only one exception, 2002, when it was 6.7%. The major limitation of this study, however, was the fact that the authors' selected and analysed data from studies that reported on drug resistance. However, they could have also included data from other studies which enrolled treatment-naïve participants to increase their sample size. This approach excluded data from other studies even though the patients were treatment-naïve.

Recently, a cross-sectional study assessed the prevalence of drug resistance across South Africa in patients initiating treatment (Steegen et al., 2016). The study assessed samples representative of the number of patients receiving antiretroviral treatment in each province between March 2013 and October 2014. The results from the study showed a moderate drug resistance prevalence of 9%.

2.9 Factors associated with HIV drug resistance

There are several factors that are associated with the development of HIV drug resistance which range from health system associated to socio-economic factors (Weinstock et al., 2004, Volberding and Deeks, 2010, Lockman et al., 2010, Bennett et al., 2008). Some of the factors associated with HIV drug resistance are outlined below.

2.9.1 Health system factors

As more HIV-infected people gain access to ART in developing countries, there is an increased chance of accumulation of drug resistance in circulating viruses in the population. Resistance occurs mainly due to lack of adherence by the patients or due to insufficient drug levels in the patient's system as a result of underlying genetic factors (Volberding and Deeks, 2010,

Lockman et al., 2010). Another cause for the emergence of drug resistance is the use of inadequate regimens such as single or double therapy instead of triple therapy (Volberding and Deeks, 2010). In addition, interactions between HIV drugs and other drugs may result in reduced amounts of antiretroviral drugs in the body system thereby triggering resistance (Volberding and Deeks, 2010). For example, there needs to be pre-treatment assessment of the drugs to be taken by patients who are co-infected with HIV and tuberculosis (TB). Another contributing factor to the development of drug resistance is the prevention of mother-to-child transmission (PMTCT) strategy. PMTCT generally involves a single dose of one drug (such as nevirapine) to pregnant women (Barth et al., 2008). This has been reported to result in nevirapine-resistance in 40-60% of mothers as well 40-50% of infected babies (Barth et al., 2008). However, this has now changed with the new PMTCT guidelines published by WHO in 2015 which recommended that all pregnant women living with HIV be immediately provided with lifelong treatment (WHO, 2015a).

Unlike in developed countries where there is individualisation of ART through resistance testing before ART initiation and close viral load and CD4 count monitoring (Williams et al., 2012, Vandamme et al., 2011, Gill et al., 2010), there is no baseline resistance testing in developing countries, including South Africa, to guide therapy and as a result there is an increased risk of patients failing therapy due to the fact that they would have been infected with a viral strain which is already resistant to the therapy that they are put on (Lessells et al., 2013). This is a major driver of circulating drug resistant viruses in developing countries.

During the early days of HIV treatment, before the highly active antiretroviral therapy era, there were high levels of drug resistance due to the use of mono and dual therapy in developed countries (Weinstock et al., 2000, Weinstock et al., 2004, Resistance, 2001, Grant et al., 2002, Little et al., 2002, Cane et al., 2005, Wensing et al., 2005).

2.9.2 Other factors

There are also socio-economic factors that the literature suggests may contribute to the development of HIV drug resistance. These include missed drug collections by patients due to family obligations and travel costs (El-Khatib et al., 2011, Bennett et al., 2008, Mills et al., 2006). Other factors that influence the transmission of HIV drug resistance include the population being studied, access to antiretroviral therapy, risk behaviours, mode of transmission and the viral strain circulating in the population (Pillay, 2004, Sagir et al., 2007).

These factors result in nonadherence to treatment which increases the risk of the development of resistance.

Some previous studies have reported associations between HIV drug resistance and age (Bianco et al., 2011, Lucas et al., 2002, Hinkin et al., 2004)as well as gender (Kan et al., 2017, Goedecke et al., 2013, Sabin et al., 2008). The association between drug resistance is mainly attributed to nonadherence to treatment in older people due to cognitive impairment which results in them forgetting to take their drugs according to protocol (Hinkin et al., 2004). Females have been shown to have higher odds of developing drug resistance compared to males (Kan et al., 2017, Goedecke et al., 2013, Sabin et al., 2008). This has been attributed the differences in the pharmacokinetics of the drugs between the two genders. Females have been documented to have higher drug concentrations in their blood compared to males despite taking similar amounts of drug (Cressey and Lallemant, 2007). This is thought to result in greater drug toxicity in females which may play a role in nonadherence, leading to drug resistance.

2.10 Summary

The extent to which drug resistance has accumulated in the South African HIV-infected population is not well established. While several studies have assessed the prevalence of drug resistance in South Africa, they were focused on specific study populations and their findings cannot be generalised nationally due to several differences that exist within South Africa such as socio-economic backgrounds and demographic genetic backgrounds (Nwobegahay et al., 2012, Parikh et al., 2013). This underscores the need for a study that includes a diverse sample from the country so as to overcome some of the limitations of the previous studies. Results from such a study would generally be more representative of the national picture.

Chapter 3: Methodology

3.1 Introduction

This chapter describes the design for the study and how the data was obtained from the Los Alamos HIV database (www.hiv.lanl.gov). The validity and limitations of the study are explained and the methods that were used to conduct the data analyses are described. Lastly, the ethics considerations that were taken into account are outlined and the fact that ethics approval was obtained from the University of the Western Cape Biomedical Research Ethics Committee.

3.2 Study Design

In this retrospective study, the investigation occurred after both the exposure and the outcome of interest occurred, and the study sought to establish the association between the exposure and the outcome. In this study, the secondary data on the exposures (i.e., age, gender, estimated year of infection) was utilised to determine their association with the occurrence of drug resistance. The study is a cross-sectional study. The advantage of this type of study is that it enables the establishment of association between exposure and outcome (Beaglehole et al., 1997).

3.3 Population and Sampling

The population of interest in this study was all HIV-infected individuals in South Africa who were estimated to have been infected from 1996 to 2011 and were not on ART. The study sample comprised of treatment naïve HIV-infected individuals who had the following data available on the Los Alamos HIV Database (www.hiv.lanl.gov);

a) HIV (RNA) sequence data for the Polymerase gene
b) Estimated date/year of infection
c) Age
d) Gender
e) Geographic location

The participants with the above data available were representative of the documented HIV-infected individuals in South Africa during the period of interest, the data came from studies published in international journals (www.hiv.lanl.gov). The study participants were stratified into two groups based on their estimated infection date/year (1996-2004 and 2005-2011). As

the aim of the study was to estimate the prevalence of transmitted drug resistance in treatment naïve individuals both before and after the introduction of antiretroviral treatment in the public sector, this stratification was very useful in determining whether there was accumulation of drug resistance mutations after treatment rollout. Ideally, it would have been much better to stratify the participants for each year of estimated infection but this would result in low numbers of those with available data (www.hiv.lanl.gov). Therefore, stratifying the participants into these two groups provided enough participants in each stratum for statistical analyses. The dates selected (1996 – 2011) gave roughly the same amount of data prior to ART rollout (1996-2004) and after ART rollout (2005-2011).

3.4 Data Collection

This study utilised data available on the Los Alamos HIV Database (www.hiv.lanl.gov) where all available information on HIV related publications that generated DNA sequences is stored, including DNA sequences, treatment history, infection or sampling dates, gender, age and sexually transmitted infections (STIs). It is a requirement for researchers to submit the data before publication in most international journals (Kuiken et al., 2003). The database made use of all HIV sequences that were published from South Africa from 1996 to 2011, which have additional data available such as year of infection, age, gender, and location. The data was be freely accessed through the Los Alamos HIV database (www.hiv.lanl.gov) , thereby eliminating the need to generate the sequence and other relevant data which would have been a costly and time-consuming process. The bulk of the data will primarily come from four studies conducted in the country (van Loggerenberg et al., 2008, Abdool Karim et al., 2010, Gray et al., 2014, Kiepiela et al., 2007).

The data which was used in this study was primarily from four main published studies;

1) the Centre for the AIDS Programme of Research in South Africa (CAPRISA) Acute Infection study (CAPRISA 002) which was aimed at identifying acute HIV infection and investigating the role of viral and immunological factors in acute and early HIV-1 infections (van Loggerenberg et al., 2008),

2) the CAPRISA 004 study which assessed the effectiveness of a 1% tenofovir gel microbicide in preventing HIV infection (Abdool Karim et al., 2010),

3) the HIV Vaccine Trial Network (HVTN) 503 study which tested the efficacy of an HIV vaccine (Gray et al., 2014), and

4) the Sinikhithemba Study designed to assess immune responses in HIV infected individuals in KwaZulu-Natal (Kiepiela et al., 2007).

The most important outcome variable for this study was the prevalence of HIV drug resistance before and after public sector antiretroviral therapy rollout in South Africa. To describe drug resistance prevalence, the important data was the HIV DNA sequence data for the Pol gene from the infected participants. The most important exposure variables in this study were; 1) year of infection, 2) geographic location of the participant, 3) age, and 4) gender. The year of infection was important as the period assessed in the study spanned from pre-ART to the ART era and the treatment coverage generally increased with time. It was, therefore, likely that the circulation of drug resistant viruses would increase with the availability of treatment. Age and gender are important exposure variables as these could indicate differences in susceptibility to drug resistant forms of HIV by age or gender.

The data analysed in this study was obtained from the Los Alamos HIV Database (https://www.hiv.lanl.gov). DNA sequences for the three HIV genes (reverse transcriptase, protease and integrase) that are targeted by antiretroviral drugs were selected on the database's Sequence Search Interface (Figure 1). The following fields were selected;

1) Virus – HIV-1
2) Sampling country – South Africa
3) Sampling year – 1996-2011
4) Subtype - C (the predominant subtype in South Africa)
5) Only drug naïve sequences
6) Genomic region – Reverse transcriptase, Protease, Integrase

The sequences were quality checked manually using the BioEdit program and sequences of low quality (i.e., incomplete, mixtures etc.) were removed from the dataset.

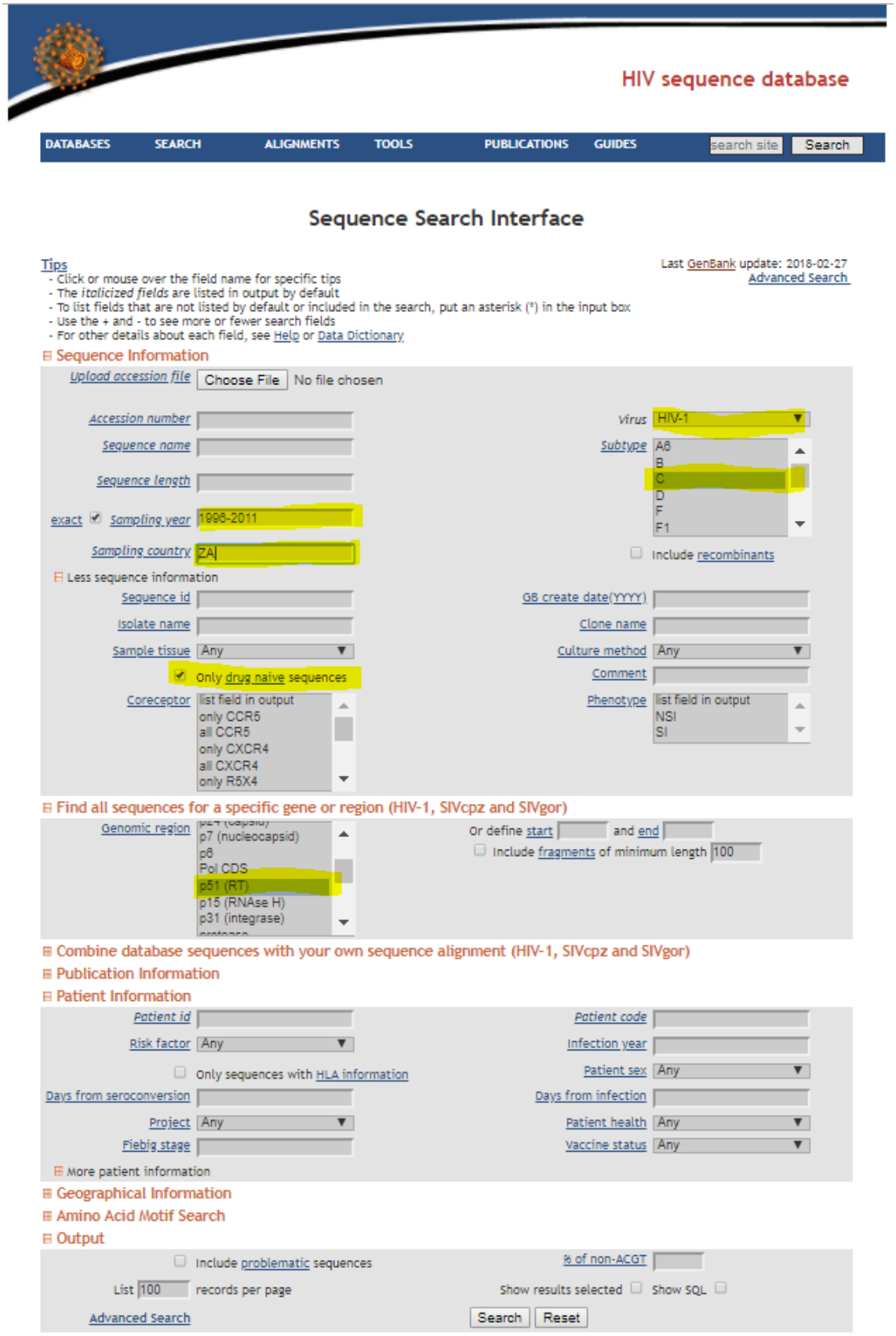

Figure 1: Snapshot of the Search Interface on the Los Alamos HIV Database. Sequences were selected based on the fields highlighted in yellow.

3.5 Detection of drug resistance mutations

The DNA sequences from each of the groups (1996-2004 and 2005-2011) will be uploaded onto the Calibrated Population Resistance Program on the Stanford University HIV Drug Resistance database (Shafer, 2006). The online software scans uploaded HIV DNA sequences for known drug resistance mutations and provides a summary of the detected mutations in a tabular format detailing the mutation and the drug associated with the mutation. Once the sequences have been loaded, the software will be prompted to run the drug resistance detection.

3.6 Validity and Reliability

This study used data from studies from South Africa which recruited ART naïve participants with an estimated year of infection which fell within the period of interest (1996 – 2011). The use of published data from different parts of the country was a fair representation of the study population. Potential bias arose from the fact that most of the studies from which the data in this study will emanate from, predominantly recruited women (e.g., the CAPRISA 002 and CAPRISA 004 studies). However, as the most important outcome variable was the prevalence of HIV drug resistance and the participants in these studies where the data came from were predominantly heterosexual, it was reasonable to assume that the prevalence of drug resistance in women was the same as in men. Some study participants had more than one DNA sequence for the Pol gene available in the Los Alamos HIV Database due to the different nature of the parent studies. Including all the DNA sequences in this study would have posed a measurement bias due to re-sampling. In order to eliminate this bias, only one DNA sequence (earliest available) was used in this study.

3.7 Data Analysis

3.7.1 Descriptive analysis

In order to determine the characteristics of the sample used in the study, the data was sorted into two groups. Data downloaded from the Los Alamos HIV Database (www.hiv.lanl.gov) was grouped by period of infection (1996-2004 and 2005-2011). The number of participants, age range and geographic distribution of the participants was calculated for the dataset.

3.7.2 Determination of prevalence of drug resistance

The output from the Calibrated Population Resistance Program analysis was used to determine the prevalence of drug resistance mutations for each of the groups (1996-2004 and 2005-2011) (i.e., the proportion of study participants who have at least one drug resistant mutation in each

group). The prevalence of drug resistance before antiretroviral therapy rollout (1996-2004) was compared to that after the rollout (2005-2011) to determine the effect of antiretroviral roll-out on the prevalence of circulating HIV drug resistance mutations by a Mann-Whitney U test. The Fishers Exact Test was used to determine the associations between drug resistance and associated risk factors (age, gender, geographical location). For all prevalence calculations, 95% confidence intervals were calculated. The statistical analyses were performed using GraphPad Prism 6.

3.8 Generalizability

The results of this study give an indication of whether antiretroviral treatment resulted in the circulation of drug resistant forms of HIV and the factors associated with it. Even though there are some biases in the data (e.g., over-representation of women in the study), the results of the study can be generalized to the study population. The groups to which the results can be generalized are;

1) the South African population,

2) the different locations where the study participants were recruited from.

3.9 Ethics

The data used in this study was published in accredited international journals. The studies where the data came from have all gone through ethical clearance from their respective institutions as this is a requirement for the data to be deposited onto the database.

As the study utilised information obtained through a publicly accessible database, there was no need for informed consent from participants in this study (this would have been obtained in the parental studies as these have been approved by the respective Ethics Committees of the relevant institutions). The data on the Los Alamos HIV Database (www.hiv.lanl.gov) is freely available and researchers are allowed to use it in their studies with no permission or approval from those who run it and make it available. The database is publicly funded with Federal funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services (www.hiv.lanl.gov). Ethics approval was sought for this study from University of the Western Cape Biomedical Research Ethics Committee (BMREC).

3.10 Summary

This study utilised secondary data obtained from the Los Alamos HIV Database (www.hiv.lanl.gov) to determine the prevalence of HIV drug resistance in South Africa prior to the public ART rollout (1996-2004) and after public ART rollout (2005-2011). DNA sequences from the database were analysed using the Calibrated Population Resistance Program on the Stanford University HIV Drug Resistance database (Shafer, 2006) to detect drug resistance mutations. The effect of ART rollout was determined by comparing the drug resistance prevalence prior to ART rollout and after ART rollout. The data from the study should be fairly generalizable as the study makes use of all published data from therapy-naïve HIV-infected people. The next chapter describes the results from the analyses.

4. Results

4.1 Introduction

The participants in this study were antiretroviral therapy-naïve HIV-infected South Africans whose HIV sequence data was available on the Los Alamos HIV database (www.hiv.lanl.gov). In order to determine the trend in prevalence of HIV drug resistance in South Africa before and after the public rollout of antiretroviral therapy, the DNA sequences were analysed for the presence of known drug resistance mutations and associations between drug resistance mutations and antiretroviral therapy rollout, age and gender were determined.

4.2 Summary of the data

DNA sequences for the three genes (reverse transcriptase, protease and integrase) that are targeted by antiretroviral drugs were selected on the database's Sequence Search Interface. After quality control, there were a total of 1701 DNA sequences for the three genes; 604 (35,5%) reverse transcriptase, 794 (46,7%) protease and 303 (17,8%) integrase sequences (Table 4).

The data obtained from the Los Alamos HIV database (www.hiv.lanl.gov) was from six of the nine provinces of South Africa (Gauteng, KwaZulu-Natal, Mpumalanga, Western Cape, Limpopo, and Free State) (Table 4). There was no data available from Eastern Cape, North West and Northern Cape provinces. For all the three genes of interest, most of the data was from the KwaZulu-Natal province with 928 (54,6%) sequences (355 [20,1%] reverse transcriptase, 329 [19,3%] protease and 244 [26,2%] integrase). There was an uneven distribution of data from the other provinces with some of the provinces having more data for

specific genes than others. For example, the Free State province had more data for reverse transcriptase (231 [38,2%] sequences) while there were 31 (3,9%) protease sequences and no integrase sequences from the same province. Similarly, there were 246 (31%) protease sequences from Gauteng while for reverse transcriptase and integrase there were 10 (1,6%) and 57 (18,8%) sequences, respectively (Table 4).

There was more data from females as compared to males. For reverse transcriptase, there were 27 (4,5%) sequences from males and 577 (95,5%) (from females). Of the 794 protease sequences, 93 (11,7%) were from males while 701 (88,3%) were from females. Similarly, for the integrase gene, 31 (10,2%) sequences were from males while 272 (89,8%) sequences were from females (Table 4).

Table 4: DNA sequence data for reverse transcriptase, protease and integrase genes from the provinces of South Africa from 1996-2011 stratified by gender.

	Reverse Transcriptase			**Protease**			**Integrase**		
Province	**Male**	**Female**	**Total**	**Male**	**Female**	**Total**	**Male**	**Female**	**Total**
Gauteng	5	5	**10**	2	244	**246**	9	48	**57**
KwaZulu-Natal	22	333	**355**	34	295	**329**	21	223	**244**
Mpumalanga	0	2	**2**	2	11	**13**	0	0	**0**
Western Cape	0	2	**2**	55	120	**175**	1	1	**2**
Limpopo	0	4	**4**	0	0	**0**	0	0	**0**
Free State	0	231	**231**	0	31	**31**	0	0	**0**
Total	**27**	**577**	**604**	**93**	**701**	**794**	**31**	**272**	**303**

Of the 604 reverse transcriptase sequences, there were 302 (50%) (23 [7,6%] male and 279 [92,4%] female) sampled prior to ART introduction (pre-ART) and 302 (50%) (4 [1,3%] male and 298 [98,7%] female) sampled post-ART introduction (Table 5). Most of the pre-ART sequence data was from KwaZulu-Natal province which had 294 (97,4%) sequences (18 [6,1%] male and 276 [93,9%] female) while most of the post-ART data was from the Free State province which had 231 (76,5%) sequences (all female) (Table 5).

For protease, there were 378 (47,6%) sequences from the pre-ART period (68[18%] male and 310 [82%] female) and 416 (52,4%) sequences from the post-ART period (25 [6%] male and 391 [94%] female) (Table 6). Similar to the reverse transcriptase data, most of the pre-ART data for protease was from the KwaZulu-Natal province which 238 (63%) sequences (22 [9,2%] male and 216 [90,8%] female). Western Cape province had 99 (26,2%) sequences which were almost evenly balanced between males and females (43 [43,4%] and 56 [56,6%], respectively). The majority of post-ART data came from Gauteng province with 236 (56,7%) sequences (all female) (Table 5).

Of the 303 integrase sequences, 246 [81,2%] (23 [9,3%] male and 223 [90,7%] female) were from the pre-ART period while only 57 (18,8%) were from the post-ART period (Table 7). The pre-ART sequences were predominantly from the KwaZulu-Natal province with 237 96,3%) sequences (18 [7,6%] male and 219 [92,4%] female) while the post-ART data was mainly from Gauteng province with 50 87,7%) sequences (five [10%] male and 45 [90%] female) (Table 5).

4.3 Prevalence of drug resistance mutations in South Africa between 1996-2011

The overall prevalence of HIV drug resistance in South Africa during the period 1996-2011 was calculated from the dataset. In order to determine if the prevalence varied by geographical location, the data was stratified by province.

Table 5: Distribution of DNA sequences for reverse transcriptase, protease and integrase from South African provinces before (pre-ART: 1996-2004) and after (post-ART: 2005-2011) ART rollout stratified by gender. The percentages indicated are relative to the total number of DNA sequences (male and female) for the respective gene pre- or post-ART.

Province	Reverse Transcriptase				Protease				Integrase			
	Pre-ART		Post-ART		Pre-ART		Post-ART		Pre-ART		Post-ART	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Gauteng	5 (1,6%)	3 (1%)	0	2 (0,7%)	3 (0,8%)	7 (18,5%)	0	236 (56,7%)	4 (1,6%)	3 (1,2%)	5 (8,8%)	45 (78,9%)
KwaZulu-Natal	18 (6%)	276 (91,4%)	4 (1,3%)	57 (18,9%)	22 (5,8%)	216 (57,1%)	11 (2,6%)	80 (19,2%)	18 (7,3%)	219 (89%)	3 (5,3%)	4 (7%)
Mpumalanga	0	0	0	2 (0,7%)	0	0	2 (0,5%)	11 (2,6%)	0	0	0	0
Western Cape	0	0	0	2 (0,7%)	43 (11,4%)	56 (14,8%)	12 (2,9%)	64 (15,4%)	1 (0,4%)	1 (0,4%)	0	0
Limpopo	0	0	0	4 (1,3%)	0	0	0	0	0	0	0	0
Free State	0	0	0	231 (76,5%)	0	31 (8,2%)	0	0	0	0	0	0
Total	**23**	**279**	**4**	**298**	**68**	**310**	**25**	**391**	**23**	**223**	**8**	**49**

For the period 1996-2011, the period prevalence of drug resistance mutations in reverse transcriptase was 7,5% (Table 6). Non-nucleoside reverse transcriptase inhibitor (NNRTI) drug resistance mutations contributed the majority of detected mutations with 37/604 (6,1%, 95% CI: 4,5-8,3) individuals presenting with at least one nucleoside reverse transcriptase inhibitor (NRTI) resistance mutation (Table 6). NRTI drug resistance was much lower with only 12/604 (12%, 95% CI: 1,1-3,4) study participants presenting with at least one NRTI resistance mutation (Table 6). The two provinces, which contributed most of the reverse transcriptase sequences were KwaZulu-Natal with 355/604 (59%) and Free State with 231/604 (38%) (Table 6). KwaZulu-Natal had a higher overall frequency of drug resistance mutations (8,5%) compared to Free State (5,6%) although this difference was not statistically different (p=0,3; data not shown). The frequency of NNRTI drug resistance mutations for the two provinces were more comparable (6,2% for KwaZulu-Natal and 5,6% for Free State). KwaZulu-Natal had higher NRTI drug resistance mutations 10/355 (2,8%, 95% CI: 1,5-5,1) compared to Free State which had 1/231 (0,4%, 95% CI: 1,02-2,4) and the difference between the two was statistically significant (p=0.05; Fishers exact test), suggesting that resistance to this class of drugs might vary by province.

The prevalence of drug resistance to protease inhibitors between 1996-2011 was 2,3% (95% CI: 1,4-3,6) representing a low level of resistance (Table 7). Most of the study participants who contributed protease data were from Gauteng with 246/794 (31%), KwaZulu-Natal with 329/794 (41%) and Western Cape with 175/794 (22%). The prevalence of protease drug resistance varied among these three provinces with KwaZulu-Natal having the highest prevalence of 3,3% (95% CI: 1,9-5,9) while Gauteng had 1,6% (95% CI: 0,6-4,1) and Western Cape had the lowest of 0,6% (95% CI: 0,03-3,2).

Table 6: Prevalence of reverse transcriptase drug resistance mutations for South African provinces with 95% confidence interval between 1996 and 2011 for the different reverse transcriptase inhibitor drugs (NRTI and NNRTI).

Province	*RT sequences N	Any *DRM N (%)	Any *DRM (95% CI)	*NRTI N (%)	*NRTI (95% CI)	*NNRTI N (%)	*NNRTI (95% CI)
Gauteng	10	0 (0%)	0-27,8	0 (0%)	0-27,8	0 (0%)	0-27,8
KwaZulu-Natal	355	30 (8,5%)	8,4-16,3	10 (2,8%)	1,5-5,1	22 (6,2%)	4,1-9,2
Mpumalanga	2	1 (50%)	2,6-97,4	0 (0%)	0-82,2	1 (50%)	2,6-97,4
Western Cape	2	1 (50%)	2,6-97,4	1 (50%)	2,6-97,4	1 (50%)	2,6-97,4
Limpopo	4	0 (0%)	0-49,0	0 (0%)	0-49,0	0 (0%)	0-49,0
Free State	231	13 (5,6%)	3,3-9,4	1 (0,4%)	0,02-2,4	13 (5,6%)	3,3-9,4
Total	**604**	**45** (7,5%)	**5,6-9,8**	**12** (2%)	**1,1-3,4**	**37** (6,1%)	**4,5-8,3**

*RT – Reverse transcriptase

DRM – Drug resistance mutations

NRTI – Nucleoside reverse transcriptase

NNRTI - Non-nucleoside reverse transcriptase

Table 7: Prevalence of protease drug resistance mutations in South Africa between 1996 and 2011

Province	*PR sequences N	PR *DRM N (%)	PR *DRM (95% CI)
Gauteng	246	4 (1,6%)	0,6-4,1
KwaZulu-Natal	329	11 (3,3%)	1,9-5,9
Mpumalanga	13	0 (0%)	0-22,8
Western Cape	175	1 (0,6%)	0,03-3,2
Free State	31	2 (6,5%)	1,1-20,7
Total	**794**	**18** (2,3%)	**1,4-3,6**

*PR – Protease

DRM – Drug resistance mutations

For integrase, the drug resistance prevalence was a moderate 5% (95% CI: 3,0-5,0) (Table 8). The KwaZulu-Natal province had an integrase drug resistance prevalence of 5,3% (95% CI: 3,1-5,3) while Gauteng had 3,5% (95% CI: 0,6-12,0). The two provinces (KwaZulu-Natal and Gauteng) contributed more than 99% of the integrase data in this study.

Table 8: Prevalence of integrase drug resistance mutations in South Africa between 1996 and 2011

Province	*INT sequences (n)	INT *DRM (n)	INT *DRM (%)	INT *DRM (95% CI)
Gauteng	57	2	3,5	0,6-12,0
KwaZulu-Natal	244	13	5,3	3,1-5,3
Western Cape	2	0	0	0-82,2
Total	**303**	**15**	**5,0**	**3,0-5,0**

*INT – Integrase

DRM – Drug resistance mutations

In order to determine the effect of ART rollout in 2004 on the level of drug resistance in therapy-naïve individuals in South Africa, the frequency of drug resistance mutations prior to the introduction of ART (1996-2004) and after the introduction of ART (2005-2011) were compared. The prevalence of drug resistance to reverse transcriptase inhibitors (combined NRTI and NNRTI) from 1996-2004 was 7,9% compared to 7% post-ART introduction (Figure 2a). The difference between pre-ART and post-ART reverse transcriptase drug resistance was not statistically significant (p>0,99; Fishers exact test). NRTI drug resistance prior to ART introduction was 2,6% compared to 1,3% post-ART (p=0.62; Fishers exact test) (Figure 2b).

For NNRTI drug resistance, there was a slight increase between pre- and post-ART prevalence (5% vs. 6,2%) although this was not significant (p>0,99; Fishers exact test) (Figure 2c).

There was also no significant difference between the frequency of protease drug resistance between 1996-2004 (2,9%) and 2005-2011 (1,9%) (p>0,99; Fishers exact test; Figure 2d). Integrase drug resistance increased from 4,9% (1996-2004) to 5,3% from (2005-2011) (Figure 2e). This was also not statistically significant (p>0,99; Fishers exact test).

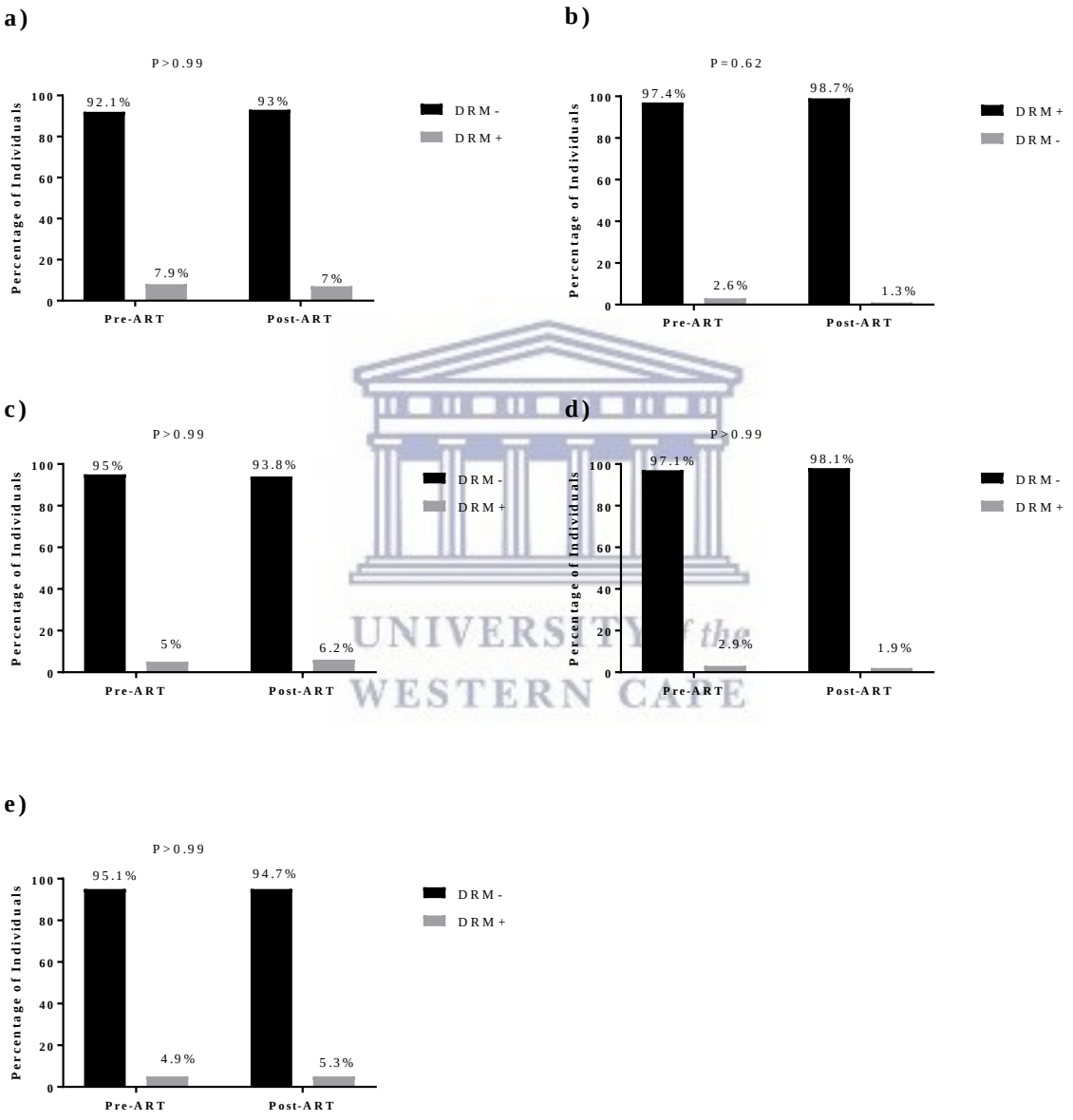

Figure 2: Comparison of prevalence of drug resistance mutations Pre- and post-ART.

a) Prevalence of drug resistance mutations to reverse transcriptase inhibitors in the Pre- (1996-2004) and Post-ART (2005-2011) period. b) Prevalence of drug resistance mutations to nucleoside reverse transcriptase inhibitors (NRTI) in the Pre- (1996-2004) and Post-ART

(2005-2011) period. c) Prevalence of drug resistance mutations to non-nucleoside reverse transcriptase inhibitors (NNRTI) in the Pre- (1996-2004) and Post-ART (2005-2011) period. d) Prevalence of drug resistance mutations to protease inhibitors in the Pre- (1996-2004) and Post-ART (2005-2011) period. e) Prevalence of drug resistance mutations to integrase inhibitors in the Pre- (1996-2004) and Post-ART (2005-2011) period. DRM- denotes drug resistance mutation negative and DRM+ denotes drug resistance positive. The p-values shown are for Fishers exact test.

The association between age and drug resistance was assessed by comparing the prevalence of drug resistance for participants under the age of 29 (median age) and those aged 29 and over (Tables 4.6 and 4.7). The ages of the study participants ranged from 19 to 39 years. Overall, there was no association between age and drug resistance mutations in all the three classes of antiretroviral drugs before (Table 9) and after the introduction ART (Table 10).

Table 9: Pre-ART comparison of drug resistance mutations by age group

Resistance Mutations	**<29 Years N (%)**		**>29 Years N (%)**		**p-value (Fishers exact test)**
	***DRM-**	**DRM+**	**DRM-**	**DRM+**	
Reverse transcriptase	131 (92,9%)	10 (7,1%)	147 (91,3%)	14 (8,7%)	0.67
Protease	197 (96,6%)	7 (3,4%)	170 (97,7%)	4 (2,3%)	0.56
Integrase	92 (96,8%)	3 (3,2%)	142 (96,6%)	5 (3,4%)	>0,99

*DRM – Drug resistance mutations

Table 10: Post-ART comparison of drug resistance mutations by age group

Resistance Mutations	**<29 Years N (%)**		**>29 Years N (%)**		**p-value (Fishers exact test)**
	***DRM-**	**DRM+**	**DRM-**	**DRM+**	
Reverse transcriptase	127 (94,1%)	8 (5,9%)	154 (92,2%)	13 (7,8%)	0.65
Protease	219 (98,6%)	3 (1,4%)	189 (97,4%)	5 (2,6%)	0.48
Integrase	23 (100%)	0 (0%)	31 (91,2%)	3 (8,8%)	0.27

*DRM – Drug resistance mutations

The association between drug resistance mutations and gender was assessed by comparing the prevalence of drug resistance in males and females in the different drug classes before (Table 11) and after (Table 12) ART rollout). There was no significant difference in prevalence of

drug resistance mutations between male and female participants during the pre-ART era (Table 11) and in the post-ART (Table 12) period. However, there were more female participants in the study compared to males (1550 vs. 151: 91%).

Table 11: Pre-ART comparison of drug resistance mutations by gender

Resistance Mutations	**Male N (%)**		**Female N (%)**		**p-value (Fishers exact test)**
	DRM-	**DRM+**	**DRM-**	**DRM+**	
Reverse transcriptase	21 (91,3%)	2 (8,7%)	257 (92,1%)	22 7,9%)	0.70
NRTI	23 (100%)	0 (0%)	271 (97,1%)	8 (2,9)	>0,99
NNRTI	21 (91,3%)	2 8,7%)	264 (94,6%)	15 (5,4%)	0.38
Protease	67 (98,5%)	1 (1,5%)	300 (96,8%)	10 (3,2%)	0.70
Integrase	22 (95,6%)	1 (4,4%)	212 (95,1%)	11 (4,9%)	>0,99

*DRM – Drug resistance mutations

Table 12: Post-ART comparison of drug resistance mutations by gender

Resistance Mutations	**Male N (%)**		**Female N (%)**		**p-value (Fishers exact test)**
	DRM-	**DRM+**	**DRM-**	**DRM+**	
Reverse transcriptase	4 (100%)	0 (0%)	277 (93%)	21 (7%)	>0,99
NRTI	4 (100%)	0 (0%)	294 (98,7%)	4 (1,3%)	>0,99
NNRTI	4 (100%)	0 (0%)	279 (93,6%)	19 (6,4%)	>0,99
Protease	24 (96%)	1 (4%)	384 (98,2%)	7 (1,8%)	0.40
Integrase	8 (100%)	0 (0%)	46 (93,9%)	3 (6,1%)	>0,99

*DRM – Drug resistance mutations

4.4 Summary

There were 1701 DNA sequences analysed in this study for the three genomic regions targeted by antiretroviral drugs (reverse transcriptase, protease and integrase). There was over-representation of data from females as well as data from the KwaZulu-Natal Province. This is mainly due to the fact that most of the studies carried out in the country were intervention studies aimed at reducing HIV incidence among females and the KwaZulu-Natal province

being the region with the highest prevalence of HIV in the country. Overall, there was no increase in the prevalence of drug resistance mutations in the drug-naïve HIV-infected population after the public sector rollout of ART in 2004. The prevalence of drug resistance mutations in the South African population seems to be driven by the high prevalence of non-nucleoside reverse transcriptase inhibitor mutations. Also, there was no association between drug resistance mutations and age or gender both pre-ART and post-ART.

Chapter 5: Discussion

This study sought to determine the prevalence of drug resistance mutations in the drug-naïve HIV-infected South African population before and after public ART rollout. Several studies have previously assessed the prevalence of drug resistance in South Africa (Nwobegahay et al., 2012, Parikh et al., 2013, Hunt et al., 2012, Parboosing et al., 2011, Manasa et al., 2012, Steegen et al., 2016). However, some of the limitations of these studies has been the small sample sizes, the fact that the samples were from one region of the country (Nwobegahay et al., 2012, Parikh et al., 2013, Hunt et al., 2012, Parboosing et al., 2011), they examined drug resistance to only one class of drugs (Manasa et al., 2012) or that they were cross-sectional in nature (Steegen et al., 2016). This study utilised DNA sequence data available on the Los Alamos HIV database which is a publicly available resource for researchers to access data from published studies (www.hiv.lanl.gov). The utilisation of data from the Los Alamos HIV Database gives this study a wide coverage of South Africa, a modest sample size and cover a long period of time (1996-2011).

Drug resistance poses a serious threat to the management of HIV infection using ART (Cohen et al., 2011, Li et al., 2011). Assessing the extent of drug resistance in treatment-naïve individuals has huge public health benefits on several fronts. Firstly, it gives an indication of whether the current drug regimens being used in the country are effective as high prevalence would indicate low adherence or easy development of resistance. Secondly, it has now been established through basic science research that there is selection of virus strains with specific characteristics during sexual transmission of HIV; the so-called 'transmission bottleneck' (Shaw and Hunter, 2012, Joseph et al., 2015). Due to the high mutation rate of HIV, an infected individual harbours several variants of the virus at any given time. During sexual transmission, it is believed that only a single variant is able to establish infection in the newly infected individual due to the barrier posed by the immune system (Shaw and Hunter, 2012). Once infection has been established, the genetically homogeneous viral population then diversifies through mutation (Shaw and Hunter, 2012, Joseph et al., 2015). Therefore, the prevalence of drug resistance in treatment-naïve individuals will indicate whether there is preferential transmission of drug resistant viruses which will need to be addressed at public health level.

The data from this study largely came from the KwaZulu-Natal province with 55% of the DNA sequences. This is due to the fact that most published studies assessing different biological correlates of HIV infection have been carried out this province. While this introduces a bias towards the region in the results, the KwaZulu-Natal province is the province with the highest

prevalence of HIV infection in South Africa and is considered the epicentre of the HIV pandemic globally (Kiepiela, 2014). This suggests that findings in KwaZulu-Natal represent the region with the highest incidence and prevalence of HIV in the country and are therefore relevant to any public health interventions. In addition to having most of the samples coming from KwaZulu-Natal, there were also more females (91%) in this study compared to males. This is because most of the published studies were designed to come up with intervention strategies to reduce infection in women, particularly young women (Abdool Karim et al., 2010). Given the fact that the HIV epidemic in South Africa is driven by heterosexual transmission, the results in women can be generalised to the population (Kiepiela, 2014). There was a fairly balanced percentage of samples from the pre-ART era (1999-2004) and post-ART era (2005-2011).

The study assessed the prevalence of drug resistance mutations for each of the drug classes in the South African population between 1996-2011. There was a moderate level of 7,5% prevalence (Jordan et al., 2012) of drug resistance in the reverse transcriptase gene, which was largely driven by non-nucleoside reverse transcriptase inhibitors (NNRTIs) which had a prevalence of 6,1%. Nucleoside reverse transcriptase inhibitor (NRTI) resistance had a prevalence of 2%. This pattern is consistent with that reported in a recently published study, which was a cross-sectional survey of the different South African provinces in 2016 where NNRTI resistance had a prevalence of 8,3% while NRTI resistance had a prevalence of 2,5% (Steegen et al., 2016). Furthermore, it is widely acknowledged that resistance from NNRTIs is the major driver of the prevalence of HIV drug resistance globally (Tang and Shafer, 2012).

There was no significant change in the prevalence of drug resistance mutations to reverse transcriptase inhibitors between the pre-ART (1996-2004) and post-ART (2005-2011) eras. However, there was an increase in NNRTI drug resistance from low level 5% to a moderate level 6,2% although this was not statistically significant. Mechanistically, it remains unclear why there is a high prevalence of drug resistance to NNRTIs (Tang and Shafer, 2012). Some studies have pointed to the possibility that drug resistance mutations to NNRTIs generally do not reduce the virus' ability to replicate efficiently (i.e., come at a low fitness cost to the virus) and are, therefore, more tolerated (Jain et al., 2011). On the other hand, drug resistance mutations to NRTIs compromise the virus' ability to replicate (Jain et al., 2011). In the latter case, development of drug resistance for the virus becomes a balance between its ability to replicate efficiently and 'escaping' recognition by the drug.

While the prevalence of drug resistance mutations to protease inhibitors was at a low level 2,3% (Jordan et al., 2012) during the period covered in this study (1996-2011), comparison of the prevalence in the pre-ART period to that during the post-ART era showed a decrease in the prevalence although this decrease was not statistically different. The levels of drug resistance mutations to protease inhibitors observed in this study are consistent with those recently reported by Steegen and others (Steegen et al., 2016). The national cross-sectional study reported a protease inhibitor drug resistance prevalence of 2%(Steegen et al., 2016). This suggests that the prevalence of protease inhibitor drug resistance has not changed appreciably due to the introduction of ART as the prevalence during the pre-ART period is comparable to the post-ART period.

The prevalence of drug resistance mutations to integrase inhibitors was 5% for the period 1996-2011. This is on the borderline between low and moderate level of resistance (Jordan et al., 2012). The resistance to integrase inhibitors in the pre-ART era was 4,9% and it marginally increased to 5,3% in the post-ART era. It is quite interesting to note that there were drug resistance mutations to integrase inhibitors prior to ART roll-out (1999-2004) as the first drug in this class of antiretrovirals, raltegravir, was only approved in 2007 (Hicks and Gulick, 2009). The presence of drug resistance mutations to integrase inhibitors prior to their introduction could be a result of the natural variation of HIV. HIV has a high mutation rate (i.e., it changes at a high rate) which is caused by its error prone replication mechanism (Peeters and Sharp, 2000). In fact, the variation in HIV is one of the major challenges in the development of an effective vaccine to the virus as it is difficult to have a vaccine that will target all possible forms of the virus (Peeters and Sharp, 2000). To put this into perspective, the influenza virus is so variable such that every year there is need for a new vaccine (Khan et al., 2017). However, the variation of the influenza virus globally is equivalent to the variation of HIV in one infected individual (Peeters and Sharp, 2000). The presence of drug resistance mutations to integrase inhibitors suggests a genetic predisposition of the virus to developing resistance and is a major threat to the use of ART in managing HIV infection and prevention of transmission.

There was no association between drug resistance and age in this study. Some previous studies reported differences between age and HIV treatment adherence (Bianco et al., 2011, Lucas et al., 2002, Hinkin et al., 2004). While older people were found to be more likely to be adherent to treatment, they are more prone to non-adherence due to being cognitively impaired than younger study participants (Hinkin et al., 2004). The lack of association between drug resistance and age observed in this study could have been due to the narrow range in age of the

study participants (19-39 years). Due to the current test and treat guidelines, it is likely that there will be a growing population of HIV-infected aged people and there is need for studies focusing on the association between age and the development of drug resistance.

This study did not find any association between drug resistance and gender. However, this result is unlikely to be a true representation of the reality in the country. Females comprised 91% of the study participants and there was limited data from males. Previous studies have reported gender differences in HIV drug resistance patterns (Kan et al., 2017, Goedecke et al., 2013, Sabin et al., 2008). The differences were attributed to factors such as barriers to treatment adherence and physiological differences between males and females.

Despite the advantages that this study has which include a modest sample size, access to DNA sequence data from several independent studies from different regions in South Africa and the fact that the data spans a wide period, there are several limitations. The major limitation of the study comes from the fact that the secondary analysis of data generated by other researchers limits the control of the parameters under study. There was a disproportionate distribution of samples across specific regions (provinces) of the country with some of the provinces not being represented in the study. This makes it difficult to generalise the results of the study to the national level. However, it is quite important that the province with the highest HIV prevalence in the country was over-represented in the study, suggesting the results could represent the 'worst case scenario'. The other limitation of the study comes from the fact that there was an over-representation of female participants, making it impossible to determine whether there is a significant difference in the prevalence of drug resistance mutations between males and females. However, due to the fact that the HIV epidemic in South Africa is driven by heterosexual transmission, it is unlikely that the prevalence of drug resistance is different between males and females.

Another limitation was the lack of data quality control when using secondary data generated by other researchers. However, the data that was utilised in this study comes from studies published in reputable international journals and has undergone rigorous checks to ensure its integrity (www.hiv.lanl.gov). It would have also been much better to stratify the participants for each year of estimated infection, but this would result in low numbers of those with available data.

Chapter 6: Conclusion and Recommendations

This study observed that the public-sector rollout of ART in 2004 did not result in an increase in the prevalence of HIV drug resistance mutations in the treatment-naïve population. The drug resistance level was low to moderate according to the World Health Organisation (WHO) classification of drug resistance levels (Jordan et al., 2012). These findings suggest that there is minimal risk of treatment failure in newly infected individuals once they are put on therapy. This is especially important given the new guidelines where treatment has to be initiated upon diagnosis of HIV infection (WHO, 2016b). While the new strategy is envisaged to significantly reduce the morbidity and mortality in HIV infected people and to contribute towards the ambitious goal of eliminating AIDS disease by 2030, it has the potential to increase the level of drug resistance, which could have a negative impact (Philips et al., 2017). Findings from this study suggest that the risk of this happening is minimal. However, there is need to ensure treatment adherence in order to further reduce the development of drug resistance.

The natural variation of HIV presents a risk to the management of HIV infection through ART as there was a low level of resistance mutations to integrase inhibitors prior to their introduction. This might negatively affect the prospects of using integrase inhibitors as part of first line regimen in HIV treatment on the African continent. Considerations for the use of integrase inhibitors for first line treatment are driven by the fact that they offer simpler dose schedules and have fewer side effects (Yombi and Pozniak, 2016). However, the fact that the viral strain predominant in South Africa (HIV subtype C) appears to be genetically predisposed to developing resistance to this class of antiretrovirals suggests that they might not be an effective option for first line treatment of HIV, at least in the Southern Africa region where HIV subtype C is predominant. This also points to the need for more studies characterising HIV infections on the African continent, as findings from studies conducted in Western countries may not always be generalizable to the African population.

The fact that the prevalence of HIV drug resistance appears to be driven by the resistance to NNRTI drugs suggests that there may be need to start thinking about introducing other drug classes in first line regimen if this trajectory of resistance patterns continues. There is also need to come up with cost-effective methods of detecting drug resistance before commencement of ART, which would ensure that the individuals initiating therapy are resistant to the treatment that they are put on.

The absence of data and underrepresentation of several provinces of South Africa in the Los Alamos HIV database suggests that there is need for HIV studies to be expanded to cover other regions of the country. This also applies to the low number of data available for males in the database. A broad coverage of the country's population will likely result in appropriate interventions in the fight against HIV.

References

Abdool Karim, Q., Abdool Karim, S. S., Frohlich, J. A., Grobler, A. C., Baxter, C., Mansoor, L. E., Kharsany, A. B., Sibeko, S., Mlisana, K. P., Omar, Z., Gengiah, T. N., Maarschalk, S., Arulappan, N., Mlotshwa, M., Morris, L., Taylor, D. & Group, C. T. 2010. Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women. *Science,* 329**,** 1168-74.

Abdool Karim, Q., Kharsany, A. B., Frohlich, J. A., Werner, L., Mlotshwa, M., Madlala, B. T. & Abdool Karim, S. S. 2012. HIV incidence in young girls in KwaZulu-Natal, South Africa--public health imperative for their inclusion in HIV biomedical intervention trials. *AIDS Behav,* 16**,** 1870-6.

Arthos, J., Cicala, C., Martinelli, E., Macleod, K., Van Ryk, D., Wei, D., Xiao, Z., Veenstra, T. D., Conrad, T. P., Lempicki, R. A., Mclaughlin, S., Pascuccio, M., Gopaul, R., Mcnally, J., Cruz, C. C., Censoplano, N., Chung, E., Reitano, K. N., Kottilil, S., Goode, D. J. & Fauci, A. S. 2008. HIV-1 envelope protein binds to and signals through integrin alpha4beta7, the gut mucosal homing receptor for peripheral T cells. *Nat Immunol,* 9**,** 301-9.

Barth, R. E., Wensing, A. M., Tempelman, H. A., Moraba, R., Schuurman, R. & Hoepelman, A. I. 2008. Rapid accumulation of nonnucleoside reverse transcriptase inhibitor-associated resistance: evidence of transmitted resistance in rural South Africa. *AIDS,* 22**,** 2210-2.

Beaglehole, R., Bonita, R. & Kjellstrom, T. 1997. Types of Study. *Basic Epidemiology.* Geneva: WHO Publications.

Bennett, D. E., Bertagnolio, S., Sutherland, D. & Gilks, C. F. 2008. The World Health Organization's global strategy for prevention and assessment of HIV drug resistance. *Antivir Ther,* 13 Suppl 2**,** 1-13.

Bianco, J. A., Heckman, T. G., Sutton, M., Watakakosol, R. & Lovejoy, T. 2011. Predicting adherence to antiretroviral therapy in HIV-infected older adults: the moderating role of gender. *AIDS Behav,* 15**,** 1437-46.

Cane, P., Chrystie, I., Dunn, D., Evans, B., Geretti, A. M., Green, H., Phillips, A., Pillay, D., Porter, K., Pozniak, A., Sabin, C., Smit, E., Weber, J., Zuckerman, M. & Resistance, U. K. G. O. T. H. D. 2005. Time trends in primary resistance to HIV drugs in the United Kingdom: multicentre observational study. *BMJ,* 331**,** 1368.

Chan, D. C. & Kim, P. S. 1998. HIV entry and its inhibition. *Cell,* 93**,** 681-4.

Clutter, D. S., Jordan, M. R., Bertagnolio, S. & Shafer, R. W. 2016. HIV-1 drug resistance and resistance testing. *Infect Genet Evol,* 46**,** 292-307.

Cohen, J. 2018. Concern as HIV prevention strategy languishes. *Science,* 359**,** 1205.

Cohen, M. S., Chen, Y. Q., Mccauley, M., Gamble, T., Hosseinipour, M. C., Kumarasamy, N., Hakim, J. G., Kumwenda, J., Grinsztejn, B., Pilotto, J. H., Godbole, S. V., Mehendale, S., Chariyalertsak, S., Santos, B. R., Mayer, K. H., Hoffman, I. F., Eshleman, S. H., Piwowar-Manning, E., Wang, L., Makhema, J., Mills, L. A., De Bruyn, G., Sanne, I., Eron, J., Gallant,

J., Havlir, D., Swindells, S., Ribaudo, H., Elharrar, V., Burns, D., Taha, T. E., Nielsen-Saines, K., Celentano, D., Essex, M., Fleming, T. R. & Team, H. S. 2011. Prevention of HIV-1 infection with early antiretroviral therapy. *N Engl J Med,* 365**,** 493-505.

Cressey, T. R. & Lallemant, M. 2007. Pharmacogenetics of antiretroviral drugs for the treatment of HIV-infected patients: an update. *Infect Genet Evol,* 7**,** 333-42.

El-Khatib, Z., Katzenstein, D., Marrone, G., Laher, F., Mohapi, L., Petzold, M., Morris, L. & Ekstrom, A. M. 2011. Adherence to drug-refill is a useful early warning indicator of virologic and immunologic failure among HIV patients on first-line ART in South Africa. *PLoS One,* 6**,** e17518.

Gill, V. S., Lima, V. D., Zhang, W., Wynhoven, B., Yip, B., Hogg, R. S., Montaner, J. S. & Harrigan, P. R. 2010. Improved virological outcomes in British Columbia concomitant with decreasing incidence of HIV type 1 drug resistance detection. *Clin Infect Dis,* 50**,** 98-105.

Goedecke, J. H., Micklesfield, L. K., Levitt, N. S., Lambert, E. V., West, S., Maartens, G. & Dave, J. A. 2013. Effect of different antiretroviral drug regimens on body fat distribution of HIV-infected South African women. *AIDS Res Hum Retroviruses,* 29**,** 557-63.

Grant, R. M., Hecht, F. M., Warmerdam, M., Liu, L., Liegler, T., Petropoulos, C. J., Hellmann, N. S., Chesney, M., Busch, M. P. & Kahn, J. O. 2002. Time trends in primary HIV-1 drug resistance among recently infected persons. *JAMA,* 288**,** 181-8.

Gray, G. E., Moodie, Z., Metch, B., Gilbert, P. B., Bekker, L. G., Churchyard, G., Nchabeleng, M., Mlisana, K., Laher, F., Roux, S., Mngadi, K., Innes, C., Mathebula, M., Allen, M., Mcelrath, M. J., Robertson, M., Kublin, J., Corey, L. & Team, H. P. S. 2014. Recombinant adenovirus type 5 HIV gag/pol/nef vaccine in South Africa: unblinded, long-term follow-up of the phase 2b HVTN 503/Phambili study. *Lancet Infect Dis,* 14**,** 388-96.

Gupta, R. K., Jordan, M. R., Sultan, B. J., Hill, A., Davis, D. H., Gregson, J., Sawyer, A. W., Hamers, R. L., Ndembi, N., Pillay, D. & Bertagnolio, S. 2012. Global trends in antiretroviral resistance in treatment-naive individuals with HIV after rollout of antiretroviral treatment in resource-limited settings: a global collaborative study and meta-regression analysis. *Lancet,* 380**,** 1250-8.

Hamers, R. L., Siwale, M., Wallis, C. L., Labib, M., Van Hasselt, R., Stevens, W. S., Schuurman, R., Wensing, A. M., Van Vugt, M., Rinke De Wit, T. F. & Pharmaccess African Studies to Evaluate, R. 2010. HIV-1 drug resistance mutations are present in six percent of persons initiating antiretroviral therapy in Lusaka, Zambia. *J Acquir Immune Defic Syndr,* 55**,** 95-101.

Health, D. O. 2015. National consolidated guidelines. *Republic of South Africa*.

Hemelaar, J., Gouws, E., Ghys, P. D. & Osmanov, S. 2006. Global and regional distribution of HIV-1 genetic subtypes and recombinants in 2004. *AIDS,* 20**,** W13-23.

Hicks, C. & Gulick, R. M. 2009. Raltegravir: the first HIV type 1 integrase inhibitor. *Clin Infect Dis,* 48**,** 931-9.

Hinkin, C. H., Hardy, D. J., Mason, K. I., Castellon, S. A., Durvasula, R. S., Lam, M. N. & Stefaniak, M. 2004. Medication adherence in HIV-infected adults: effect of patient age, cognitive status, and substance abuse. *AIDS,* 18 Suppl 1**,** S19-25.

Hunt, G. M., Ledwaba, J., Basson, A. E., Moyes, J., Cohen, C., Singh, B., Bertagnolio, S., Jordan, M. R., Puren, A. & Morris, L. 2012. Surveillance of transmitted HIV-1 drug resistance in Gauteng and KwaZulu-Natal Provinces, South Africa, 2005-2009. *Clin Infect Dis,* 54 Suppl 4**,** S334-8.

Jain, V., Sucupira, M. C., Bacchetti, P., Hartogensis, W., Diaz, R. S., Kallas, E. G., Janini, L. M., Liegler, T., Pilcher, C. D., Grant, R. M., Cortes, R., Deeks, S. G. & Hecht, F. M. 2011. Differential persistence of transmitted HIV-1 drug resistance mutation classes. *J Infect Dis,* 203**,** 1174-81.

Johnson, L. F. 2012. Access to antiretroviral treatment in South Africa, 2004-2011. *South. Afr. J. HIV Med. ,* 13**,** 1-8.

Johnson, L. F., Dorrington, R. E. & Moolla, H. 2017. Progress towards the 2020 targets for HIV diagnosis and antiretroviral treatment in South Africa. *South Afr J HIV Med,* 18**,** 694.

Jordan, M. R., Bennett, D. E., Wainberg, M. A., Havlir, D., Hammer, S., Yang, C., Morris, L., Peeters, M., Wensing, A. M., Parkin, N., Nachega, J. B., Phillips, A., De Luca, A., Geng, E., Calmy, A., Raizes, E., Sandstrom, P., Archibald, C. P., Perriens, J., Mcclure, C. M., Hong, S. Y., Mcmahon, J. H., Dedes, N., Sutherland, D. & Bertagnolio, S. 2012. Update on World Health Organization HIV drug resistance prevention and assessment strategy: 2004-2011. *Clin Infect Dis,* 54 Suppl 4**,** S245-9.

Joseph, S. B., Swanstrom, R., Kashuba, A. D. & Cohen, M. S. 2015. Bottlenecks in HIV-1 transmission: insights from the study of founder viruses. *Nat Rev Microbiol,* 13**,** 414-25.

Kan, W., Teng, T., Liang, S., Ma, Y., Tang, H., Zuohela, T., Sun, G., He, C., Wall, K. M., Marconi, V. C., Liao, L., Leng, X., Liu, P., Ruan, Y., Xing, H. & Shao, Y. 2017. Predictors of HIV virological failure and drug resistance in Chinese patients after 48 months of antiretroviral treatment, 2008-2012: a prospective cohort study. *BMJ Open,* 7**,** e016012.

Khan, A., Mushtaq, M. H., Ahmad, M. U. D., Nazir, J., Farooqi, S. H. & Khan, A. 2017. Molecular Epidemiology of a novel re-assorted epidemic strain of equine influenza virus in Pakistan in 2015-16. *Virus Res,* 240**,** 56-63.

Kharsany, A. B. M., Cawood, C., Khanyile, D., Lewis, L., Grobler, A., Puren, A., Govender, K., George, G., Beckett, S., Samsunder, N., Madurai, S., Toledo, C., Chipeta, Z., Glenshaw, M., Hersey, S. & Abdool Karim, Q. 2018. Community-based HIV prevalence in KwaZulu-Natal, South Africa: results of a cross-sectional household survey. *Lancet HIV,* 5**,** e427-e437.

Kiepiela, P., Ngumbela, K., Thobakgale, C., Ramduth, D., Honeyborne, I., Moodley, E., Reddy, S., De Pierres, C., Mncube, Z., Mkhwanazi, N., Bishop, K., Van Der Stok, M., Nair, K., Khan, N., Crawford, H., Payne, R., Leslie, A., Prado, J., Prendergast, A., Frater, J., Mccarthy, N., Brander, C., Learn, G. H., Nickle, D., Rousseau, C., Coovadia, H., Mullins, J. I., Heckerman, D., Walker, B. D. & Goulder, P. 2007. CD8+ T-cell responses to different HIV proteins have discordant associations with viral load. *Nat Med,* 13**,** 46-53.

Kiepiela, P. M., J.; Moosa, M.; Moodley, P.; Gordon, M.; Parikh, U.; Sunpath, H.; De Oliveira, T.; Ramjee, G. 2014. HIV Drug Resistance Patterns at the Epicentre of the HIV-1 Epidemic in Kwazulu-Natal, South Africa 2003-2013. *Journal of AIDS & Clinical Research,* 5**,** 294-299.

Kost, G. J. 1995. Guidelines for point-of-care testing. Improving patient outcomes. *Am J Clin Pathol,* 104**,** S111-27.

Kuiken, C., Korber, B. & Shafer, R. W. 2003. HIV sequence databases. *AIDS Rev,* 5**,** 52-61.

Lange, J. 1995. Combination antiretroviral therapy. Back to the future. *Drugs,* 49 Suppl 1**,** 32-7; discussion 38-40.

Lee, G. Q., Bangsberg, D. R., Muzoora, C., Boum, Y., Oyugi, J. H., Emenyonu, N., Bennett, J., Hunt, P. W., Knapp, D., Brumme, C. J., Harrigan, P. R. & Martin, J. N. 2014. Prevalence and virologic consequences of transmitted HIV-1 drug resistance in Uganda. *AIDS Res Hum Retroviruses,* 30**,** 896-906.

Lessells, R. J., Avalos, A. & De Oliveira, T. 2013. Implementing HIV-1 genotypic resistance testing in antiretroviral therapy programs in Africa: needs, opportunities, and challenges. *AIDS Rev,* 15**,** 221-9.

Li, J. Z., Paredes, R., Ribaudo, H. J., Svarovskaia, E. S., Metzner, K. J., Kozal, M. J., Hullsiek, K. H., Balduin, M., Jakobsen, M. R., Geretti, A. M., Thiebaut, R., Ostergaard, L., Masquelier, B., Johnson, J. A., Miller, M. D. & Kuritzkes, D. R. 2011. Low-frequency HIV-1 drug resistance mutations and risk of NNRTI-based antiretroviral treatment failure: a systematic review and pooled analysis. *JAMA,* 305**,** 1327-35.

Little, S. J., Holte, S., Routy, J. P., Daar, E. S., Markowitz, M., Collier, A. C., Koup, R. A., Mellors, J. W., Connick, E., Conway, B., Kilby, M., Wang, L., Whitcomb, J. M., Hellmann, N. S. & Richman, D. D. 2002. Antiretroviral-drug resistance among patients recently infected with HIV. *N Engl J Med,* 347**,** 385-94.

Lockman, S., Hughes, M. D., Mcintyre, J., Zheng, Y., Chipato, T., Conradie, F., Sawe, F., Asmelash, A., Hosseinipour, M. C., Mohapi, L., Stringer, E., Mngqibisa, R., Siika, A., Atwine, D., Hakim, J., Shaffer, D., Kanyama, C., Wools-Kaloustian, K., Salata, R. A., Hogg, E., Alston-Smith, B., Walawander, A., Purcelle-Smith, E., Eshleman, S., Rooney, J., Rahim, S., Mellors, J. W., Schooley, R. T., Currier, J. S. & Team, O. a. S. 2010. Antiretroviral therapies in women after single-dose nevirapine exposure. *N Engl J Med,* 363**,** 1499-509.

Lucas, G. M., Gebo, K. A., Chaisson, R. E. & Moore, R. D. 2002. Longitudinal assessment of the effects of drug and alcohol abuse on HIV-1 treatment outcomes in an urban clinic. *AIDS,* 16**,** 767-74.

Manasa, J., Katzenstein, D., Cassol, S., Newell, M. L., De Oliveira, T., Southern Africa, T. & Resistance, N. 2012. Primary drug resistance in South Africa: data from 10 years of surveys. *AIDS Res Hum Retroviruses,* 28**,** 558-65.

Massyn, N. D., C.; Dombo, M.; Barron, P.; English, R.; Padarath, A. 2013. *District Health Barometer 2012/13,* Durban, Health Systems Trust.

Mccutchan, F. E. 2000. Understanding the genetic diversity of HIV-1. *AIDS,* 14 Suppl 3**,** S31-44.

Meintjes, G., Moorhouse, M. A., Carmona, S., Davies, N., Dlamini, S., Van Vuuren, C., Manzini, T., Mathe, M., Moosa, Y., Nash, J., Nel, J., Pakade, Y., Woods, J., Van Zyl, G., Conradie, F. & Venter, F. 2017. Adult antiretroviral therapy guidelines 2017. *South Afr J HIV Med,* 18**,** 776.

Mills, E. J., Nachega, J. B., Bangsberg, D. R., Singh, S., Rachlis, B., Wu, P., Wilson, K., Buchan, I., Gill, C. J. & Cooper, C. 2006. Adherence to HAART: a systematic review of developed and developing nation patient-reported barriers and facilitators. *PLoS Med,* 3**,** e438.

Muula, A. S. 2008. South Africa's national response to HIV and AIDS treatment: popular media's perspective. *Croat Med J,* 49**,** 114-9.

Nosyk, B., Montaner, J. S., Yip, B., Lima, V. D., Hogg, R. S. & Group, S. H. S. 2014. Antiretroviral drug costs and prescription patterns in British Columbia, Canada: 1996-2011. *Med Care,* 52**,** 362-9.

Nwobegahay, J., Selabe, G., Ndjeka, N. O., Manhaeve, C. & Bessong, P. O. 2012. Low prevalence of transmitted genetic drug resistance in a cohort of HIV infected naive patients entering antiretroviral treatment programs at two sites in northern South Africa. *J Med Virol,* 84**,** 1839-43.

Palella, F. J., Jr., Delaney, K. M., Moorman, A. C., Loveless, M. O., Fuhrer, J., Satten, G. A., Aschman, D. J. & Holmberg, S. D. 1998. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. *N Engl J Med,* 338**,** 853-60.

Parboosing, R., Naidoo, A., Gordon, M., Taylor, M. & Vella, V. 2011. Resistance to antiretroviral drugs in newly diagnosed, young treatment-naive HIV-positive pregnant women in the province of KwaZulu-Natal, South Africa. *J Med Virol,* 83**,** 1508-13.

Parikh, U. M., Kiepiela, P., Ganesh, S., Gomez, K., Horn, S., Eskay, K., Kelly, C., Mensch, B., Gorbach, P., Soto-Torres, L., Ramjee, G., Mellors, J. W., Team, M. T. N. P. & Taskforce, I. P. 2013. Prevalence of HIV-1 drug resistance among women screening for HIV prevention trials in KwaZulu-Natal, South Africa (MTN-009). *PLoS One,* 8**,** e59787.

Peeters, M. & Sharp, P. M. 2000. Genetic diversity of HIV-1: the moving target. *AIDS,* 14 Suppl 3**,** S129-40.

Pillay, D. 2004. Current patterns in the epidemiology of primary HIV drug resistance in North America and Europe. *Antivir Ther,* 9**,** 695-702.

Plantier, J. C., Leoz, M., Dickerson, J. E., De Oliveira, F., Cordonnier, F., Lemee, V., Damond, F., Robertson, D. L. & Simon, F. 2009. A new human immunodeficiency virus derived from gorillas. *Nat Med,* 15**,** 871-2.

Popp, D. & Fisher, J. D. 2002. First, do no harm: a call for emphasizing adherence and HIV prevention interventions in active antiretroviral therapy programs in the developing world. *AIDS,* 16**,** 676-8.

Ragonnet-Cronin, M., Lee, B. E., Plitt, S. S., Zahariadis, G., Merks, H., Sandstrom, P. A. & Brooks, J. I. 2013. Baseline clinical HIV genotypes are a valid measure of transmitted drug resistance within the treatment-naive population. *J Acquir Immune Defic Syndr,* 64**,** 443-7.

Rerks-Ngarm, S., Pitisuttithum, P., Nitayaphan, S., Kaewkungwal, J., Chiu, J., Paris, R., Premsri, N., Namwat, C., De Souza, M., Adams, E., Benenson, M., Gurunathan, S., Tartaglia, J., Mcneil, J. G., Francis, D. P., Stablein, D., Birx, D. L., Chunsuttiwat, S., Khamboonruang, C., Thongcharoen, P., Robb, M. L., Michael, N. L., Kunasol, P., Kim, J. H. & Investigators, M.-T. 2009. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. *N Engl J Med,* 361**,** 2209-20.

Resistance, U. K. C. G. O. M. T. T. O. H. D. 2001. Analysis of prevalence of HIV-1 drug resistance in primary infections in the United Kingdom. *BMJ,* 322**,** 1087-8.

Roberts, J. D., Bebenek, K. & Kunkel, T. A. 1988. The accuracy of reverse transcriptase from HIV-1. *Science,* 242**,** 1171-3.

Robertson, D. L., Anderson, J. P., Bradac, J. A., Carr, J. K., Foley, B., Funkhouser, R. K., Gao, F., Hahn, B. H., Kalish, M. L., Kuiken, C., Learn, G. H., Leitner, T., Mccutchan, F., Osmanov, S., Peeters, M., Pieniazek, D., Salminen, M., Sharp, P. M., Wolinsky, S. & Korber, B. 2000. HIV-1 nomenclature proposal. *Science,* 288**,** 55-6.

Sabin, L. L., Desilva, M. B., Hamer, D. H., Keyi, X., Yue, Y., Wen, F., Tao, L., Heggenhougen, H. K., Seton, L., Wilson, I. B. & Gill, C. J. 2008. Barriers to adherence to antiretroviral medications among patients living with HIV in southern China: a qualitative study. *AIDS Care,* 20**,** 1242-50.

Sagir, A., Oette, M., Kaiser, R., Daumer, M., Fatkenheuer, G., Rockstroh, J. K., Knechten, H., Schmutz, G., Hower, M., Emmelkamp, J., Pfister, H., Haussinger, D. & Team, R. S. 2007. Trends of prevalence of primary HIV drug resistance in Germany. *J Antimicrob Chemother,* 60**,** 843-8.

Shafer, R. W. 2006. Rationale and uses of a public HIV drug-resistance database. *J Infect Dis,* 194 Suppl 1**,** S51-8.

Shaw, G. M. & Hunter, E. 2012. HIV transmission. *Cold Spring Harb Perspect Med,* 2.

Steegen, K., Carmona, S., Bronze, M., Papathanasopoulos, M. A., Van Zyl, G., Goedhals, D., Macleod, W., Sanne, I. & Stevens, W. S. 2016. Moderate Levels of Pre-Treatment HIV-1 Antiretroviral Drug Resistance Detected in the First South African National Survey. *PLoS One,* 11**,** e0166305.

Stevens, W., Kaye, S. & Corrah, T. 2004. Antiretroviral therapy in Africa. *BMJ,* 328**,** 280-2.

Tang, M. W. & Shafer, R. W. 2012. HIV-1 antiretroviral resistance: scientific principles and clinical applications. *Drugs,* 72**,** e1-25.

Tanser, F., Barnighausen, T., Grapsa, E., Zaidi, J. & Newell, M. L. 2013. High coverage of ART associated with decline in risk of HIV acquisition in rural KwaZulu-Natal, South Africa. *Science,* 339**,** 966-71.

Unaids. 2016a. *Global AIDS Update* [Online]. Available: http://www.unaids.org/en/resources/documents/2016/Global-AIDS-update-2016 [Accessed 2 June 2017].

Unaids. 2016b. *Guideline on when to start antiretroviral therapy and on pre-exposure prophylaxis for HIV* [Online]. Available: http://www.who.int/hiv/pub/guidelines/earlyrelease-arv/en/ [Accessed 2 June 2017].

Unaids. 2017. *Ending AIDS: progress towards the 90–90–90 targets* [Online]. Available: http://www.unaids.org/en/resources/documents/2017/20170720_Global_AIDS_update_2017 [Accessed 18 February 2018].

Unaids. 2018. *Global AIDS Monitoring 2018: Indicators for monitoring the 2016 United Nations Political Declaration on Ending AIDS* [Online]. Available: http://www.unaids.org/sites/default/files/media_asset/global-aids-monitoring_en.pdf [Accessed August 2018].

Van Loggerenberg, F., Mlisana, K., Williamson, C., Auld, S. C., Morris, L., Gray, C. M., Abdool Karim, Q., Grobler, A., Barnabas, N., Iriogbe, I., Abdool Karim, S. S. & Team, C. a. I. S. 2008. Establishing a cohort at high risk of HIV infection in South Africa: challenges and experiences of the CAPRISA 002 acute infection study. *PLoS One,* 3**,** e1954.

Vandamme, A. M., Camacho, R. J., Ceccherini-Silberstein, F., De Luca, A., Palmisano, L., Paraskevis, D., Paredes, R., Poljak, M., Schmit, J. C., Soriano, V., Walter, H., Sonnerborg, A. & European, H. I. V. D. R. G. P. 2011. European recommendations for the clinical use of HIV drug resistance testing: 2011 update. *AIDS Rev,* 13**,** 77-108.

Venter, W. F., Kaiser, B., Pillay, Y., Conradie, F., Gomez, G. B., Clayden, P., Matsolo, M., Amole, C., Rutter, L., Abdullah, F., Abrams, E. J., Casas, C. P., Barnhart, M., Pillay, A., Pozniak, A., Hill, A., Fairlie, L., Boffito, M., Moorhouse, M., Chersich, M., Serenata, C., Quevedo, J. & Loots, G. 2016. Cutting the cost of South African antiretroviral therapy using newer, safer drugs. *S Afr Med J,* 107**,** 28-30.

Volberding, P. A. & Deeks, S. G. 2010. Antiretroviral therapy and management of HIV infection. *Lancet,* 376**,** 49-62.

Weinstock, H., Respess, R., Heneine, W., Petropoulos, C. J., Hellmann, N. S., Luo, C. C., Pau, C. P., Woods, T., Gwinn, M. & Kaplan, J. 2000. Prevalence of mutations associated with reduced antiretroviral drug susceptibility among human immunodeficiency virus type 1 seroconverters in the United States, 1993-1998. *J Infect Dis,* 182**,** 330-3.

Weinstock, H. S., Zaidi, I., Heneine, W., Bennett, D., Garcia-Lerma, J. G., Douglas, J. M., Jr., Lalota, M., Dickinson, G., Schwarcz, S., Torian, L., Wendell, D., Paul, S., Goza, G. A., Ruiz, J., Boyett, B. & Kaplan, J. E. 2004. The epidemiology of antiretroviral drug resistance among drug-naive HIV-1-infected persons in 10 US cities. *J Infect Dis,* 189**,** 2174-80.

Wensing, A. M., Van De Vijver, D. A., Angarano, G., Asjo, B., Balotta, C., Boeri, E., Camacho, R., Chaix, M. L., Costagliola, D., De Luca, A., Derdelinckx, I., Grossman, Z., Hamouda, O., Hatzakis, A., Hemmer, R., Hoepelman, A., Horban, A., Korn, K., Kucherer, C., Leitner, T., Loveday, C., Macrae, E., Maljkovic, I., De Mendoza, C., Meyer, L., Nielsen, C., Op De Coul, E. L., Ormaasen, V., Paraskevis, D., Perrin, L., Puchhammer-Stockl, E., Ruiz, L., Salminen, M., Schmit, J. C., Schneider, F., Schuurman, R., Soriano, V., Stanczak, G., Stanojevic, M., Vandamme, A. M., Van Laethem, K., Violin, M., Wilbe, K., Yerly, S., Zazzi, M., Boucher, C. A. & Programme, S. 2005. Prevalence of drug-resistant HIV-1 variants in untreated individuals in Europe: implications for clinical management. *J Infect Dis,* 192**,** 958-66.

Who 2015a. Guideline on when to start antiretroviral therapy and on pre-exposure prophylaxis for HIV.

Who 2015b. HIV drug resistance surveillance guidance: 2015 Update.

Who 2017. HIV drug resistance report 2017.

Williams, I., Churchill, D., Anderson, J., Boffito, M., Bower, M., Cairns, G., Cwynarski, K., Edwards, S., Fidler, S., Fisher, M., Freedman, A., Geretti, A. M., Gilleece, Y., Horne, R., Johnson, M., Khoo, S., Leen, C., Marshall, N., Nelson, M., Orkin, C., Paton, N., Phillips, A., Post, F., Pozniak, A., Sabin, C., Trevelion, R., Ustianowski, A., Walsh, J., Waters, L., Wilkins, E., Winston, A. & Youle, M. 2012. British HIV Association guidelines for the treatment of HIV-1-positive adults with antiretroviral therapy 2012. *HIV Med,* 13 Suppl 2**,** 1-85.

Wood, E. & Montaner, J. S. 2011. Time to get serious about HIV antiretroviral resistance. *Lancet Infect Dis,* 11**,** 723-4.

Www.Hiv.Lanl.Gov. Available: www.hiv.lanl.gov [Accessed].

Wyatt, R. & Sodroski, J. 1998. The HIV-1 envelope glycoproteins: fusogens, antigens, and immunogens. *Science,* 280**,** 1884-8.

Yombi, J. C. & Pozniak, A. L. 2016. Is It Time for Integrase Inhibitors to be the Preferred Regimen for the First-Line Treatment of HIV-1-Infected Naive Patients? *AIDS Rev,* 18**,** 89-100.

Appendices

Appendix 1: Ethics Certificate

UNIVERSITY of the WESTERN CAPE

OFFICE OF THE DIRECTOR: RESEARCH
RESEARCH AND INNOVATION DIVISION

Private Bag X17, Bellville 7535
South Africa
T: +27 21 959 2988/2948
F: +27 21 959 3170
E: research-ethics@uwc.ac.za
www.uwc.ac.za

09 November 2017

Mr DR Chopera
School of Public Health
Faculty of Community and Health Sciences

Ethics Reference Number: BM17/9/5

Project Title: Exploring the trends in prevalence of Human Immunodeficiency Virus drug resistance in South Africa over the course of the HIV epidemic.

Approval Period: 27 October 2017 – 27 October 2018

I hereby certify that the Biomedical Science Research Ethics Committee of the University of the Western Cape approved the scientific methodology and ethics of the above mentioned research project.

Any amendments, extension or other modifications to the protocol must be submitted to the Ethics Committee for approval.

Please remember to submit a progress report in good time for annual renewal.

The Committee must be informed of any serious adverse event and/or termination of the study.

Ms Patricia Josias
Research Ethics Committee Officer
University of the Western Cape

PROVISIONAL REC NUMBER -130416-050

FROM HOPE TO ACTION THROUGH KNOWLEDGE.