THE LEVEL OF ANTIRETROVIRAL DRUG RESISTANCE AT NKHENSANI HOSPITAL HUMAN IMMUNODEFICIENCY VIRUS AND ACQUIRED IMMUNE DEFICIENCY SYDROME TREATMENT SITE.

by

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DISSERTATION

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DECLARATION

I declare that the dissertation hereby submitted to the University of Limpopo, for the degree of master of pharmacy in pharmacology has not previously been submitted by me for a degree at this or any other university; that it is my work in design and in execution, and that all material contained herein has been duly acknowledged.

_____ 15 September 2014

Machethe K.F (Miss) Date

DEDICATION

This work is dedicated to:

- My late parents: Masilu Albert Machethe and Mamoyahabo Frenika Ragolani, who gave me unconditional love;
- My late sister, Mankwana Hilda Mongwe, who loved me, as though I was her own child and encouraged me to study;
- My sisters: Selaelo, Mapula, Mmatlala and Madjadji;
- My nieces: Matlatsi, Phumzile, Rhulani, Amukelani, Pretty, Nikiwe, Dineo, Zanele, Zandile and Oratilwe;
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- My uncles: Steve, Joseph, Sello, Biza and Raphalela; and
- My aunts: Maria and Madira.

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ABBREVIATIONS/ACRONYMS

APP	Annual Performance Plan
ART	Antiretroviral therapy
ARVs	Antiretrovirals
AIDS	Acquired immune deficiency syndrome
AZT	Zidovudine
CNS	Central Nervous System
DDI	Didanosine
DNA	Deoxyribonucleic acid
DOH	Department of Health
DOTS	Directly observed treatment short course
D4T	Stavudine
EFV	Efavirenz
EWI	Early warning indicator
FBC	Full blood count
FDC	Fixed Dose Combination
GGLM	Greater Giyani local municipality
HCT	HIV counseling and testing
HIV	Human immunodeficiency virus
HIVDR	Human immune deficiency virus drug resistance
HAART	Highly active antiretroviral therapy
HSV	Herpes Simplex Virus
LFT	Liver Function Test
MTCT	Mother to Child Transmission
NGO	Non governmental organisation
NHLS	National health laboratory services
NRTIs	Nucleoside reverse transcriptase inhibitors
NNRTIs	Non-nucleoside reverse transcriptase inhibitors
NVP	Nevirapine
OIs	Opportunistic Infections
PLWHA	People Living With HIV/AIDS
PIs	Protease Inhibitors
PMTCT	Prevention of Mother to Child Transmission

PSM	Procurement supply management
RNA	Ribonucleic acid
STI	Sexual transmitted infection
TB	Tuberculosis
T/F	Transfer
WHO	World Health Organization

ABSTRACT

BACKGROUND

Drug resistance is a problem that complicates the efficacy of drugs used against infectious organisms such as the human immunodeficiency virus (HIV). Resistance is one of the principal obstacles to the success of antiretroviral therapy (ART) and efforts to prevent resistance are crucial to optimal drug design and development. In order for practitioners to provide effective pharmaceutical care to HIV patients, it is essential that they appreciate the level of resistance as well as the various factors that may contribute to its emergence. It was against this background that the study was carried out.

The purpose of the study was: To identify early-warning indicators that could be used to prevent and manage antiretroviral drug resistance at Nkhensani Hospital HIV/AIDS treatment site.

The objectives of the study were then:

- To establish whether or not there was antiretroviral drug resistance at Nkhensani Hospital HIV/AIDS treatment site,
- To determine the level of drug resistance,
- To identify the factors that promote drug resistance and
- To identify the genotype drug resistance mutation at Nkhensani Hospital HIV/AIDS treatment site.

Methodology

The study design combined both qualitative and quantitative descriptive approaches. The study was conducted at Nkhensani Hospital which is a district hospital in Mopani district and the district catered for the majority of HIV/AIDS patients on ART in Limpopo province. The ARV treatment facility at the hospital had two thousand two hundred and sixty-two (n=2262) HIV/AIDS patients in June 2009 whose files were screened, for the purpose of recruiting patients into the study. Several factors were considered to develop selection criteria. These included viral load above 1000 cps/ml. After screening the files 86 patients met the criteria and only 34 patients consented to the study. A structured interview using a pretested questionnaire was conducted to collect data on patients' characteristics, in other words demographic information, patients' satisfaction with the service provided and their rate of adherence. Blood samples were obtained from these 34 patients who agreed to take part in the study after their informed consent was obtained. The collected blood samples were sent to the National Health Laboratory Service (NHLS) for genotype testing. During the study participant observation was also carried out where patients were observed while interacting with the health care provider during service provision.

Results: The study showed that the majority of patients were female, unemployed, single and aged from 30 to 35 years with low level of education. All 34 patients were on ART for less than 2 years and 94% of patients were on regimen 1a. The following factors that contributed to early-warning indicator of ARV drug resistance were observed during participants' observation: ARV drug supply problems; some patients either did not comply with the prescribed regimen or forgot to take their medication as prescribed; some patients misunderstood the health care providers and changed their initiating ARV treatment times. The results of the study appeared to be no ARV drug resistance, but instead a low rate of virological failure at 11.6% and good immunological response. The results of the study also indicated that the patients were satisfied with the service rendered by health care providers.

Conclusion: The study appeared to show no ARV drug resistance at Nkhensani Hospital ARV treatment site. However, a low rate of virological failure at 11.6% and good immunological response were observed. The results of the study revealed that there were good patients / health care interactions which might have contributed to low level of ARV drug resistance because patients were satisfied with the service that they were provided. Continued effort is required to maintain a high level of patient satisfaction that was seen at Nkhensani Hospital ART site.

Problems with ARV drug supply seem not to have had any effect on the level of drug resistance among the sample patients in the study. This, however, has to be interpreted with caution since a small number of patients were used.

In spite of the limitation of the small number of patients who participated in the study, it is reasonable to conclude that the ART programme in facilities such as Nkhensani Hospital appeared to have effective patient management and support to achieve acceptable immunological and virological outcomes.

CHAPTER 1
INTRODUCTION AND BACKGROUND

1.1 Introduction

Human immunodeficiency virus (HIV) is a retrovirus which was identified in 1984 as the cause of a widespread epidemic of severe immune suppression called AIDS (Acquired immune deficiency syndrome) (Lachman, 1999). It is an RNA virus and its hallmark is the reverse transcriptase enzyme; a unique characteristic of retroviruses. HIV infection is a dynamic process characterized by vigorous viral replications and CD4 lymphocyte depletion which leads to profound immune deficiency. This profound immune deficiency is characterized by opportunistic infections (OIs), malignancies, neurologic dysfunction and a variety of other syndromes. AIDS is the most severe manifestation of a spectrum of HIV conditions (Hardman & Limbird, 2001).

OIs are the cause of considerable morbidity and mortality in HIV-infected individuals. Some of the OIs decrease quality of life, without being life threatening, e.g., candidiasis of oesophagus/trachea, cryptosporidiosis, herpes simplex virus(HSV) ulsers, histoplasmosis, isosporiasis and microsporidosis, while others are immediately life threatening, e.g., bacterial pneumonia, cytomegalovirus (CMV), non-tuberculous mycobacterial infection (disseminated), pneumocystis pneumonia (PCP), progressive multifocal leukoencephalopathy, salmonella bacteraemia, tuberculosis (TB), toxoplasmosis, HIV-associated kaposi's sarcoma (KS), cryptococcosis, HIV-associated nephropathy and HIV dementia (Makiwane & Regensberg, 2009). Multiple OIs can occur concurrently in individuals with advanced disease and should be treated as quickly and effectively as possible. The diagnosis and treatment of intercurrent illnesses is the first concern in the medical management of HIV. Thereafter, the need for prophylactic therapy needs to be assessed (Rossouw & Botes, 2006).

The most common OIs are Tuberculosis (TB), bacterial pneumonia, candidiasis, Herpes simplex virus and Cryptosporidiosis. TB is a very serious OI and it is the leading cause of illness and death among people living with HIV/AIDS (PLWHA) in South Africa. About 40-50% of TB patients in South Africa are also HIV positive.

Prophylaxis with trimethoprim-sulphamethoxazole (cotrimoxazole) reduces the incidence of OI's such as pneumocystis pneumonia (PCP), toxoplasmosis, isosporiasis, bacteraemia and bacterial pneumonia. It is indicated for all HIV-infected patients who are immune suppressed either clinically according to WHO stages 3 and 4 or immunologically with CD4+ count <200 cells/mm^3. Now that there is greater access to the ARV treatment, HIV/AIDS has become like any other manageable, chronic illness (Martin, 2004).

According to Rossouw and Botes (2006), there are two types of HIV, namely, HIV-1 and HIV-2, both of which are transmitted by sexual contact, through blood and other body fluids or from mother to child. They appear to cause clinically indistinguishable AIDS. The period between infection and illness is longer for HIV-2, which is also less easily transmitted. It is HIV-1 that is responsible for the pandemic, because HIV-2 is rare and concentrated in West Africa (Mark, 2004). The strains of HIV-1 can be classified into the following three genetic groups, M (major group) is responsible for the HIV pandemic; O (outlier group) relatively rare and N (new group) most common found in West Africa. The predominant M group consists of eleven genetic subtypes (clades) designated A to K. Subtype C is largely predominant in South Africa, South Asia and Ethiopia (Rossouw & Botes, 2006).

The treatment of HIV began in the late 1980's with the introduction of the nucleoside reverse transcriptase inhibitors (NRTI) such as zidovudine (AZT). Subsequently, in the early 1990's, more drugs in the same class became available and combination therapy with two agents was shown to be more effective than monotherapy. In the mid 1990's, new and more powerful drugs such as the protease inhibitors (PIs) and the non-nucleoside reverse transcriptase inhibitor (NNRTIs) were also developed (Martin, 2004). These anti-retroviral drugs suppress the replication of HIV by blocking the enzymes HIV uses to replicate. The suppression allows the immune system to regain its strength and combat opportunistic infections. Antiretroviral (ARV) treatment is not a cure; it prolongs and enhances the quality of life of the PLWHA. ARVs must be taken for life in order to prevent the virus from further multiplication, thereby decreasing the amount of virus in the blood and allowing the CD4 cells to increase. It is now standard practice that ARVs should be given in

combination of at least three drugs. This is called highly active antiretroviral therapy (HAART). This is done to prevent emergence of HIV resistance to ARV drugs.

ARV drugs are divided into different classes according to where they act in the lifecycle of HIV. At the moment, we have three classes available in South Africa as reflected in Table 1.1 (Spencer, 2005) and there are other several classes of drugs registered in developing countries (see Table 1.2) (Makiwane & Regensberg, 2009). Nucleoside reverse transcriptase inhibitors (NRTIs) act by competitive inhibition of HIV reverse transcriptase and can also be incorporated into the growing viral DNA chain, thereby blocking the conversion of viral RNA into proviral DNA. NRTIs impair mitochondrial function by inhibiting mitochondrial DNA polymerase-y, resulting in the class side effects of hyperlactaemia and steatohepatitis. (See Table 1.3). Nucleotide reverse transcriptase inhibitors (NtRTIs) act the same as nucleoside. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) block HIV production by binding directly onto reverse transcriptase and preventing the conversion of RNA to DNA. Protease inhibitors (PIs) bind to the viral protease enzymes and prevent it binding and cleaving its natural polyprotein substrate, thus halting production of mature, infective virions. Fusion inhibitor and chemokine receptor antagonists (CCR5) block the entry of the virus into the cell. Integrase inhibitors prevent integration of viral DNA into the host nucleus.

Table 1.1: Examples of ARV drugs available in South Africa

NRTIs	**NtRTIs**	**NNRTIs**	**PIs**
Abacavir	Tenofovir	Delavirdine	Amprenavir
Didanosine		Efavirenz	Indinavir
Lamivudine		Nevirapine	Lopinavir
Stavudine			nelfinavir
Zalcitabine			Ritonavir
Zidovudine			Saquinavir

Table 1.2: Examples of ARV drugs available in South Africa and developing countries

NRTIs	**NtRTIs**	**NNRTIs**	**PIs**	**Fusion inhibitor**	**CCR5 inhibitor**	**Integrase inhibitor**
Abacavir	Tenofovir	Delavirdine	Amprenavir	Enfuvirtide	Maraviroc	Raltegravir
Didanosine		Efavirenz	Atazanavir			
Lamivudine		Etravirine	Darunavir			
Stavudine		Nevirapine	Indinavir			
Zalcitabine			Lopinavir			

Zidovudine			Nelfinavir			
			Ritonavir			
			Saquinavir			
			Tipranavir			

Despite the fact that ARV's prolong the quality of life, they have side effects like all medicines. These are due to the fact that the body adapts to taking ARV's and the immune system strengthens. Some side effects that PLWHA experience on ARV drugs can be mild and disappear over a short time are nausea, vomiting, headaches, skin rash, stomach pain, insomnia and diarrhea, while others can be severe, e.g., hepatic steatosis, hyperlactaemia, peripheral neuropathy, pancreatitis, haematological toxicity, lipodystrophy, hypersensitivity, hyperlipidaemia and lactic acidosis (Martin, 2004). The side effects can be treated, but if PLWHA continue to experience severe side effects in the regimen, the offending drug can be replaced with another drug that does not have the same side effects, e.g., substitution of d4T for AZT (for anaemia), tenofovir for stavudine (for peripheral neuropathy), NVP for EFV (for CNS toxicity or pregnancy). If change in regimen is needed because of treatment failure, a new second-line regimen becomes necessary (WHO, 2004).

Table 1.3: ARV drugs and common or severe side effects

Common or severe side effects	**ARV's drugs**
Abdominal pain	Ritonavir and saquinavir
Anaemia	Zidovudine
CNS effects	Efavirenz
Diarrhea	Darunavir, indanavir, ritonavir, saquinavir, nelfanavir and lopinavir / ritonavir
Dyslipidaemia	Atazanavir, darunavir, indanavir, ritonavir, saquinavir, nelfanavir and lopinavir / ritonavir
Fatigue	Zidovudine
Hair loss	Indanavir
Headache	Zidovudine and lamivudine
Hepatitis	Nevirapine
Hyperpigmentation	Emtricitabine
Hypersensitivity	Abacavir
Insulin resistance	Darunavir, indanavir, ritonavir, saquinavir, nelfanavir and lopinavir / ritonavir
Kidney stones	Indanavir
Lipodystrophy	Stavudine
Myalgia	Zidovudine
Nausea	Zidovudine, didanosine, lamivudine, darunavir, indanavir,

	ritonavir, saquinavir, and lopinavir / ritonavir
Nephrotoxicity	Tenofovir
Neutropaenia	Zidovudine
Pancreatitis	Didanosine
Peripheral neuropathy	Stavudine and didanosine
Rash	Nevirapine and darunavir
Rash teratogenic	Efavirenz
Symptomatic Hyperlactaemia	Stavudine and didanosine
Unconjugated hyperbilibinaemia	Atazanavir

(AFA, 2009)

Drug resistance

Generally, drug resistance is a problem that complicates the efficacy of drugs used against infectious organisms. Resistance is one of the principal obstacles to the success of antiretroviral therapy and efforts to prevent resistance are crucial to optimal drug design and development. The way to eliminate drug resistance would be to devise agents targeted against components of HIV that are common to all strains and absolutely essential to the viral life cycle (Lachman, 1999). Factors influencing drug resistance in HIV are the high genetic variability of HIV and the relative 'fitness' of these variants in the presence of one or more drugs (Martin, 2004). Other factors that promote HIV drug resistance are socio-economic (e.g., poverty), lifestyle, culture, poor patient compliance, sub-therapeutic blood levels of antiretroviral drugs and inappropriate choice of antiretroviral drugs. Pharmaceutical factors that can also contribute to emergence of resistance include poor absorption after oral administration and alteration of drug metabolizing enzymes by other drugs. In addition, the error-prone nature of HIV reverse transcriptase promotes rapid evolution of genetic diversity and remarkable propensity to develop resistance to antiretroviral.

Resistance may be broadly defined as any change, relative to a "wild-type" virus, i.e., a variant with a normal genetic constitution, which is detected in the presence of an ARV agent and results in an improved replicative capacity. Resistance is not an "all-or-none" event, since it may be overcome, invitro, by increasing drug concentration; therefore, the term "reduced susceptibility" seems to be more appropriate (WHO, 2000).

Despite much progress, many patients do not benefit from antiretroviral due to emergence of viral resistance, adverse effects of chronic therapy or inability to adhere to complex regimens. Incomplete therapeutic control of replication inevitably selects for drug-resistant mutants (Havlir & Richman, 1996; and Molla et al., 1996). The mutated virus is less fit and the wild type virus dominates as long as the patient is on treatment. Resistance arises from the change in the three dimensional structure of either of the two enzymes, reverse transcriptase or protease. This resistant mutation can be carried over when the person carrying the virus infects someone else, and the end result is resistant mutations even in treatment naïve patients (Rossouw & Botes, 2006).

Resistance to zidovudine (AZT), a reverse transcriptase inhibitor was first documented in 1989; two years after the drugs became widely available, in subjects treated with monotherapy for 6 or more months (WHO, 2000). These individuals experienced viral rebound to pre-therapy levels and viral isolates were 100 times less susceptible to zidovudine than isolated pre-treatment strains. Resistance to zidovudine is associated with the mutations at reserve transcriptase codons 41, 67, 70, 215 & 219. Those at codons 41, 215 & 219 are most important. Mutations accumulated gradually and resistance developed in one-third of patients after one year of zidovudine monotherapy (Hardman & Limbird, 2001). Resistance of NRTIs develops slowly with the exception of lamivudine, a drug with a low genetic barrier, whose resistance occurs with the single mutation, M184V. The principal site involved in NRTI cross-resistance (defined as resistance to one drug that causes resistance to one or more agents within the same pharmacological class), is codon 151.

In addition, the Q151M mutation alone confers high level resistance to all NRTIs but, also impairs viral replication, so further changes occur as compensatory mutations restoring replicative capacity to the highly resistant virus (WHO, 2000). Only one amino acid substitution needs to take place for the virus to develop resistance to both lamivudine and the NNRTIs. Although non-nucleoside reverse transcriptase inhibitors are very effective antiretroviral drugs, a single mutation at reverse transcriptase codon 103 confers high level resistance. HIV resistance to NNRTIs is associated with the appearance of mutations in the reverse transcriptase enzyme.

Major NNRTI mutations responsible for 20-50 fold resistance result in treatment failure (e.g., K103N mutation). On the other hand, protease inhibitors require multiple substitutions for drug resistance. Resistance amongst protease inhibitors develops also because of mutations with amino acid substitutions at both the active enzymatic site and other regions (Rossouw & Botes, 2006). Resistance to ARVs can be induced.

Primary resistance

Primary resistance occurs when HIV is resistant to one or more antiretroviral drugs in the absence of antiretroviral exposure. Antiretroviral resistant mutations in primary resistance persist for much longer (a year or more) in the absence of antiretroviral drug selection pressure than in the patients who have developed resistance after exposure to antiretroviral therapy, eventually back mutations occur and create the 'wild type'. Studies carried out in industrialized countries show that 5% to 20% of patients have primary resistance to one or more antiretroviral classes. The prevalence of primary resistance in Southern Africa is currently low, but it is likely to increase with the burgeoning use of antiretroviral therapy (Douglas, 2008).

Induced resistance

Most antiretroviral resistance is induced or can be described as secondary resistance. This means that a patient could be infected with the 'wild type' HIV and mutations are selected following the use of antiretroviral drug. The resistance in this situation is a consequence of continuing an antiretroviral regimen while the viral load is not suppressed and selects for resistant mutations that accumulate, eventually convey resistance to all antiretroviral drugs. Once the resistance mutations have been selected, they confer a survival advantage for the virus, and the mutant strain becomes dominant in the HIV population. However, if the antiretroviral that have been selected for the resistance mutations are removed the 'wild type' HIV population becomes the dominant population because it is fit. The antiretroviral resistant mutant population will disappear from the circulation in weeks to months, and will no longer be detectable by resistance, but if the antiretroviral drugs are reintroduced, the resistant mutant HIV rapidly becomes dominant again, because the resistant mutations are archived in long-lived populations of CD4+ T-cells. (Douglas, 2008)

Methods for testing antiretroviral resistance

Drug resistance of HIV-1 is assessed in plasma virions that are the progeny of recent, active infection and represents the form of the virus with best replicative capacity. Tests for drug resistance depend either on genotype or phenotype of the virus. Genotyping tests identify specific mutations in the consensus sequence of circulating RNA from viral genomes associated with drug resistance. The relative drug resistance of the virus is deduced from the mutations present in circulating virus. Phenotypic tests directly measure the enzymatic activity of viral gene products or the replication of the virus in the presence of increasing concentration of drug (WHO, 2000).

Genotypic testing of HIV-1 drug resistance is an application of the recent advances in DNA sequencing and data analysis techniques. Genotypic tests are designed to flag specific mutations in HIV genetic makeup that have been linked with drug resistance. These tests are relatively inexpensive and comparatively rapid, with a turnaround time of about 5 to10 days. Genotypic tests are more likely to be used as a sentinel to warn of emerging drug resistance, while phenotypic assays would be more appropriate for complex situations, as in the case of patients exposed to several antiretroviral drugs who may have a strain of virus with many mutations. Genotyping can be used as an early warning sign when the genetic basis of resistance to a drug is known. Because genotypic assays provide information only about the specific mutations they were designed to detect, the tests will fail to signal a potential resistance problem stemming from unidentified mutations. Genotypic tests also generally are less likely to detect resistance mutations in samples from patients with low viral loads. Moreover, when genotypic testing identifies multiple resistance mutations in a single strain, it is difficult to interpret what effect these possibly interacting mutations will have on the patient's response to a drug regimen (Lachman, 1999).

Phenotypic tests of ARV drug susceptibility were initially developed using cultured virus isolates (WHO, 2000). Phenotypic tests measure how well a patient's strain of HIV will grow in the presence of antiretroviral drugs. They are easier to interpret than genotypic assays because they provide a more direct measure of how a particular

strain of HIV will respond to a particular drug regimen. These assays also are quantitative, indicating whether resistance to an agent is relatively strong or weak. Phenotypic assays are more complex than genotypic assay, taking 2 to 5 weeks on average. They are also substantially more expensive. Phenotypic testing has its own limitations, such as a lack of standardization of tests from different laboratories, which makes it impossible to compare results from different assays. Phenotypic test also may fail to detect evolving resistance in a patient with low viral load (Lachman, 1999).

Both genotypic and phenotypic assays are technically complex and require expert interpretation. In the case of complex resistance patterns or when the genetic basis of resistance is unknown, a phenotypic assay is likely to be more useful and easier to interpret than a genotypic test (Lachman, 1999).

There are 38.8 million PLWHA globally, of whom it is estimated that 6.1 million are living in South Africa, with almost 950 AIDS deaths occurring every day (worldwide HIV/AIDS statistics, 2012). The majority of PLWHA are between 15 to 34 years old. At Nkhensani Hospital, Limpopo Province in South Africa there were 4091 PLWHA registered and 2262 patients were on ART, between September 2005 and June 2009. The number of infected patients is increasing on a daily basis; and of 2262 patients on ARV treatment, 1843 patients were on regimen 1a, 223 patients are on regimen 1b, 155 patients are on regimen 1c, 31 patients are on regimen 1d and 10 patients are on second line regimen and 219 patients are deceased (Nkhensani Hospital HIV / AIDS treatment site statistics, 2009).

The South Africa National Antiretroviral Treatment Guidelines (2004) reflects the following criteria for initiation antiretroviral treatment for adults and adolescents:

- The patients with WHO stage IV disease (clinical AIDS defining illness) should not be dependent on a CD4 cell count determinations;
- Patients with CD4 cell count less than 200 cells/$mm^{3,}$ irrespective of stage; and
- The patient must express a willingness and readiness to take ART adherently.

The clinical criteria for initiation of ART in children are as follows:

- Confirmation of diagnosis of HIV- infection;
  Recurrent (>2 admissions per year) hospitalizations or prolonged hospitalization (>4 weeks) for HIV- related illness;
- The patient satisfies the provisional WHO stage III/IV disease; and
- For relatively asymptomatic patients, one can consider CD4 percentage <20% if under 18 months or <15% if over 18 months.

The 2010 South Africa National Antiretroviral Treatment guideline reflect the following criteria for initiating ART for adults and adolescents:

- Patients with CD4 count ≤ 200 cells/mm^3 irrespective of clinical stages;
- CD4 count ≤ 3500 cells/mm^3 in pregnant women and patients with TB/HIV;
- WHO stage iv irrespective of CD4 cell count and
- MDR/ XDR irrespective of CD4 cell count.

The criteria for initiating ART regimens for infants and children are as follows:

- All children less than one year of age;
- Children 1 – 5 years with clinical stage III or IV or CD4 ≤ 25% or absolute CD4 <750 cells/mm^3 and
- Children 5 to 15 years with clinical stage III or IV or CD4 ≤ 350 cells/mm^3

There are two ART regimens recommended for use in the South Africa public sector, namely: first- line regimens (1a and 1b) and second-line regimen (2). Regimens 1a and 1b are offered to patients who are antiretroviral naïve and regimen 2 is offered to those who fail their primary treatment regimen.

Regimen 1a

Stavudine (d4T) + Lamivudine (3TC) + Efavirenz (EFV)

This is suitable for all men and women on reliable contraception.

Regimen 1b

Stavudine (d4T) + Lamivudine (3TC) + Nevirapine (NVP)

This is suitable for women who may fall pregnant or already pregnant.

Regimen 2

Zidovudine (AZT) + Didanosine (ddi) + Lopinavir/Ritonavir (LPV/r)

This is suitable for patients who become resistant to first-line regimens or have severe side effects (Spencer, 2005).

1.2 Statement of the Problem

Nkhensani Hospital is a district hospital in Mopani District of Limpopo Province, South Africa. It has 322 beds. ARVs are offered as a priority programme at the Nhlamulo Clinic (HIV/AIDS treatment site). Treatment failure has been observed at Nkhensani Hospital in some patients receiving ARV treatment and worsening some quality life of the patient. Between September 2005 and June 2009, 219 patients were reported dead. The causes and magnitude of treatment failure at Nkhensani Hospital were not established.

1.3 Purpose of the Study

To identify early-warning indicators that can be used to prevent and manage ARV resistance at Nkhensani Hospital.

1.4 Broad Objective

To establish whether or not there is ARV drug resistance at Nkhensani Hospital HIV/AIDS site.

1.5 Specific Objectives

1.5.1. To determine the level of drug resistance at Nkhensani Hospital HIV/AIDS treatment site.

1.5.2. To identify factors that promotes drug resistance at Nkhensani Hospital HIV/AIDS treatment site.

1.5.3. To identify the genotype drug resistance mutations at Nkhensani Hospital HIV/AIDS treatment site.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

In this chapter, literature regarding ARV drug resistance and what determine drug resistance will be reviewed. The chapter is divided into the following section: the studies that have been done in other countries and South Africa (SA), adherence to the prescribed treatment and factors that contribute to ARV drug resistance.

2.2 ARV Drug Resistance Studies in other Countries and South Africa

2.2.1 Primary drug resistance

ARV drug resistance is a major challenge to treatment programmes for both developed and developing countries. By 2004, approximately 10% of new HIV-1 infections in the USA and Europe involved viral strains exhibiting resistance to at least one drug (WHO, 2004). A worldwide surveillance program (Worldwide Analysis of resistance Transmission over time of Chronically and acute infected HIV-1 (WATCH)) found the rate of resistance to ARV drugs among treatment naïve patient to be 5.5% in Africa, 7.4% in East Asia, 5.7% in Southeast Asia and 6.4% in Latin America, lower than in North America 11.4% and Europe 10.6% (Agency for Health care Research and Quality (AHRQ), 2007)

According to the studies carried out by AHRQ (2007) found that, the study carried out in India in 2007 among treatment naïve patients reported no drug resistance. However, in a study carried out in Northern India amongst 60 persons 80 percent were found resistance to AZT; in South India amongst 50 persons fourteen percent were resistant to NNRTIs, 6 percent resistant to NRTIs, and 20 percent PI resistant. A study of 128 persons in Mumbai found that only two percent were resistant to NRTIs (AHRQ, 2007). Only three studies were found that reported on the rates of resistance in other developing regions of Asia. None reported NNRTI resistance, NRTI resistance range from four to seven percent, primary resistance to PIs range from two to three percent (AHRQ, 2007).

The 14 studies carried out in the Sub-Saharan African region among treatment-naïve patients found that the resistance to NNRTI ranged from 0% to 7.7%, were

associated with infection with most HIV clades and to NRTI ranged from 0% to 8% for all clades. Primary resistances to PI mutations were less than three percent among Africans; however, there were high levels of secondary PI mutations in most studies (AHRQ, 2007).

2.2.2 Secondary drug resistance

ARVs resistance is now a worldwide phenomenon, occurring in areas where ART has only recently been introduced (WHO, 2000). In Uganda, in the context of the drug access initiative treatment programme, after a mean of 3 months from starting therapy, 63% of 107 patients receiving 2 NRTIs or HAART regimens showed high phenotypic resistance to more than 1 drug, intermediate resistance was observed in 11% and 7% of the specimens had multidrug resistance (WHO, 2000). More than 10 years ago at the 12th World AIDS conference in Geneva 2004, it was reported that up to 54% of patients that were taking these antiretroviral drug combinations (NRTI and PI) developed resistance to the drugs (Lachman, 1999). In a study of 12000 US-based patients on treatment during 1999, 27% of the patients had virus that was resistant to all three classes of ARV drugs, 29% had virus resistant to two classes of drugs and 22% had virus resistant to a single class of ARVs (Lachman, 1999).

In eight studies carried out in Latin America, resistance to NRTIs ranged from 2 to 14% among treatment naïve groups and that to NNRTIs ranging from 0 to 2%. In a handful of studies that were carried out, which included the Brazilian and Argentine country, the rates of secondary PI mutations were found to be high. Presumably, HIV subtype found among the study population was clade B (AHRQ, 2007).

ARV resistance has been found in children born to mothers on the PMTCT programmes, in most studies, infected infants were tested for resistance at 6 or 7 weeks of age. Rate of NNRTI resistance among untreated HIV positive infants of mothers receiving only SD-NVP ranged from 36 percent to 50 percent. One study found that detectable resistance fades over time in infants exposed to SD-NVP (AHRQ, 2007).

The study carried out by Pascal (2009) at Mankweng Hospital, Limpopo Province, South Africa, found that after the phylogenetic analysis the HIV-1 subtype was clade

C. None of the samples investigated had mutations coding for resistance to the NRTIs. However, one sample (1/17) from a female patient aged 28 years had mutations to the NNRTI (A98G and K103N). No major or minor mutations to protease inhibitors were observed. However, protease polymorphisms such as K20R/S, M36I/L, L63P/Q/V/S and I93L typical in HIV-1 subtype C had a frequency of 10/17, 12/17, 14/17 and 17/17(Pascal, 2009)

2.3 HIV genotypes and its effect on management of ARV drug resistance

The efficacy of an ARV treatment regimen depends on the activity of the regimen's individual ARV drug and the number of HIV-1 mutations required for the development of resistance to each ARV – the genetic barrier to resistance. Genotypic resistance testing is used to identify transmitted drug resistance, provide insight into the reasons for virological failure in treated patients and help to guide second – line and salvage therapies (Tang and Shafer, 2012).

The VIRADAPT study was carried out in Europe found that patient submitted to genotyping presented a higher reduction in viral load than that of those who had no such test results. The tests were developed to help attenuate the impact of viral resistance and able to detect mutations associated with the phenotypic resistance of HIV to ARV (Meynard, Vray, Morand-Joubert, et al, 2002).

Multivariate analysis, the variables associated with a greater drop in viral load in the first assessment were the patients whose switching was based on genotyping. The genotypic resistance testing was beneficial for making decisions about changes in treatment and it helps to choose more effective regimens in patients failing previous ARV regimen. In addition, genotyping test and adherence were found to be independent factors for success in the management of patients who failed treatment (Brazilian journal, 2006).

Development of resistance of ARV drugs is a major impediment to optimum treatment of HIV-1 infection. Although resistance testing can help to select subsequent regimens when virologic failure occurs, cross-resistance, which affects all classes of ARVs, may make it more difficult to achieve optimum control of HIV (Kuritzkes, 2004).

2.4 ARV Adherence

Adherence means that the patient must take more than 95% of their doses (i.e., missing less than 3 doses in a month). It is more important to know what type of patient has a disease than what type of disease a patient has (Osler, 2001). The appropriate use of medicines to ensure maximum outcomes is, however, challenging and requires input and support from all role players including the patient, the health care providers, family members/ the community and the health system where the patient is receiving care.

In antiretroviral treatment (ART) patient adherence includes, taking medications, keeping appointments, undertaking recommended preventive measures such as dieting, exercise, substance use and changing possibly deep-seated behavioural patterns. Research shows that patients are more likely to benefit from their prescribed medication when they:

1. Understand and accept the diagnosis;
2. Agree with the treatment proposed; and
3. Have had their concerns about the medicines specifically and seriously addressed.

Adherence is the extent to which a patient's behaviour coincides with the prescribed health care regimen as agreed upon through a shared decision making process between the client and the health care provider (Barker & Burton et al., 1999).

On the other hand, concordance describes the agreement between a patient and a health care professional about whether, when and how medicines are to be taken and it is fundamentally different from either compliance or adherence in two important areas:

1. It focuses on the consultation process rather than a specific patient behaviour; and
2. It has an underlying ethos of a shared approach to decision-making rather than paternalism (Weiss & Britten, 2003).

In a two-way communication between patients and health-care providers about medicines, that led to improved satisfaction with care; knowledge of the condition

and treatment; adherence, health outcomes and few medications-related problems (Cox & Stevenson, 2003).

Support by healthcare providers was another variable that significantly predicted ARV adherence. Participants were generally satisfied with the information, care, support and respect they received from their healthcare providers (Weidle et al., 2006; and Kip et al., 2009).

In the treatment of HIV and AIDS, adherence to antiretroviral drugs varies between 37% and 53% depending on the drug under study and the demographic characteristics of patient populations. Poor adherence has been reported to result in incomplete suppression of HIV replication, as well as emergence of resistance to ARV (Cox & Stevenson, 2003).

2.5 Factors that Contribute to the Development of HIV /AIDS Drug Resistance

2.5.1 Virus

They are several factors related to the life cycle and replication of HIV which are key contributors toward the rapid and widespread emergence of resistance that is seen with this organism:

1. The HIV reverse transcriptase (RT) enzyme has notoriously ‘low fidelity’.
2. It is prone to errors when copying viral RNA into DNA.
3. HIV RT makes one error in each HIV genome per round of replication.
4. Has an exceptionally high rate of replication.
5. The high rate of replication coupled with the high rate of error for RT results in numerous HIV ‘variants’ which are rapidly formed and propagated.

Patients who are infected with HIV, therefore can have multiple variants of the virus present in their system. These variants can have greatly different sensitivities to antiretroviral drugs, a factor that can significantly complicate the selection of drugs and course of therapy (Bartex, 2000).This means that a proper assessment of the patient’s current health–related behaviour, such as medication taking and non-drug treatment and background information that defines the patient’s educational needs must be carried out. Several factors related to the host/patient need to be taken into consideration (Weiss & Britten, 2003).

2.5.2 The host/patient

Patient's knowledge, attitudes and beliefs about a condition and it management are important to understand. When dealing with infection such as HIV infection. It is the patient's perceptions of the illness experience, the treatment and management that correlate with adherence more than the objective realities. Patients often have their own models of disease and treatment, and this may be based on cultural beliefs. If this model conflicts with the regimen prescribed for the patient, non-adherence is likely to occur. For example, if a patient believes that HIV and AIDS is a curse from family members or friends, such a patient is most likely not going to adhere to treatment. The patient's value system may also differ from that of the provider. For example, a full-blown AIDS patient may rather prefer to die than start ART, which is a lifelong treatment (Cox & Stevenson, 2003).

In addition, psychological factors such as immaturity, impulsivity, hostility, fear of dependence, denial and type A personality have been found to correlate with non-adherence. Interestingly, it has been reported that socio-demographic variables such as age, sex, race, education, occupation, income and marital status do not correlate with non-adherence (Cox & Stevenson, 2003).

This means that patients who have stable support systems and stable family situations tend to be more adherent to the prescribed treatment, as compared to patients having disinterested family members or friends, which can contribute to the patients developing drug resistance. A patient's previous experiences with similar disease, convenience and access to the health-care facility and appointment scheduling around the patient's schedule also have an effect on adherence (Cox & Stevenson, 2003).

2.6 HIV/AIDS Characteristics

Patients who are experiencing symptoms such as pain, lethargy and palpitations are more likely than asymptomatic patients to adhere to treatment recommendations, especially if the symptoms are relieved by the treatment (Weiss & Britten, 2003). On the other hand, the patients may be so overwhelmed and debilitated by their symptoms and they may be unable to follow through with aspects of treatment regimen. Co-morbidity may either create problems for the patients or provide the

potent through experiencing a set of self-management skills (DOH, 2004). A TB patient, who has been taught how to use pillboxes and the importance of adherence, is mostly going to carry through, if he or she is to start on ART (Cox & Stevenson, 2003). However, patients with underlying psychiatric disorders or factors that affect the ability to comprehend such as retardation, dementia or having other addictive behaviours such as alcoholism or drug abuse, are most likely to develop drug resistance (Cox & Stevenson, 2003).

2.7 Treatment Regimen

According to Weiss and Britten (2003) long duration of treatment and requirement for significant behaviour changes has been reported to lead to ARV drug resistance. Side effects with significant symptoms or interference may also cause ARV drug resistance. ARV drug resistance may increase the potential for regimen failure, compromise future treatment options and lead to increase the risk of mortality (Weiss & Britten, 2003).

Drug interactions may interfere with the hepatic metabolism and some of the ARV drugs (e.g., PIs and NNRTIs) are metabolized by the liver, this means that drugs that induce or inhibit by hepatic enzymes will affect the levels of PIs and NNRTIs (Aid for Aids, 2009). Enzyme induction may lead to sub-optimal drug levels, especially when it involves ARV drugs and it could lead to the development of ARV drugs resistance, e.g., TB and ARV drugs (Regensberg & Makiwane, 2009).

However, drug interactions have become an increasingly complex challenge for clinicians treating HIV-infected patients and there is an equally significant number of interactions that can cause a decrease in patient clinical outcomes, therapeutic failures, mild to moderate toxicity and severe to life threatening toxicities (Spencer, 2005). Clinically significant drug interactions are generally those that produce at least a 30% change in pharmacokinetic parameters. Drug interactions occur in almost all patients who are being treated for HIV/AIDS due to the high average number of drugs (for HIV and opportunistic infections), food interactions, and vitamins, complementary and herbal or traditional medicines that the patient may be taking (DOH, 2004).

CHAPTER 3
METHODOLOGY

3.1 Study Setting

The study was carried out at the Nkhensni Hospital, Giyani, Mopani District in the Province of Limpopo, South Africa. As shown in Figure 3.1, Limpopo Province consists of five districts, Mopani, Vhembe, Sekhukhune, Waterberg and Capricorn Districts. In the Mopani District, there are 9 hospitals, three of which did not have ARV's programme (Department of Health pharmaceutical services statistics, 2009/2010). Nkhensani Hospital was one of the six hospitals that had an ART facility and was chosen for the study. It also had a high number of patients on antiretroviral treatment.

Figure 3.1 Districts in Limpopo Province; Nkhensani Hospital is situated in the Mopani District

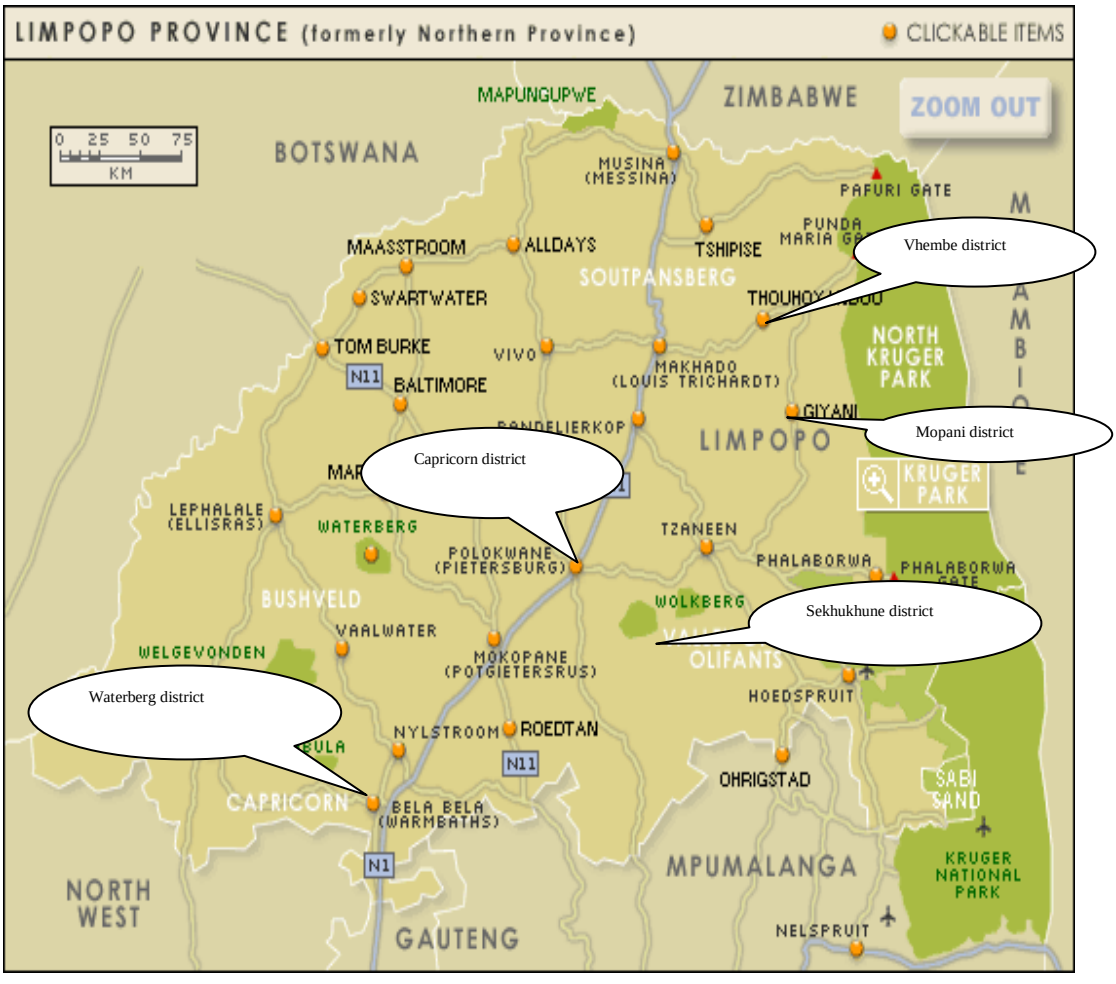


Figure 3.2 shows the map of all hospitals in Mopani district.

3.2 Study Design

The study design combined both quantitative and qualitative descriptive approaches. The combined approaches were used to examine the level of ARV drug resistance at Nkhensani Hospital HIV/AIDS treatment site. The structured questionnaire was used to collect data among the targeted populations in relation to demographic data, participant and non participant observation, patient interaction with the health care provider, physical environment of the facility and adherence. The results from NHLS were used to analyse the treatment outcome (immunological and virological).

3.3 Study Population and Ethical Considerations

After obtaining ethical clearance from the University of Limpopo's Turfloop Research and Ethics Committee, the Limpopo Provincial Department of Health and permission from the CEO at the Nkhensani Hospital, two thousand two hundred and sixty-two (n=2262) HIV/AIDS patients' files were screened in June 2009. For the purpose of recruiting patients into the study, several factors were considered for the development of selection criteria as shown in Table 3.1 below.

Table 3.1 Exclusions and inclusions criteria used for recruitment

Exclusions	Inclusions
CD4 count above 200 cells/mm^3 in adult	CD4 count below 200 cells/mm^3 in adult
CD4 count above 20% in children	CD4 count below 20% in children
Age above 45 years	Age between 1 to 45 years
Viral load less than 1000 copies/ml	Viral load above 1000 copies/ml
Patients not on ART	Patients must be on ART
Resistant testing must not be done on previous ARV drugs	Resistant testing must be done on current ARV drugs
Patients more than 2 years on ARV treatment	Patients less than 2 years on ARV treatment

Note: The CD4 count below 200 cells/mm^3 was the requirement of initiating patients on ARV drug according to South Africa National Antiretroviral Treatment Guideline (2004)

Only 86 patients met the criteria to be enrolled into the study. The identified patients were contacted and given a date to come to the hospital. When they came they were informed about the study and their consent sought to participate. Only after agreeing to participate in the study was each patient given another appointment and assured that the expenses incurred would be reimbursed. Out of the 86 patients who had met the criteria, only thirty-four (n=34) patients agreed to participate in the study.

3.4 Sampling Method

Sampling is described as taking a portion of a population or universe and considering it is representative of that population or universe (De Vos, 2002). According to Polit and Beck (2006) quantitative research designs should have large samples in order to increase representativeness and reduce the possibilities of sampling errors. The sample frame was 2262 patients, 86 of which met the inclusions criteria as seen in table 3.1. Only 34 patients consented to participate in the study. The convenience sampling method was used in this study.

3.5 .1 Non-Participant Observation

Patient / health care interaction

During the screening of patient's records, a modified covert participant observation method was used at the ART facility, to observe at least 20 patients daily for 10 days. This involved watching how patients interacted with a health-care provider; who could be a physician, nurse, pharmacist, dietician or counsellor. The aim of observation was to determine whether or not i) the patients adhered to the prescribed regimen, ii) what information was provided to the patients by the health-care provider, iii) the prescribing practice, and iv) to study patients access to ARV drugs.

3.5.2 Participant Observation

Drug supply chain at NKhensani Hospital

A participant observation study was carried to understand how the drug supply chain was managed at the hospital. The pharmacist responsible for procurement was observed while selecting and quantifying ARV drugs to be procured, receiving, storing and how the drugs were then distributed from main storeroom at Nkhensani Hospital pharmacy to the ARV treatment site. The observation was also done at the treatment site in order to determine whether or not the patients received the correct medication as prescribed by the physicians. The observation included checking whether each patient had received the correct strength, dosage form and information about duration of treatment. This involved talking to the patient and also to the responsible pharmacist.

3.6 Validity

Polit and Beck (2006) define “validity” in terms of whether the measuring instrument measures what it is supposed to measure. To ensure that the questionnaire measures what it was supposed to measure in order to draw meaningful scores from it, its accuracy was determined by looking at face validity (Creswell, 2009).

Face- validity

This type of validity ensured that the questionnaire looks valid and clear to be read by anyone and also that the content was relevant to the study (Brink, 2006; and Creswell, 2009). In order to measure validity, the questionnaire was assessed by an expert and, in this case, the supervisor and co-supervisor of this research project were considered to have expertise in assessing the question variables under study.

3.7 Reliability

Reliability is defined as the accuracy or precision of an instrument; degree of consistency or agreement between two independently derived sets of scores and the extent to which independent administrations of the same instrument yield the same or similar results under reliable conditions (De Vos, 2000). In this study, the structured questionnaire was administered to 10 patients who did not participate in the study. This assisted the researcher to determine the time taken to complete the interview and consistency in the responses.

3.8 Data Collection

Interviews

A pre-tested questionnaire was administered to the thirty four patients who accepted to participate in the study. The questionnaire gathered information regarding (a) gender, age, level of education, marital status, home language, employment status and as to whether one was a bread winner or not, (b) perception about interpersonal relationship between the patients and health-care provider (c) issues concerning adherence, (d) accessibility/convenience and physical environment of the facility.

In addition to the interview the NHLS results (the first and second CD4 count and viral load) were obtained from Nkhensani Hospital patients’ clinical record to analyse

treatment outcome, i.e., immunological and virological responses. The patient clinical records were examined as a follow-up after the second CD4 count and viral load.

Following the interview, about 15 ml whole venous blood was collected from each patient. The blood samples were collected into clearly marked EDTA tubes. About 4 ml for CD4 count were collected into a tube with a purple stopper and for viral load determination, 5 ml were collected into a tube with a white stopper. The tubes were labelled accordingly. For genotype testing, 6 ml of blood were collected, spun and aliquoted into 1 ml tubes which were also labelled accordingly and stored at -80^{0}C. The blood sample and first viral load were eventually sent to Johannesburg National Health Genotype Laboratory Service.

Blood specimen handling

CD4 count determination

When performing CD4 enumeration, which is used for monitoring immunological response following ART, two different concepts are conventionally applied thus:

- A dual platform procedure comprising of two instruments, a haematology analyzer for absolute lymphocyte counting and a flow cytometer for defining the percentage of CD4 cells in a matching lymphoid populations; and
- A single platform that includes volumetric counting or addition reference beads in known number to samples.

Equipment required:

- Epics XL-MCL flow cytometer
- T-Q prep station
- Automated hematology analyzer for performing white cell counts for dual platform method with adequate internal and external quality assurance procedures.
- Vortex mixer
- Blood mixer
- Calibrated automated pipettes
- Biohazard class II or dead air cabinet for preparing samples.
- Refrigerator at 2-8^{o}C for storing prepared samples and reagents.

Materials needed:

- 12 X 75 mm blue plastic test tubes for use on XL-MCL flow cytometer
- Appropriate pipette tips
- Test tube racks
- Gauze swabs
- Printer paper

Procedure

In the present study, the samples were prepared on receipt and those that were received after working hours would be Q-prepped and kept for the next working day until analysed. The 5 ml EDTA samples were kept at room temperature 20-25^{o}C. Generally, the samples were found to be stable for 5 days as long as they were kept away from direct sunlight, not exposed to extreme temperatures or dramatic temperature changes.

Before commencing with preparation of samples, the T-Q-prep and XL-MCL instruments were opened and appropriate cleaning procedures and controls were run. The samples, once they arrived at the lab, were checked against the following shipping list:

1. Each blue tube was labelled with the laboratory number using a barcode.
2. The blood samples were placed on the blood mixer to ensure adequate mixing of the blood before pipetting.
3. 10 mcl of the PLG CD4 monoclonal antibodies were pipetted into the bottom of each labelled sample tube.
4. 100 µl of the well mixed, whole blood sample or immunotrol control were added to the bottom of appropriately labelled tube, containing the monoclonal antibodies.
5. Each tube was gently vortexed for a few seconds and incubated in the T-Q-prep machine in the in the dark for 10-15 minutes at 20-25^{o}C.
6. After incubation, the tubes were loaded into the T-Q-prep carousel, placed in the T-Q- prep workstation
7. Once the samples had been prepared, they were checked to see whether lysis had taken place properly. This was then logged on the daily

maintenance worksheet. Applying the same pipette techniques used for adding blood or using a stepper pipette that had been checked for accuracy, 100 µl of flow count beads were added. The carousel was loaded onto XL-MCL and analysis carried out using the 'CDARV' protocol or stored capped in the refrigerator at 2-8^{o}C until analysis.

8. Analysis would stop automatically once a total of 5000 lymphocytes had been acquired and a report printed automatically.

Viral load

Generally, the virus levels in the peripheral blood can be quantified either by,

1. measurement of the HIV P_{24} antigen in serum,
2. quantitative culture of HIV from plasma, or
3. direct measurement of viral RNA in plasma using nucleic acid amplification, polymerase chain reaction (PCR) or signal amplification technologies.

In the present study, direct measurement of viral RNA in plasma was done using the COBAS^{R}Ampliprep/COBAS^{R}TaqMan^{R}HIV-1 test, v2.0. This is a nucleic acid amplification test for the quantification of human immunodeficiency virus type 1 (HIV-1) RNA in human plasma. The COBAS^{R}Ampliprep/COBAS^{R}TaqMan^{R}HIV-1 test, v2.0 is based on three major processes, namely:

1. Specimen preparation to isolate HIV-1 RNA;
2. Reverse transcription of the target RNA to generate complementary DNA (cDNA), and
3. Simultaneous PCR amplification of target cDNA and detection of cleaved dual-labelled oligo nucleotide detection probe specific to the target.

Procedure

- Blood was collected in sterile tubes with lavender tops using EDTA as the anticoagulant and mixed adequately according to the tube manufacturer's instructions;
- Whole blood was stored at 2-25^{o}C for no longer than 24 hours. Plasma was separated from whole blood within 24 hours of collection by centrifugation at

800-1600 xg for 20 minutes at room temperature and transferred to a sterile polypropylene tube;

- Plasma specimens were stored at room temperature (25-30^{o}C) for up to 1 day or at (2-8^{o}C) for up to 6 days. Plasma specimens were shown to be stable for six weeks if frozen at -20^{o}C to -80^{o}C in 1100-1200 µl aliquots contained in sterile 2.0 ml polypropylene screw-cap tubes such as Sarstedt 72.694.006;
- All reagent cassettes were removed from 2-8^{o}C storage and immediately loaded onto the COBAS^{R}Ampliprep Instrument and allowed to equilibrate to ambient temperature on the instrument HIV-1 v2.0 CS1 for at least 30 minutes before the first specimen was to be processed;
- HIV-1 v2.0 CS1 was placed onto a reagent rack. HIV-1 v2.0 CS2, HIV-1 v2.0 CS3, HIV-1 v2.0 CS4 was placed onto a separate reagent rack. The reagent containing HIV-1 v2.0 CS1 was loaded onto rack position A of the COBAS^{R}Ampliprep instrument and the other reagent racks containing HIV-1 v2.0CS2, HIV-1 v2.0 CS3, HIV-1 v2.0 CS4 were loaded onto rack position B, C, D or E of the COBAS^{R}Ampliprep instrument.;
- Sample racks were prepared as follows: A barcode label clip was attached to each sample rack position where a specimen (S- tube) was to be placed. One of the specific barcode label clips for the controls [CTM (-) C, HIV-1(+) C,v2.0 and HIV-1 H (+) C, v2.0] was attached to each sample rack position where the controls (S-tube) were to be placed. The barcode label clips for controls had the same control lot number as the lot number on the control vials in the kit. The position was assigned with the appropriate control barcode clip and one input S-tube was placed into each position containing a barcode label clip. Using the AMPLILINK software specimen orders were created for each specimen order and order report was printed and used as a worksheet;
- Specimen and control racks were prepared in the designated area for specimen and control addition as follows; each specimen and control [CTM (-) C, HIV-1 L (+) C, v2.0 and HIV-1 H (+) C, v2.0] was vortexed for 3 to 5 seconds. 1000 to 1050mcl of each specimen and control [CTM (-) C, HIV-1 L (+) C, v2.0 and HIV-1 H (+) C, v2.0] were transferred to the appropriate barcode labelled input S-tube using a micropipettor with an aerosol barrier or positive displacement RNase-free tip. Specimens and controls were

transferred to tube positions as assigned and recorded on the worksheet. The bar code label clips for controls had the same control lot number as the lot number on the control vials in the kit. The right control was assigned to the position with the appropriate control barcode clip; and

- The COBAS^{R}Ampliprep instrument was started using the AMPLILINK software. Flags or error messages were checked and processed specimen sand controls were removed from the COBAS^{R}Ampliprep instrument on either sample racks (for COBAS^{R}TaqMan^{R}Analyser without Docking Station) or K-carrier racks (for COBAS^{R}TaqMan^{R}48 Analyser) depending on the workflow and was started within 120 minutes following completion of specimen and control preparation.

At completion, the results report was printed. The HIV-1 RNA concentration was expressed in copies (cp)/ml. It appeared that plasma specimens were frozen and thawed up to five times without a significant loss of HIV-1 RNA.

3.9 Data Analysis

The raw data that were obtained was structured and meaning that provided answers to the research questions. The study design combined both quantitative and qualitative designs. The analysis of the data used several strategies which were not mutually exclusive in order to make stronger case in answering the research questions. Descriptive statistics were used by converting and condensing the data into an organized, visual representation. Pie charts and histograms, for example, were used for nominal data collected.

Data which was non-numerical involved mainly from the observation studies and interviews that were carried was analysed as early as the start of data collection. It was a ‘hands on process”. Initially data was calculated manually using statistical method or formula and then later it was entered into the SPSS 21 programme in order to verify the data.

The nominal scale measurement was used in the demographic data. Data such as gender, age distribution, level of education, marital status, employment, home language, bread winner distribution and participants staying near ARV treatment site,

the nominal scale measurement was used, and then the ratio data was applied in order to determine the percentage.

Patient and health care provider interaction were presented by the numeric frequency distribution which summarises data into intervals of equal width and each interval shows how many numbers (data values) fall within the interval. Data such as level of patient satisfaction with health care providers, service delivery among different ages and general attitude towards the treatment environment were analysed in relation to the response which range from strongly disagree to strongly agree and it was then represented in the table and histogram indicating the percentage of satisfaction (factors).

Adherence measurement tools and factors that influenced patients adherence such as side effects, changing regimen, taking treatment as instructed, co-morbidity with TB, difficult to store treatment and forget to take ARV treatment, nominal scale measurement were used and then presented in a table.

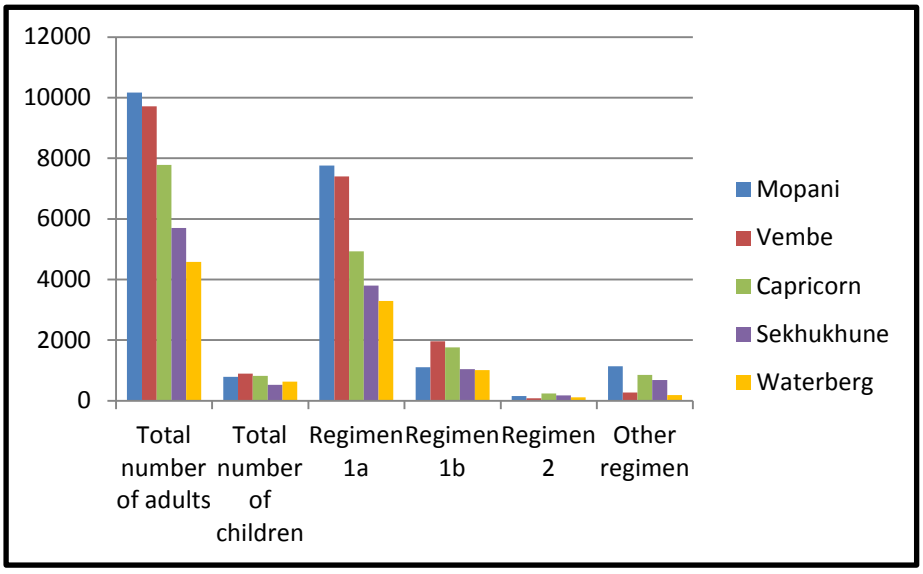
Treatment outcomes with viral load and CD4 count results from genotype laboratory and NHLS, data (laboratory results) was presented in a table. Data was analysed using South African National Department of Health treatment guidelines 2004, the following criteria was used: for immunological response persistent CD 4 Count below 100 cells/mm^3, a drop of CD4 count below baseline pre-treatment level and for virological response, viral load below 400 copies/ml, viral load between 400 to 5000 copies/ml and viral load above 5000 copies/ml switch treatment regimen. Then the frequency distribution and ratio scale measurement were used to determine the level of ARV drug resistance, immunological response and the percentage of virological failure.

CHAPTER 4
RESULTS

4.1 Introduction

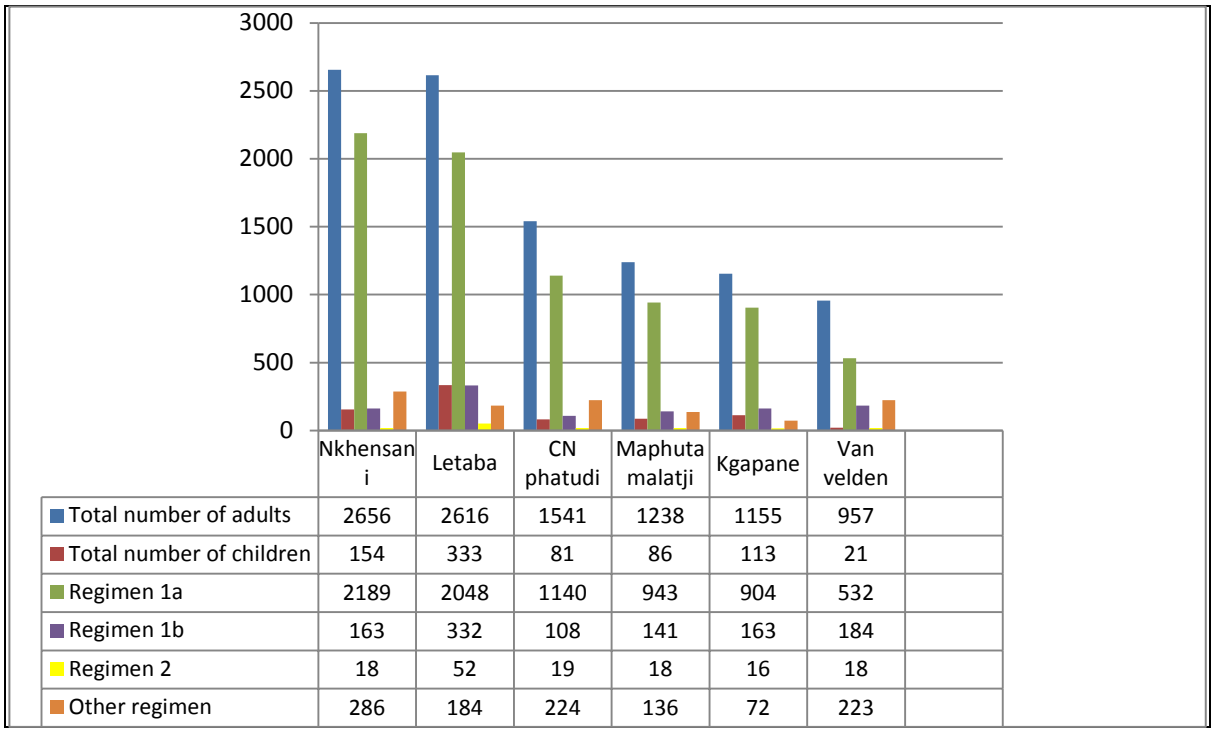
This chapter is divided into sections. These are, namely, an HIV/AIDS overview of the situation in Limpopo Province as of 2009/2010 statistics from the Department of Health; demographic data of the participants in the study; results of the participants observation; prescribing practice; drug supply; patient/ health system interaction; and treatment outcomes.

Figure 4.1 The average statistics of ARV's patients for Limpopo Province as of 2009/2010



As can be seen from the above figure 4.1, the district that had the highest number of patients who were on ARV's treatment in Limpopo Province was Mopani for the financial year 2009/2010.The district had 10163 adults and 788 children, the Vembe District had 9711 adults and 890 children; the Capricorn District had 7776 adults and 818 children; the Sekhukhune District had 5701 adults and 522 children; while the Waterberg District had the lowest average number of 4585 adults patients and 633 children. From the records obtained from the Limpopo Department of Health.

Figure 4.2: The average number of patients on ARV's in Mopani District



	Nkhensani	Letaba	CN phatudi	Maphutamalatji	Kgapane	Van velden
Total number of adults	2656	2616	1541	1238	1155	957
Total number of children	154	333	81	86	113	21
Regimen 1a	2189	2048	1140	943	904	532
Regimen 1b	163	332	108	141	163	184
Regimen 2	18	52	19	18	16	18
Other regimen	286	184	224	136	72	223

As shown in figure 4.2, Nkhensani Hospital had the highest number of adults' patients who were on ARVs treatment in the Mopani District for the financial year 2009/2010. Nkhensani Hospital had 2656 adults and 154 children, Letaba Hospital had 2616 adults and 333 children; Dr CN Phatudi Hospital had 1541 adults and 81 children; Maphutha Malatji Hospital had 1238 adults and 86 children; Kgapane Hospital had 1155 adults and 113 children; while Van Velden had the lowest average number of 957 adults and 21 children.

4.2 Participant Observation at Nkhensani Hospital

From the participant observation of the patients attending the ART facility, during their interaction with the physician, nurse, pharmacist, dietician or counsellor, over a period of 2 weeks it was discovered that some of the patients:

- either did not comply with the prescribed regimen or forgot to take their medication as prescribed;
- lacked information about their disease and treatment;
- totally misunderstood the health care providers and changed their initiating ARV treatment times; or
- took home wrong medication dispensed by the health care provider

Most of the patients observed seemed to have financial problems, just from their appearance indicating poverty. Some complained about lack of food and money for transport to come to the clinic for their medical appointment. Some patients already on ARV treatment and getting food parcels were heard say to the dietician; *"The food you are giving me is not enough. I need more".*

And also, interestingly, some of the patients indicated lack of support from family and friends. Some patients did not see the reason for change of life style. Some indeed admitted to continuing to have unprotected sexual intercourse, because they felt it was safe since they were taking ARV drugs and getting STI treatment. One patient was heard bragging to the pharmacist about his sexual activity. He was heard say, *"I am a lion and I eat meat".*

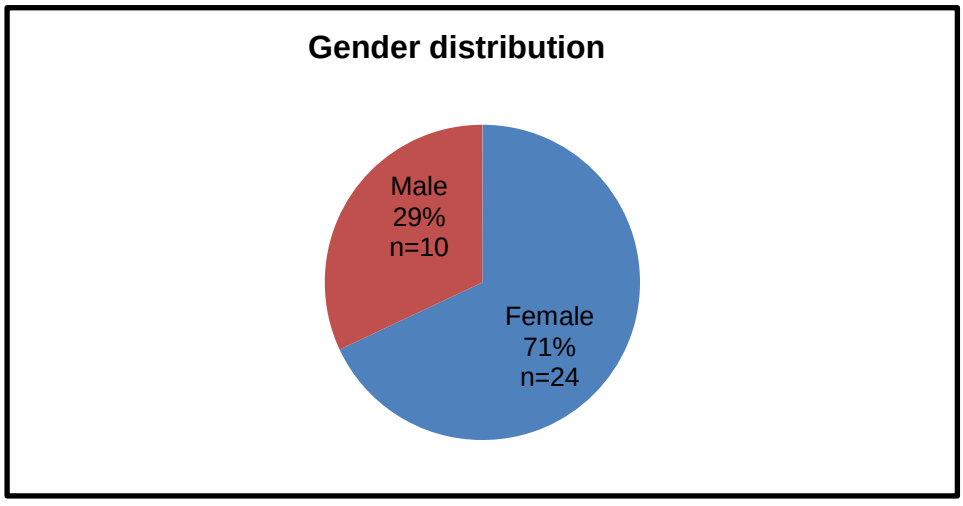
Even though some patients were coming to the ART facility, it was observed that they still concurrently consulted the community traditional healer. It was discovered that some of them actually started taking ARV medication when they were in pain and would stop taking the medication when the pain was gone.

4.3 Structured Interviews with the Patients

4.3.1 Demographic data

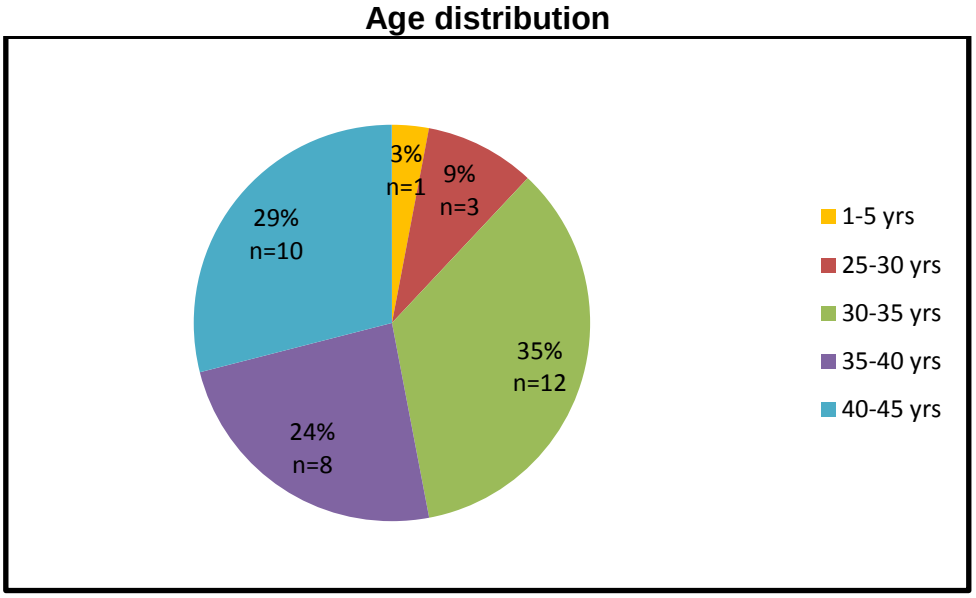
Figures show the demographic profiles of participants in the study.

Figure 4.3 Shows gender distribution of the participants



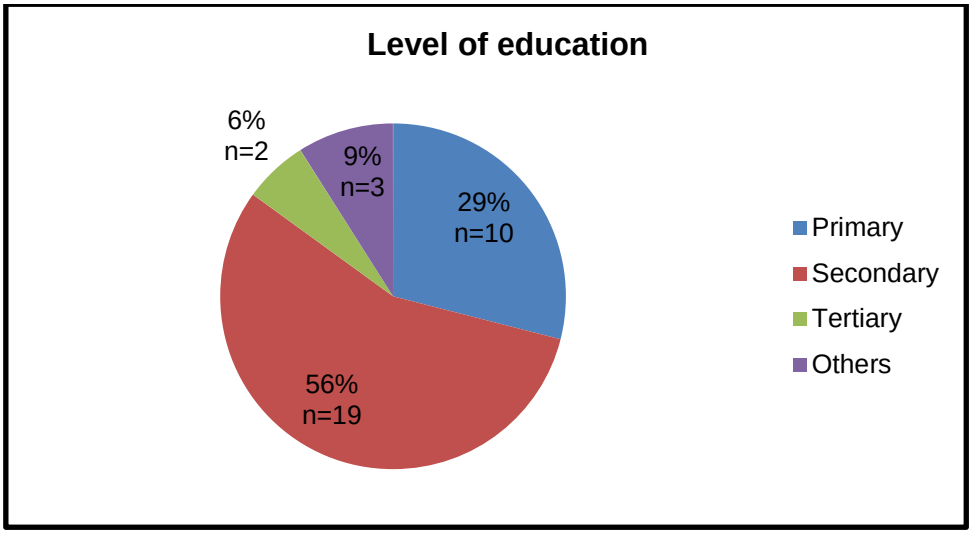
The above figure shows that there were more women participants than men in the study.

Figure 4.4 shows the age distribution



As shown in Figure 4.4, the age groups of participant were as follows 1-5 years, 25-30 years, 30-35 years, 35-40 years and 40-45 years representing 3%, 9%, 35%, 24% and 29% respectively

Figure 4.5 Shows the level of education of the participants.



Among the participants, as shown in Figure 4.5, n=10 (29%) attended primary school level, n=19 (56%) secondary level, only n=2 (6%) went to school up to tertiary level and n=3 (9%) others are 2 adults participants that never went to school and 1 child was attending crèche.

Figure 4.6 Shows the participants' marital status

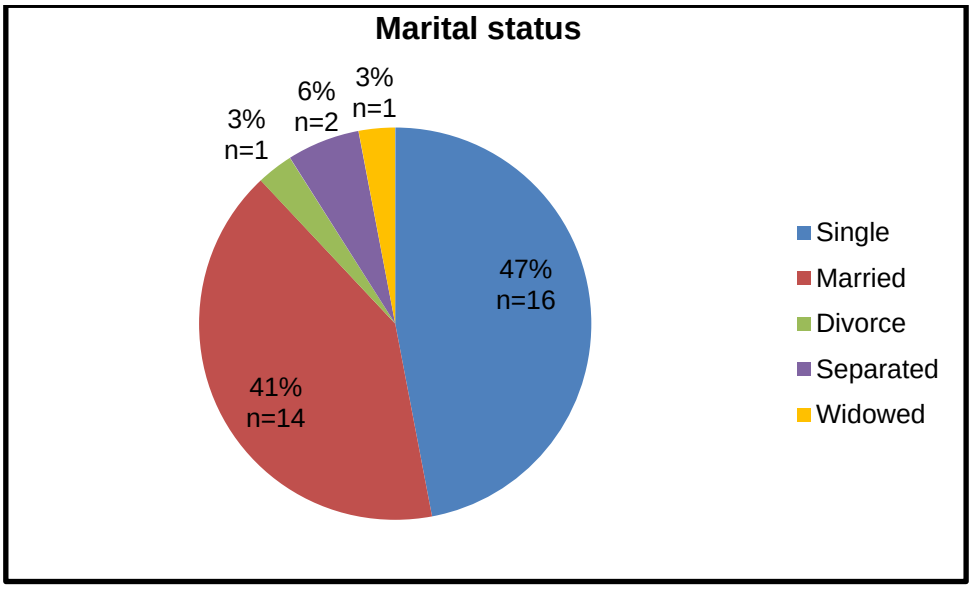


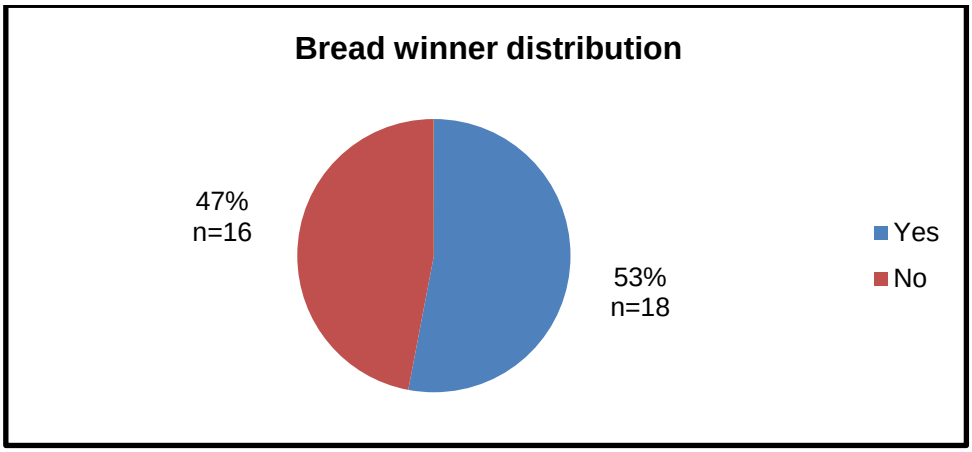
Figure 4.6 shows that 47% participants were single, 41% were married, and 12% of the participants were divorced, separated or widowed.

Table 4.1 Employment data

Type of employment	**Frequency of participants (N=34)**	**Percentage**
Self-employed	1	3%
Full-time	2	6%
Part-time	1	3%
Pensioner	6	18%
Unemployed	24	70%

As shown in Table 4.1, most of the participants were unemployed (i.e., n=24 (70%)), n=6 (18%) were pensioners, n=2 (6%) were full-time workers, n=1 (3%) was self-employed, and n=1 (3%) was a part-time worker.

Figure 4.7 Bread winner



As can be seen from Figure 4.7 above, the majority of the participants were the one who provided for their families (bread winner) (at 53%) and 47% of participants were not.

Table 4.2 Shows the language spoken in the home by the participants

Home language	**Frequency of participants (N=34)**	**Percentage**
Tsonga	27	79%
Sepedi	6	18%
Others(Zulu)	1	3%

As shown in Table 4.2, most of the participants spoke the Xitsonga Language n=27 (79%), while n=6 (18%) spoke the Sepedi and only n=1 (3%) spoke the Zulu Language.

Figure 4.8 The percentage of the participants who lived near or far away from ARV treatment site

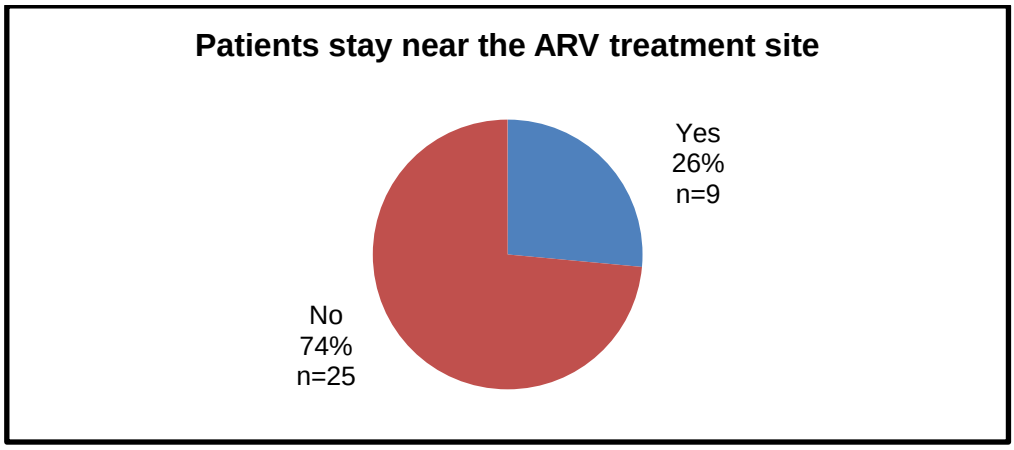


Figure 4.8 shows that the majority of participants n=25 (74%) were staying more 30 km away from HIV/AIDS treatment site at Nkhensani Hospital and the other n=9 (26%) were staying less than 10 km to the Hospital.

4.3.2. Patient and health care provider interaction

From the results obtained, as shown in Table 4.3, it would appear that patients generally felt that health-care providers at the centre were:

- friendly during counselling and even provision of treatment;
- respectful of cultural values and recognized the need for privacy and confidentiality;
- encouraging adherence and keeping of appointments;
- provided and explained all the information and allowed them to ask questions and promise the participants that all the information discussed during the sessions would be confidential; and
- able, where necessary, to use appropriate language.

Table 4.3 The level of satisfaction with health-care providers among different ages

Factors	**Age 25-30 (N=3)**	**Age 30-35 (N=13)**	**Age 35-40 (N=7)**	**Age 40-45 (N=10)**
Encouraging	93%	92.3%	85.7%	90%
Friendly	93%	93.8%	91.4%	94%
Respect	86.7%	92.3%	97.1%	92%
Language	93%	95.4%	97.1%	90%
Cultural value	93%	89.2%	97.1%	86%
Opportunity to ask questions	93%	93.8%	91.4%	90%
Right & confidentiality	93%	87.7%	94.3%	92%
Privacy	93%	93.8%	94.3%	92%
Average	**92%**	**92%**	**93.5%**	**90.75%**

Patient and the health system interaction

As reported earlier in Figure 4.8, the majority of the participants lived far from the treatment centre. During the interview, they, however, were generally satisfied with a number of service delivery factors as shown in Table 4.4.

Table 4.4: Level of Satisfaction with Service Delivery Factors

Factors	Age 25-30 (N=3)	Age 30-35 (N=13)	Age 35-40 (N=7)	Age 40-45 (N=10)
Appointment	73%	84.6%	91%	84%
Time acceptable	93%	80%	82.8%	92%
Easy to reach the hospital	80%	80%	80%	76%
Period of scheduled session	93%	73.8%	91%	90%
Treatment sessions	93%	78.5%	82.8%	94%
Average	**86.4%**	**79.4%**	**85.5%**	**87.2%**

As shown in Table 4.4, the participants were satisfied with the time taken by the health-care providers to scheduled their appointments and treatment sessions. It was easy for the participants to reach the HIV/AIDS clinic. The least satisfied were those between the age group 30-35 years old. It would appear that was the same age group that was least happy with physical environment of the facility as shown in Figure 4.9.

Figure 4.9 Shows the participants' attitude towards the treatment environment by age group

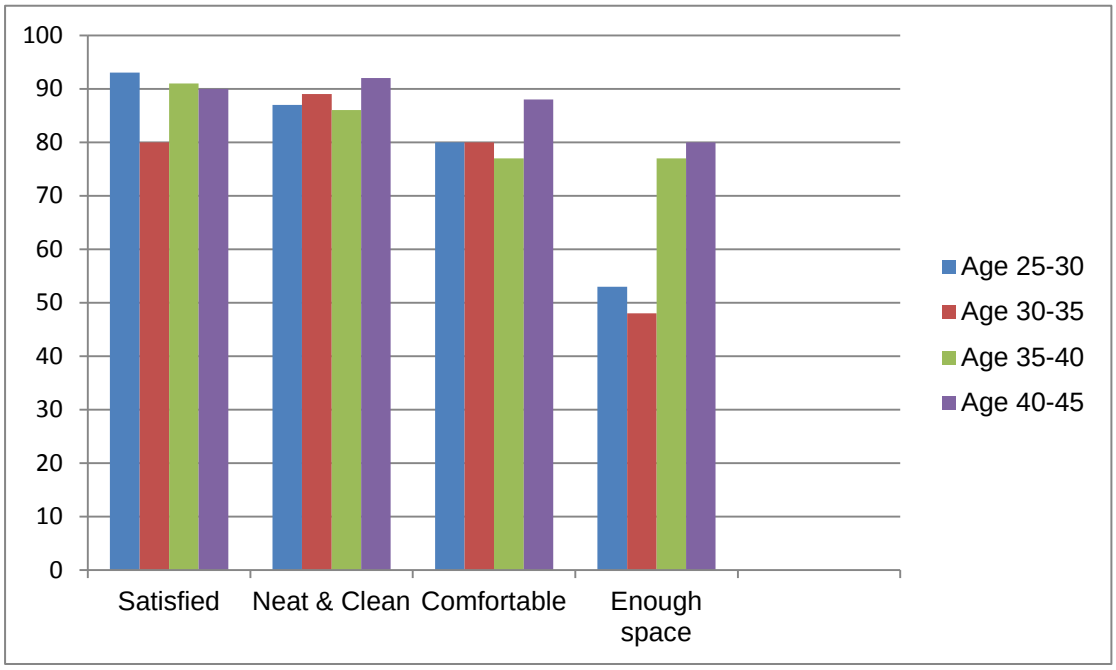


Figure 4.9 shows that the participants were satisfied with cleanliness and were comfortable with the environment at HIV/AIDS treatment site. Generally, as can be

seen from the figure, patients were not happy about the space provided at the centre, especially aged group 25-30 and age group 30-35 years.

4.4 The Prescribing Practices at Nkhensani Hospital HIV/AIDS Treatment Site

There were two ART regimens that were used at Nkhensani Hospital for adult patient, namely, First-line regimens (1a and 1b) and Second-line regimen (2).

Table 4.5 The ARVs prescribed at Nkhensani Hospital according to South Africa National Antiretroviral Treatment guidelines (2004)

Regimen 1a	**Regimen 1b**	**Regimen 2**
Stavudine (d4T)	Stavudine (d4T)	Zidovudine (AZT)
Lamivudine (3TC)	Lamivudine (3TC)	Didanosine (ddl)
Efavirenz (EFV)	Nevirapine	Lopinavir/Ritonavir

;

Table 4.6 Two first-line regimens (1c and 1d) that had been introduced at Nkhensani Hospital treatment site due to severe side effects for the financial year 2008/2009

Regimen 1c	**Regimen 1d**
Zidivudine (AZT)	Zidovudine (AZT)
Lamivudine (3TC)	Lamivudine (3TC)
Efavirenz (EFV)	Nevirapine

Table 4.7 ARV drugs of choice for children at Nkhensani Hospital HIV/AIDS treatment site

Regimen	**6 Months up to 3 years**	**Over 3 years and >10kg**
First –line	Stavudine (d4T) Lamivudine (3TC) Lopinavir/Ritonavir (LPV/r)	Stavudine (d4T) Lamivudine (3TC) Nevirapine (NVP)/Efavirenz
Second-line	Zidovudine (AZT) Didanosine (ddi) Nevirapine (NVP)/Efavirenz	Zidovudine (AZT) Didanosine (ddi) Lopinavir/Ritonavir (LPV/r)

Note: The children formulation depended on the age and the weight of the child.

Table 4.8 The first line regimens that had been introduced in 2010 at Nkhensani Hospital ARV treatment site due to stavudine side effects.

Regimen	Adults	Infants and children	
		Infants and children under 3 years	Children 3 years and over
First- line	Tenofovir (TDF) Lamivudine (3TC) Efavirenz (EFV)/ Nevirapine (NVP)	Abacavir (ABC) Lamivudine (3TC) Lopinavir/Ritonavir (LPV/r)	Abacavir (ABC) Lamivudine (3TC) Efavirenz (EFV)

Choice of ART regimen consisted of two NRTIs as backbone and a third drug that was used was usually an NNRTI either nevirapine or efarvirenz (e.g., AZT, 3TC and EFV). PI use was often kept in reserve for second-line treatment in view of the toxicities associated with the PIs and the multiple drug-drug interaction. Patients started treatment with first-line regimen and changed to second-line regimen if there were serious side effects or suspect drug resistance. Selection of ARV treatment for first-line regimen was reported to be based on:

- Potency;
- Side effect profile;
- The potential for maintenance of future treatment options;
- The anticipated adherence of the patient population with a regimen;
- Coexistent conditions (e.g., co-infections, metabolic abnormalities);
- Pregnancy or the risk thereof;
- The use of concomitant medications (i.e., potential drug interactions); and
- The potential to acquire resistant viral strains (Spencer, 2005 and WHO, 2002).

At Nkhensani Hospital ARV drugs were prescribed in accordance with the WHO and South African National Antiretroviral Treatment Guidelines on both first-line regimen 1a and b and second-line regimen 2. Normally, patients were started on regimen 1a except if they were pregnant (regimen 1b) or have co-morbidity such as TB and HIV. If the patient was on TB treatment, the ARV treatment would be started after the patient had completed the initial phase of TB treatment (2 months), if the patient had CD4+ count of less than 200 cells/mm^3.

If the patient had CD4+ count of less than 50 cells/mm^3, the clinicians introduced the ART as soon as the patient was stabilized on TB therapy (no less than 2 weeks between starting TB therapy and Starting ART). The health care provider considered the willingness, ability and the readiness of the patient to begin therapy; the degree of existing immunodeficiency determined by the CD4+ T cell count and viral load. The health care provider counselled and educated the patients on adherence to the prescribed regimen.

Table 4.9 The different ARV drugs formulation for adults and paediatric patients

Number	Description and strength	Pack size	Price
1.	Stavudine 15 mg capsules	60	R11.540
	Stavudine 20 mg capsules	60	R12.282
	Stavudine 30 mg capsules	60	R11.816
	Stavudine solution1mg/ml	200 ml	R9.678
2.	Lamivudine 150 mg tablets (scored)	60	R18.710
	Lamivudine 150 mg tablets	60	R18.710
	Lamivudine 300 mg tablets	30	R21.652
	Lamivudine 10 mg/ml	240 ml	R16.470
3.	Nevirapine 200 mg tablets	60	R22.090
	Nevirapine suspension 50 mg/ml	100 ml	R9.704
	Nevirapine suspension 50 mg/ml	240 ml	R15.333
4.	Zidovudine 300 mg tablets	60	R58.441
	Zidovudine10 mg/ml solution	200 ml	R16.626
5.	Efavirenz 50 mg capsules	30	R16.020
	Efavirenz 200 mg capsules	90	R51.180
	Efavirenz 600 mg capsules	30	R38.179
6.	Tenofovir 300 mg tablets	30	R51.449
7.	Didanosine 25 mg tablets	60	R39.933
	Didanosine 50 mg tablets	60	R79.536
	Didanosine 100 mg tablets	60	R61.349
8.	Abacavir 300 mg tablets	60	R103.877
	Abacavir 20 mg/ml	240 ml	R69.368
9.	Lopinavir / Ritonavir 200/50 mg tablets	120	R202.540
	Lopinavir / Ritonavir 80/20 mg solutions	5’s bottles	R216.510

Table 4.10 shows the cost of treatment with each regimen per month

Regimen	Patient profile	Drugs	Monthly cost per patient
1a	All men, women on reliable contraception (1843)	d4T/3TC/EFV	R68.705*
1b	Women who may fall pregnant (223)	d4T/3TC/NVP	R52.616*
1c	All patient who have severe side effects of d4T(155)	AZT/3TC/EFV	R115.33*
1d	All patient who have severe side effects of d4T(31)	AZT/3TC/NVP	R99.241*
2	Patient not responding to regimen 1 (10)	AZT/ddl/ Lopinavir/Ritonavir	R343.463*

Note: The value in bracket is the number of patients who were on that regimen at Nkhensani Hospital and * source of information was from the hospital computer (PDSX) and the price used were government prices for ARV drugs.

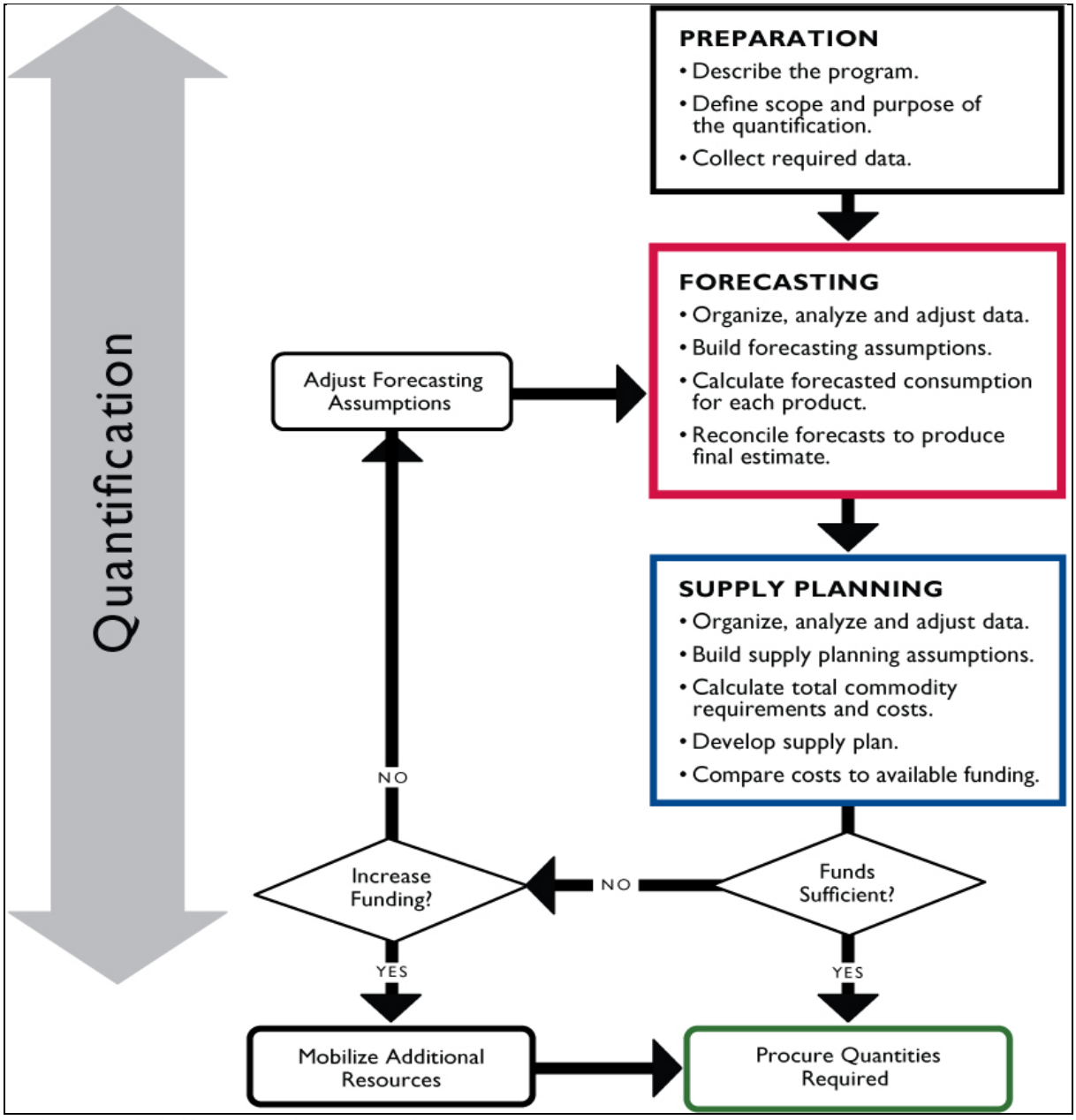
The combination of AZT, 3TC and EFV has been shown to be highly effective and was well tolerated as first line treatment of antiretroviral naive patients. The first line treatment recommendation for patients with TB and HIV co-infection was AZT or d4T+ 3TC + EFV 600 mg or 800 mg/day. The 800 mg dose of EFV was reported to achieve higher drug levels in the absence of rifampicin. It appeared that more money was spent on regimen 1a.

During the participant observation exercise, it was seen that the medicines dispensed to patients had no labels. It was reported that the labels were out of stock. In addition, at the time of study, most of the antiretroviral drugs were found to be out of stock. When paediatric formulations were out of stock, it was found that adult formulations were used instead. For example, a capsule of either stavudine or efevarenz would be opened and mixed with the correct amount of water in a measuring cylinder. A syringe was provided to determine the dose to be administered. For other ARVs, other alternatives would have to be used such as cutting / crushing the tablets.

However, in the case of adults, at times the right strength of medication, for example, 150 mg lamivudine would be out of stock and patients would then be provided 300

mg from another supplier. Change of strength; e.g., lamivudine 300 mg daily instead of 150 mg twice a day, because of lamivudine 150 mg was out of stock from the supply, was quite common. This confused some patients. At times, patients ended up not receiving enough treatment for a month and had to come to the treatment site after two weeks to collect the remaining treatment. It was quite common to hear some patients saying to the pharmacist, when told that a particular drug was out of stock: *"I have had to borrow money in order to come and pick up my medication. I cannot afford to come again".*

Figure 4.10: Steps in Quantification of ARV medication



(USAID/ deliver project 2009)

Figure 4.10 shows the ideal way of quantification of ARV drug supply as proposed by USAID/ Deliver Project (2009). From the results obtained, it would appear that it was not possible to follow all the steps in the quantification process at Nkhensani Hospital and even at the central depot. All medicines were centrally procured and at HIV/AIDS treatment facility there were stock outs on several items such as efevarenz, lamivudine and stavudine. In addition, during the study period it was observed that there were big challenges being faced in relation to the distribution of ARV drugs from the supplier to the hospital. It appeared that the supplier could not supply enough stock as per order.

Drugs at Nkhenasni Hospital were supplied from the depot Pharmaceutical Service Company based at Seshego. The drugs were ordered according to the minimum and maximum stock level and by checking the stock movement for the specific drugs based on the patients that were already on ARV treatment and the number of treatment naive patients in attendance. Then, the health-care provider (e.g., Pharmacist) estimated the correct amount of drugs required. At the time the study was carried out, they were facing big challenges in relation to the distribution of ARV drugs from the supplier because sometimes when they placed the order they did not receive enough stock. There were several reasons for this, which included change in packaging and strength of the drugs originally ordered. For example, lamivudine 300 mg, instead of 150 mg, would be supplied as per tender agreement. Issues concerning payment of the supplier; lack of necessary ingredients for manufacture; and orders made from other suppliers were responsible for such problems in the reliable supply of ARVs.

4.5 Adherence

When the drugs were available and provided to the patients, the health care provider measured patients' adherence by checking their appointment date, CD4 cell count and viral load. The health care provider also checked by asking the patient questions related to the medicine use; what they did to remember to take medication; storage of their medications; what time they took their medication and how many and how often per day and tablet or capsule counting as seen in Table 4.11

Table 4.11 The adherence measurement tools and factors that influenced patients adherence

Tools / Factors	Less than 50 cell/mm^3	50-100 cell/mm^3	100-150 cell/mm^3	150-200 cell/mm^3	Yes	No
CD4 count	39.2%	15.2%	24.2%	21.2%		
Improved					33 (100%)	
Side effects					26 (79%)	7 (21%)
Regimen	1a-31(94%)	1b-1(3%)	1c-1(3%)			
Regimen change					1 (3%)	32 (97%)
Other treatment					6 (18%)	27 (82%)
TB treatment					5 (15%)	28 (85%)
Other disease except TB					2 (6%)	31 (94%)
Miss drug dose					1 (3%)	32 (97%)
Take medication as instructed					33 (100%)	
Benefit from ARV drugs					33 (100%)	
Difficult to take ARV in the presence of other people					1 (3%)	32 (97%)
Difficult to store the medication						33 (100%)

From the results obtained, as shown in Table 4.11, 33 adults participants indicated that they had benefited from the medication and 26 participants had experienced some side effects. In spite of the side effects they had all remained on the original regimen they had started with. Only one child had been enrolled in the study and had benefited from the medication.

4.6. Treatment outcome, i.e., immunological and virological responses

Table 4.12

					CD4 count and viral load (Medical records)		**Viral load from resistance testing lab**	**CD4 count and viral load (Medical records)**		**Follow-up**	
Patient number	**Regimen**	**Gender**	**Employment status**	**Marital status**	**First CD4 count**	**First Viral load (cps/ml)**	**Viral load**	**Second CD4 count**	**Second Viral load (cps/ml)**	**CD4 count**	**Viral load**
1	1a	F	Unemployed	Single	197	170000 cps/ml	120cps/ml		No second viral load	977	3590 cps/ml
2	1c	F	Unemployed	Single	169	18000 cps/ml	Lower than detectable limits	345	No second viral load	T/F	T/F
3	1a	F	Unemployed	Married	118	34000 cps/ml	Lower than detectable limits	414	3000 cps/ml	T/F	Lower than detectable limits T/f
4	1a	M	Unemployed	Married	143	330000 cps/ml	2600000 cps/ml	348	52000 cps/ml	T/F	T/F
5	1a	F	Unemployed	Single	13	54000 cps/ml	Lower than detectable limits		91000 cps/ml	Default	Default
6	1a	F	Unemployed	Married	86	28000 cps/ml	Lower than detectable limits	199	No second viral load	T/F	T/F
7	1a	M	Pensioner	Single	6	34000 cps/ml	Lower than detectable limits		No second viral load	Died	Died
8	1a	M	Unemployed	Married	47	7100 cps/ml	Lower than detectable limits	243	No second viral load	T/F	T/F

					CD4 count and viral load (Medical records)		Viral load from resistance testing lab	CD4 count and viral load (Medical records)		Follow-up	
Patient number	**Regimen**	**Gender**	**Employment status**	**Marital status**	**First CD4 count**	**First Viral load (cps/ml)**	**Viral load**	**Second CD4 count**	**Second Viral load (cps/ml)**	**CD4 count**	**Viral load**
9	1a	F	Unemployed	Single	30	93000 cps/ml	Lower than detectable limits	296	Lower than detectable limits		Lower than detectable limits T/F
10	1a	F	Unemployed	Separated	49	1500000 cps/ml	Lower than detectable limits	61	No second viral load	T/F	T/F
11	1a	M	Full-time	Single	182	189460 cps/ml	Lower than detectable limits	T/F	T/F	T/F	T/F
12	1a	F	Unemployed	Widowed	158	74000 cps/ml	Lower than detectable limits		No second viral load	T/F	T/F
13	1a	F	Part-time	Married	165	13000 cps/ml	Lower than detectable limits		No second viral load	405	Lower than detectable limits
14	1a	M	Unemployed	Single	103	180000 cps/ml	Lower than detectable limits	139	790 cps/ml	Died	Died
15	1a	F	Unemployed	Single	30	3100 cps/ml	Lower than detectable limits	Default	Default	Default	Default

					CD4 count and viral load (Medical records)		Viral load from resistance testing lab	CD4 count and viral load (Medical records)		Follow-up	
Patient number	**Regimen**	**Gender**	**Employment status**	**Marital status**	**First CD4 count**	**First Viral load (cps/ml)**	**Viral load**	**Second CD4 count**	**Second Viral load (cps/ml)**	**CD4 count**	**Viral load**
16	1a	F	Full-time	Single	30	35000 cps/ml	Lower than detectable limits	373	Lower than detectable limits	589 T/F	Lower than detectable limits T/F
17	1a	F	Unemployed	Married	74	95000 cps/ml	Lower than detectable limits	204	310 cps/ml	Died	Died
18	1a	F	Unemployed	Married	3	41000 cps/ml	Lower than detectable limits	32	24 cps/ml	180 T/F	T/F
19	1a	M	Unemployed	Married	44	11000 cps/ml	Lower than detectable limits	98	2500 cps/ml	148	Lower than detectable limits
20	1a	F	Unemployed	Divorce	14	300000 cps/ml	Lower than detectable limits	255	No second viral load	309	168 cps/ml T/F
21	2	M	Pensioner(government child grant)	Single	2330	32000 cps/ml	Lower than detectable limits		No second viral load	Died	Died
22	1a	F	Unemployed	Married	101	110000 cps/ml	Lower than detectable limits	115	550 cps/ml	Died	Died
23	1a	F	Self-employed	Single	168	430000 cps/ml	Lower than detectable limits	186	550 cps/ml	307	Lower than detectable limits T/F

					CD4 count and viral load (Medical records)		Viral load from resistance testing lab	CD4 count and viral load (Medical records)		Follow-up	
Patient number	**Regimen**	**Gender**	**Employment status**	**Marital status**	**First CD4 count**	**First Viral load (cps/ml)**	**Viral load**	**Second CD4 count**	**Second Viral load (cps/ml)**	**CD4 count**	**Viral load**
24	1a	F	Pensioner	Married	78	8700 cps/ml	Lower than detectable limits	112	Lower than detectable limits		Lower than detectable limits
25	1a	F	Unemployed	Married	139	56000 cps/ml	Lower than detectable limits	347	650 cps/ml	T/F	T/F
26	1a	M	Unemployed	Single	41	28000 cps/ml	Lower than detectable limits	312	T/F	T/F	T/F
27	1a	F	Unemployed	Married	173	5200 cps/ml	Lower than detectable limits	601	No second viral load	779	40 cps/ml T/F
28	1a	M	Unemployed	Married	105	67000 cps/ml	Lower than detectable limits	348	No second viral load	T/F	T/F
29	1a	F	Pensioner	Separated	113	4700 cps/ml	Lower than detectable limits	312	1200 cps/ml	T/F	363 cps/ml T/F
30	1a	M	Pensioner	Married	80	510000 cps/ml	Lower than detectable limits	342	2000 cps/ml	T/F	T/F

					CD4 count and viral load (Medical records)		Viral load from resistance testing lab	CD4 count and viral load (Medical records)		Follow-up	
Patient number	**Regimen**	**Gender**	**Employment status**	**Marital status**	**First CD4 count**	**First Viral load (cps/ml)**	**Viral load**	**Second CD4 count**	**Second Viral load (cps/ml)**	**CD4 count**	**Viral load**
31	1a	F	Unemployed	Single	18	37000 cps/ml	Lower than detectable limits		No second viral load	T/F	T/F
32	1a	F	Pensioner	Single	431	5000 cps/ml	Lower than detectable limits	197	Lower than detectable limits	T/F	Lower than detectable limits T/F
33	1a	F	Unemployed	Single	14	48000 cps/ml	Lower than detectable limits	291	67000 cps/ml	478	T/F
34	1b	F	Unemployed	Single	178	6000 cps/ml	Lower than detectable limits	141	7300 cps/ml	Default	Default

The first viral load for each patient in Table 4.12 was obtained from the hospital patient medical record and followed by the viral load from the Genotype testing laboratory. The genotyping tests were not done because the results of retested viral loads from genotype laboratory were lower than 1000 cps/ml. In spite of the fact that most of the second viral load was not done at the hospital, the majority of the cases, however, as can be seen from the table above, had an improvement in CD4 count, except for the female patient number 04 who seemed to have had a rebound of the viral load and she defaulted from the treatment. It would appear there were immunological improvement and low (11.76%) virological failures as can be seen from the table in the last column. Though the viral load determination were not done again at the hospital, it was reasonable to assume that the majority of the 34 patients whose blood samples were sent to the genotype laboratory had responded well immunologically to medication; including patient number 04, as he was transferred to the nearest clinic. The majority of patients were, at the time of study on stavudine, lamivudine and efavirenz regimen. Only one person, a single male pensioner, was on zidovudine, didanosine and lopinavir / ritonavir regimen.

Some of the reasons why some of the determinations were not done were that some patients either defaulted or had transferred to another clinic or hospital and thus could not be followed up.

CHAPTER 5
DISCUSSION

5.1 Introduction

This chapter interprets the results of the present study and discusses their significance to the level of ARV drug resistance observed at the Nkhensani District Hospital. Reasons why the Mopani District in which the hospital is located had highest HIV cases are given. The chapter also addresses why there were 4 regimens for first line treatment at the hospital and why patients were changed from regimen 1a to 1c.

5.2 Population characteristics

During the study period (April 2009 to March 2010), it was observed that the Nkhensani Hospital had 2656 patients on antiretroviral treatment (ART) according to the Limpopo Province Pharmaceutical Statistics (2009/2010). Of the five districts in Limpopo Province, the Mopani District had the highest number of patients on ART as shown in Figure 4.1, when compared to other districts in the province. This was mainly because the district has many farms and mines to where many people have migrated from other districts and provinces in South Africa, and also from neighbouring countries such Mozambique and Zimbabwe. It would be reasonable to assume that most of the people who migrated to the district were strong young adults prepared to work on the farms and the mine and, obviously still sexually active.

Most of the people who had migrated have settled on the farms and mine where living conditions were much better compared to squatter and patches of informal and illegal settlements in and around the study area, greater Giyani Local Municipality (GGLM). Informal settlements during the study were found around section 14C Dzumeri, Dzingidzingi, Giyani section F and between Gazabeef and Sekhunyani. The majority of the population regarded as locals in GGLM lived in areas that would be classified as rural. They were very poor and those who were employed worked on the farms and mines together with migrants. On these farms and mines they were many illegal alcohol outlets called *shebeens* and beer taverns found. These alcohol outlets had been reported to be responsible for many people leading reckless

lifestyles and the spread of HIV invariably through having contracted one type of sexually transmitted infection (STI). There is now evidence to support a relationship between alcohol abuse and increased risk of infection of HIV and other Sexually Transmitted infections. These observations were made in studies carried out by Parry (2005), Kalichman et al., (2008) and Avalos et al., (2009), and the systematic review of 42 studies that confirmed the association between alcohol consumption and STI (Cook, 2005). Consumption of alcohol is one of the pastime activities in many informal settlements where little or no awareness on HIV/AIDS have always been carried out mainly because of safety issues. The Mopani District health team, however, has always been aware of the public health consequences of people migrating to the district.

To cater for so many people who had migrated to the district and the majority of whom were leading one form or the other of irresponsible and reckless life, the district health team started a community social mobilization campaign around HIV/AIDS issues. This was called the HIV counselling and testing (HCT) campaign. They were going from door to door and conducting road shows, which included visiting mines and taxi ranks (District health plan (DHP), 2012/2013). The result of this campaign, it would appear, was that a large number of people were coming for voluntary counselling and testing at Nkhensani Hospital. This led to the number of people that would be initiated on ART also increasing and therefore the present study would have expected a larger sample of people willing to participate. As more patients are initiated on ART, more will inevitably develop HIV drug resistance (HIVDR), necessitating a switch to more expensive and often less tolerated second-line regimen.

Though the number of people who took part in the ARV drug resistance study was small, it would appear that the campaign had made some people more treatment literate and more educated about the virus and how it was transmitted. In this exercise, the Department of Health had partnered with the non-governmental organisation (NGO), Brothers for Life, which in its study, had also found out that of all districts in Limpopo Province, Mopani District had the highest prevalence of HIV infection (Brothers for Life, 2010).The main emphasis of the NGO was to encourage

men to come for voluntary counselling and testing, which appeared to have been working. From the hospital records more men were indeed coming to be tested.

The present study, though, demonstrated that women were more proactive than men in the HCT campaign. Several studies have shown that women are at high risk of contracting HIV due to the interplay of biological, economic and cultural factors (UNAIDS, 2006). Culturally men tend to assume a position of power and control over issues concerning sex and this minimises the amount of input and consent from women who are usually the ones who take care of the dying, sick family members (Mark, 2010). According to the UNAIDS report (2006), women's powerlessness, dependence and poverty tend to diminish women's ability to protect themselves from unsafe sex. A woman's choices are often limited by her inability to negotiate when or with whom to have sex or whether to use a condom. At the same time the society in general appears to accept that men can have sex before or outside marriage. The other limiting factor is the need for financial support from men (UNAIDS, 2006).

In addition, the physical difference between men and women has been reported to make it more likely that women will contract the virus from men than vice versa (Mark, 2010). In most African cultures, women are not free to refuse sex or to insist that their partners practise safe sex by using condoms (UNAIDS 2006). The African man, on the other hand, is expected to have multiple sexual partners. In some African societies, if not all, a woman, on the other hand is expected to have a relationship with or marry an older man (Mark, 2010). All this has contributed to the risk of women being infected with the HIV. A lot of HIV/AIDS preventive activities, therefore, for a long time, have been targeted at women, most of whom have low education, are unemployed, single and, in most cases, are the bread winners for some families, as seen from the present study. The results of the study indicated there was a high prevalence of STI among women of reproductive age on any day in the study district in KwaZulu/Natal. This is a result that could have been obtained in Mopani District. They also found that most STIs in the women remained untreated. Many studies have reported that girls have their sexual debut at a very young age (Lam, Marteleto and Ranchhod, 2009). This is usually with older men who do not know their HIV status and might be carrying an HIV strain resistant to ARV in use leading to possible transmission.

The study found that most patients were women aged between 30 to 35 years and of child-bearing age. In spite of what has been mentioned earlier these were women who were expected to be in some stable relationship. This agreed with the results of an epidemiological profile study that reported HIV prevalence being the highest among women of this age group (APP vote 2011/12–2014/15) and, as a result, most HIV/AIDS programmes, such as the PMTCT, targeted women of this age group. As a result, women in general have always been keen to seek medical attention and to utilize health care services than men (Klea and Bertakis 1999; and O'Connell 2011). In South Africa, most women get to know their status when they attend antenatal clinic because they are required to test for HIV as part of the PMTCT programme. Therefore, it was not surprising that more women on ARV treatment than men were willing to take part in the in-depth study of ARV drug resistance.

5.3 Treatment and Care

Many of the patients who were on ARVs as a result of the HCT campaign were found to live within 30 kilometres of Nkhensani Hospital. Distance from the hospital is an important factor to consider when trying to target the several early warning indicators (EWIs) that have now been internationally accepted to help monitoring the emergence HIVDR. Most of the patients during an in-depth interviews reported that the hospital had a friendly environment. The hospital, unfortunately, was found to be not able to support all the patients with Home Based Care. This would have negative impact on follow-ups because patients get lost. The WHO recommends that a loss to follow up, as an early warning indicator, should be less than 20% of patients on ARV (WHO 2010). The hospital however, has been training some of the patients' relatives on how to take care of their sick relative, the disease, its treatment and prevention in the hope that would also assist in on-time drug pick up. The WHO recommended target for on-time drug pick, as early warning indicator, has been set at greater than 90% of patients. To investigate how this was achieved at the hospital, it was important in the present study to covertly observe how the healthcare providers were interacting with such a large number of HIV/AIDS patients and their relatives. Health care settings are often the first point of contact for people with HIV/AIDS, but, unfortunately, they have been found to be a common locus for stigma and discrimination against people with or suspected HIV or AIDS (Mahendra and Gilborn,

2004). In every country, much more in South Africa, and every social setting since the disease was first identified, individuals who are assumed to be HIV-positive have been subject to a variety of negative reactions including physical and verbal abuse; loss of employment and rejection by families, spouses and friends. This would invariably have a negative impact on some of the HIVDR early warning indicators in individuals starting ART.

During the interviews with patients, the results obtained seemed to indicate that the majority of patients were generally satisfied with the information, care and respect they received from the health-care providers. In addition, the patients felt that the health care provider were friendly, encouraging during counselling and even provision of treatment.

Some of the patients, even though they lived within 30 kilometres of the hospital, complained about lack of money for transport and food. ARVs bioavailability can be affected by the presence or absence of food. There is still not much evidence that link food supplementation with reduced mortality or weight gain. However there are known food-ARV interactions. For example, didanosine, one of the drugs in regimen 2 at Nkhensani Hospital, toxicity may increase owing to increased bioavailability after a high fat meal. Therefore, the drug had to be taken on an empty stomach and then the patient would be allowed to eat food and supplements provided. If not provided, some patients had to feed themselves out of their pocket, which was always not possible, especially if there was no support from family. Some ARVs need to be consumed with food (Douglas, 2008). Infact all ARVs are better tolerated in adequately nourished patients. While ART should not be delayed for patients in deteriorating health, the regimen is harder for the food-insecure patients like those seen at Nkhensani Hospital. The study showed that some patients indeed complained about lack of support from their families, thus putting them in a difficult economic situation. According to several studies, being on ART had hidden costs (Van Wyk 2008; Kagee 2008; and Nachega 2009) even though it appeared that some patients in the study had coping mechanisms. It was important to see the level of appreciation of the healthcare providers because lack of coping mechanisms has been reported to contribute to poor adherence, an early-warning indicator for HIVDR. ART is life and its success depends on optimal adherence.

Furthermore, it has been reported that good patient and healthcare provider interaction is particularly important in chronic diseases (Addulla et al., 2006). It is said to enhance favourable patient outcomes such as understanding and adherence to medical regimens and overall satisfaction (Beck et al., 2002). In the present study when non-participatory observation was carried out, it could be assumed that there could have been a certain degree of bias, as all observations though supposedly covert, were made by the investigator, alone. Theoretically, two independent covert observers might have produced more reliable information. This is because, in such cases, the performance of the health care providers may be affected by the fact that someone is making observations positively or negatively regardless of how sensitively observations are made (Last, 2000). It is hoped that the bias was minimum in the present study in which a modified covert method previously described by Brink (2006) was used.

The observation, seemed to indicate that some patients who were supposedly on ART, mainly the combination of stavudine / lamuvidine / efavirenz, referred to as regimen 1a, were not properly using the medicine. These were the first line regimen during the study. Improper use of ARVs, in particular unnecessary treatment interruption, by patients is also one of the early warning indicators for HIVDR resistance. A number of viral mutations have been reportedly associated with stavudine, a thymidine analogue, reduced susceptibility. The predominant mutations are M41L, D67N, K70R, L210W, T215Y/F and K219O. zidovudine which was reserved for regimens 1c, 1d and 2, and stavudine have been reported to select for the same set of thymidine analogue resistance mutations (Kuritzkes, Bassett, Hazelwood, Barret, Rhodes, Young and Johnson, 2004). lamivudine therapy has been reported to rapidly select for the M184V mutation in regimens that are not fully suppressive. This mutation has been said to confer high-level resistance as well as reduction in susceptibility to abacavir. Conversely, the M184V mutation restore phenotypic susceptibility to zidovudine, indicating that the two-drug combination regimen may be particularly beneficial (Douglas, 2008). Be that as it may, there is need to monitor these mutations in an environment such as Nkhensani hospital where many people are going as the roll out is continuously scaled up with the use of fixed dose combinations (tenofovir / emtricitabine / efavirenz). There is always a

possibility of HIVDR transmission particularly among individuals who are HIV positive but unaware of their status (Sinha, Ahmad, Shekhar, Kumar, Dar, Samantaray, Sharma, Bhargava, Pandey, Mitsuyasu and Fahey, 2012) HIV drug-resistance transmission and its emergence in treatment would therefore need to be routinely assessed. Mathematical modelling and experience in resource-rich countries suggest HIVDR transmission will increase as ART coverage increase, but its rise will be limited initially (Bennett, 2006). Standardize surveillance of transmitted and treatment-associated HIVDR is critical to the success of ART expansion.

During the observation of the interaction between the patients and health care provider, it was firstly seen that patients either had not complied with the prescribed regimen or had forgotten to take their medication as prescribed in the previous consultation resulting in unnecessary treatment interruption. It was observed that they appeared to have lack of information about the disease and treatment which to an extent contradicted the belief about the benefits and success of the HCT campaign. There were some factors identified that could have contributed to what was observed. These included things such as the patient's perception of the illness experience based on cultural beliefs like HIV/AIDS being a curse from a family member or someone in the community. According to Cox and Stevenson (2003), patient's knowledge, attitude, beliefs about the condition and its management are important to understand when dealing with infection such as HIV. Beliefs and assumption about how HIV is contracted are based and rooted in culture and morality. Some African traditional healers see HIV/AIDS as a 'polluted disease'' because it is transmitted sexually (Kalichman and Simbayi, 2005). Religious doctrine and morality also see HIV/AIDS as 'punishment' from God for those infected and so they are said to deserve it (Cox and Stevenson, 2003). This will inevitably influence how the patient perceives his or her illness and the treatment used resulting in negative impact on HIVDR EWI targets that may have been set up at the ART site. These targets should be similar to those set by WHO as shown in Table 5.1

Table 5.1 Early-warning indicators of HIV drug resistance

HIV DR EWI	WHO Suggested target
First-line ART prescribing practice	100 %
Patients lost to follow-up during the first 12 months of ART	< 20%
Patient retention on first-line ART at 12 months	>70 %
On-time drug pick up	>90%
Drug supply continuity	100%

(WHO, 2010)

Generally, if there is a conflict with the prescribed regimen non-adherence is likely to occur as might have been the case with some of the patients in the covert observation study. Subsequent investigation also showed that medication was not properly used if any other healthcare provider and not the responsible pharmacist had been involved in the interaction after the ARVs had been dispensed. It can be argued that even though there were Standard Treatment Guidelines for HIV/AIDS at Nkhensani Hospital, their consistent use and patient counselling on their medication, may not have been reinforced. The involvement of everybody in the ARV supply chain at local level cannot be over emphasized.

Secondly, the observation seemed to suggest that some patients totally misunderstood the healthcare providers, in spite of the fact that most information, where possible, was given in Xitsonga, the language most patients were comfortable with. The study found that some medical terms were translated in Xitsonga for examples the immune system – *masochayamiri*, virus – *xitsongatsongwani* and side effects –*switandzaku.* This made patients, generally happy with the way they were treated as was seen with the in-depth interviews with those who participated in the ARV drug resistance study. The participants reported that the healthcare providers gave them the opportunity to ask questions. May be this applied only to the small sample of patients who willingly participated in the ARV drug resistance study. The rest of the patients, if indeed this was the case, would have not totally misunderstood the health care providers who were reported to be culturally sensitive. May be the

misunderstanding came about when some medical terms that did not have Xitsonga equivalent were translated or explained in a manner that ended up confusing the patients. This could explain what was observed in the covert observation study.

Some of the patients were found to still concurrently consult the community traditional healer which probably added to the confusion. In fact, it was also observed that some of these patients would only start taking ARV dispensed when they were in some discomfort (pain) and stop when any discomfort that may have been due to the infection disappeared. This resulted in treatment interruption. This meant that to certain extent ARVs use in the community was in accordance to traditional belief of disease causation. Proper adherence was also found to be interfered with, if there was lack of support from family and friends. Some patients were observed not even see the reason to change their sexual behaviour now that they were being treated for STIs and were also taking ARVs. One patient was heard bragging to the pharmacist saying "*I am a lion and I eat meat".* This implied that he was not going to change his behaviour simply because he was on ART and therefore continue to spread the disease in the community. What these observations suggested was that even though it would have appeared that there was no ARV drug resistance at Nkhensani Hospital the result of the present study has to be interpreted with caution. If certain men had such an attitude it must therefore mean there certainly was going to be some HIVDR transmission which would one day be quite prevalent if not monitored

Thirdly, it was observed that when patients returned for review, they were found to have taken home the wrong medicine dispensed by the health care provider they had interacted with, previously. How this could have happened was not clear. Further investigation during the study found that it was indeed a pharmacist who had made the mistake since post basic pharmacist assistant at the time were not allowed to dispense ARVs. The question that arose was if a pharmacist could make such a mistake in an environment where there is a shortage of pharmacists in the province, should post basic pharmacist assistants or professional nurses be allowed to dispense ARVs. Furthermore, these two categories should be allowed to dispense ARV drugs only when they have been trained on the drug regimen and the professional nurse should have been trained on dispensing course and nurse

initiative management antiretroviral treatment (NIMART). This correlate with the National Department of health policy (2007) which states that providing quality care to patients requires training skilled health workers and establishing a cultural that values lifelong learning. The result showed that some patients who had experience severe side effects from exposure to stavudine had been put on either regimen 1c, zidovudine / lamivudine / efavirenz or 1d in which efavirenz was replaced by nevirapine. This was still first line treatment according to the South African National Antiretroviral Treatment Guidelines. Furthermore, in 2010 the majority of patients at Nkhensani hospital HIV/AIDS treatment site were switched from stavudine to tenofovir, lamivudine and efavirenz or nevirapine. The substitution of drugs required someone who had been well trained in being able to pick up problems associated with a particular drug in a regimen especial tenofovir is contraindicated to patients with history of renal disease. Maybe, the above-mentioned cases of the patients taking wrong medicine home were not an everyday occurrence at Nkhensani Hospital. However, such cases emphasize the need for strict supervision at ART facility

It would, however, appear that there was due care being taken to monitor problems at the hospital. Other problems related to dispensing, which would have impact on the ARV drug supply in general and consumption in particular, were not being paid attention to, if the observation was anything to go by. It is, however, interesting to note that given such a situation during the studied period, only 11.76% virological failure and good immunological responses were found in the medical records examined and the sample tested for genotype resistance as seen table 4.12 of treatment outcome. This was in agreement with what has been reported in literature and also found at one of the referral hospital in the province (Lekoloane 2012).

5.4 Treatment outcome: immunological and virological response

The study indicates that patients who started taking antiretroviral treatment with a CD4 count of more than 100 cells/mm^3 had a significant influence on treatment outcome such as no drug resistance and adherence to the prescribed regimen during the first 6 months of treatment for example improvement on their virological and immunological, as compared to those who started with CD4 count such as 6 cells/mm^3 & 13 cells/mm^3 as a results that they ended up not improving from their

health due to psychological problem, depression and compliance to prescribed regimen, some patients passed away and others defaulted from the ARV treatment. These confirmed by the results from South African study done by Wouters, Van Dammeb & Van Loon (2009) who found that the baseline health (CD4 count) significantly influenced treatment outcomes during the first 6 months of ART.

The genotype determination depends on the viral load more than 1000 cps/ml to be tested, but in this study the genotype testing could not be done because when genotype laboratory retested the viral load from the blood samples that were sent, it was found that the viral load were lower than detectable limit, except for patients number 1 and 4 with viral load 120 and 2600 000 cps/ml, but when the second viral load from hospital patient medical record examined showed a good immunological response and low (11.76%) virological failure. Furthermore, the patient medical record were examined as a follow-up after second viral load, the results indicated a good virological improvement except patient number 1 who had virological failure and patients number 5 and 34 defaulted from the ARV treatment. This means that there is no ARV drug resistance at Nkhensani HIV/AIDs treatment site and it correlates with the study done by Pascal (2009) at Mankweng Hospital found that after the phylogenetic analysis the HIV-1 subtype was clade C. None of the samples investigated had mutations coding for resistance to the NRTIs. However one sample (1/17) from a female patient age 28 years had mutations to the NNRTI (A98G and K103N).

5.5 ARV Drug Supply

In spite of the fact that there was no high level of virological failure and no apparent ARV drug resistance at Nkhensani Hospital, the ART facility, like many other facilities in South Africa was found to run out of ARVs. For example, when a paediatric formulation was out of stock, adult formulation, especially capsules were opened and used for treating children. The results showed a stavudine 20 mg capsule meant for any child who was able to swallow the capsule, would be opened and mixed with 20 ml of water to give a 1 mg/ml concentration. The required millilitres would then be used and the rest thrown away. For example if the child weight was 10 kg, 10 millilitres would be used and the other 10 millilitres would be thrown away. This was approximately three bottles of 200 ml of stavudine

suspension which cost R29.034 per month and it was a wasteful and fruitless expenditure.

Luckily, there appeared to be not many children who were on ART at Nkhensani Hospital which made the building for forecasting assumptions easier when it came to paediatric patients. This was not the case, though when it came to the right strength of medication for adult which were also sometimes out of stock. For example, as was seen from the results that when 150 mg tablet of lamivudine was out of stock adults patients would be given a 300 mg tablet from another supplier. The instruction in terms of how to take the higher strength would obviously be different. This would cause confusion among patient who had become used to a particular strength and were now familiar and comfortable with a particular treatment plan or dosage schedule. It was interesting that a low rate of virological failure, good immunological responses and no ARV drug resistance were found given that interruption of ARV drug supply, another early warning indicator for ARV drug resistance, appeared to be a challenge at Nkhensani Hospital.

ARV drug supply, it would appear, is going to continue to be a challenge when scaling up ART to cater for all HIV/AIDS patients coming to Nkhensani Hospital. The WHO reports that despite the impressive growth, ART coverage of the population in need calculated on basis of 2010 guidelines is 37% for adult and 28% for children in eastern and in southern Africa 41% for adults and 32% for children (WHO 2010). As the HIV/AIDS cohort at the hospital increases procurement and supply chain management is going to have to improve. It is reasonable to assume that, there definitely is going to be HIVDR as the number of people on ART increases. This is going to lead to switching to second line drugs whose supply must be guaranteed. The fact that the hospital caters for people who live within reach will not matter, if the drugs are not available when needed.

Interrupted supply of ARV plus individual patients at risk of disease progression and death jeopardize the public health due to development of ARV drug resistance. It also hampers progress towards universal access to ART programme in the eyes of patients, the community and healthcare providers (Pasque et al., 2010). An increase and spread of HIV drug resistance, as previously mentioned will necessitate a

change of the first line ARV regimens and these are more expensive. Despite the fact that they were few patients on second line regimen, these drugs on average remain four times more expensive than first line regimen as seen in Table 4.10 and it indicate the price of ART per participant. These drugs use a large portion of the overall drug expenditure in the hospital, district and province. This has been shown to be the case by the WHO drug pricing catalogue (2009). The WHO reported that Brazil Ministry of Health spent 80% of its budget on imported drugs, even though this represented only a small proportion of drug used. In Limpopo Province and indeed the whole of South Africa in future ARV procurement is no longer to be centralized. Each hospital is now expected to procure its own medicines directly from the supplier. If this is not properly done stocks-outs are going to be the order of the day.

The stock-out of drugs at the hospital were reported to be due to weakness in the Procurement and Supply Management system (PSM) in the provincial Health Department. This was bound to change the situation observed with the small sample of patient that took part in the resistance study. The PSM situation at Nkhensani Hospital confirmed what was generally a country wide challenge, as reported in a review done by the UK Government's Department of International Development Rapid Response Health Fund (2010). The report commissioned by the Minister of Health after a serious shortage of ARVs in the Free State Province in 2000 noted that most provincial supply chain processes – including proactive stock control and distribution – needed improvement.

ARVs at Nkhensani Hospital were supplied from a central medical depot unlike in Malawi where they found that such a central medical store to be unreliable (Schouten et al., 2011). In Malawi, a push system and ceilings for the first-line ART drugs for facilities use a starter pack and continuation pack kits. The patient's outcome and ARV drug stocks are monitored on a monthly basis at the facility level. The procurement and distribution system outside central medical store is used because the Central Medical Store in Malawi, the equivalent to Depot in Limpopo that supplies drugs to Nkhensani Hospital, was found to be unreliable (Schouten et al., 2011). A World Bank mission advised that HIV/AIDS supplies with funding from the Global Fund to Fight AIDS and Tuberculosis and Malaria should be procured in a parallel PSM system (Mtonya and Chizimbi, 2006). The national scale-up of ART in

Malawi, like in South Africa, is based on the public health approach with principles and practice borrowed from the successful tuberculosis DOTS strategy (directly observed treatment short course). While that might be the case there should be a proper procurement plan since ARV drugs have several characteristics that affect their management. They pose unique challenges in quantification in order to avoid stock-outs, seen at Nkhensani Hospital. Stock-outs, like adherence which includes proper use, are an early warning indicator for resistance.

The following are ARV challenges at the facility level. They include, but not limited to

- ARV requires lifelong treatment. As previously mentioned, adherence to the treatment is therefore extremely important. Adherence is also an early warning indicator for drug resistance. The results obtained from the study seem to indicate that those who took part in the study were adhering to the prescribed regimen. Their viral load measurements were lower than detectable limit. The genotype determination that would have been carried in the study, had there been any ARV drug resistance at Nkhensani Hospital, depended on 1000 cps/ml of the virus. However, as the number of patients on ARV at Nkhensani Hospital increases, this could change because of a number of reasons among which include patient ARV treatment literacy. A high level of ARV treatment literacy among HIV/AIDS patients is important when ARV treatment guidelines are updated from time to time. During the period of the study there were indications that a revised ART guideline which was going to include fixed-dose combination, (FDC) a combination of three drugs tenofovir / emtricitabine / efavirenz was on the cards in the Department of Health.

- The second challenge is that a single ARV regimen requires a combination of at least three different drugs, often from different manufacturers, to be available concurrently. The planned introduction a fixed-dose combination would not make any difference because single drugs will still have to be required in case some patient experience some problem with a particular drug in the combination. Indeed there is going to be better adherence which may help in containing any development in resistance. However, single-drug

formulations must be available for substitution within the first and second line regimen because some patients may experience side effects or toxicity to individual drugs. Three completely different ARV drugs for second line regimens must be available for patients who develop resistance to the first line drugs. Luckily, this was not found to be the case at Nkhensani Hospital during the study period.

- The choice of regimen includes consideration of toxicity, side effects, cost of treatment and these factors are unique to individual patients. It appeared that at Nkhensani Hospital, data on these individual patient characteristics were not often being captured and therefore they could not effectively be utilized for forecasting. They created a ripe environment for ARV drug resistance to develop. Unpredictability of ARV requirements is particularly true for paediatric patients, for whom changes in weight vary significantly even within a population and body weight is a factor in calculating the dose of ARV treatment. Luckily, in the present study there were not many children enrolled. To avoid resistance emerging at Nkhensani Hospital among paediatric patients, however, forecasting demand consumption of paediatric ARVs is more complex than forecasting demand for adult ART. The level of detail required for a specific number of patients reflects the general complexity and sophistication required for diagnosis, care and treatment of paediatric ART patients (USAID 2009).

Key factors that ought to be taken into consideration to avoid development of resistance among paediatric patients whose number is certain to increase are as follows:

- Prescribing and dispensing of paediatric ARV drugs is complicated by the combined use of liquid, capsule, tablet, and FDC formulations. For example, at Nkhensani Hospital the following formulations such as suspension and capsules were often used.
- Formulations need to be changed and dosages need to be adjusted over time as the child grows.

- Adult ARV drug formulations can be used for children and may need to be cut or crushed to meet paediatric dosing requirement as was the case with stavudine when the paediatric formulation was in short supply.
- Paediatric adherence is going to be difficult because of the complicated dosing for children, large volumes, and the palatability of liquid formulations, and young children inability to swallow pills.
- The cost of paediatric formulations is relatively high when compared to adult formulations. For example, paediatric stavudine / lamivudine / nevirapine R92.313 and adult R52.616 per month.
- Some paediatric formulations are bulky liquids that require additional storage space and refrigeration. This could explain why paediatric formulations were always in short supply at Nkhensani Hospital.
- Paediatric ARV drugs are not packaged according to dosing schedules, which complicates prescribing and dispensing particularly if those dispensing are not well trained to pay particular attention to this factor. If some patients could go home with wrong medicine dispensed by the pharmacist, as was seen in the study, this could easily be a regular occurrence if other poorly trained healthcare providers were to be involved in dispensing paediatric formulations (Nkhensani Hospital advent events report, 2012). This is in agreement with national department of health policy states that advents events reporting identify errors and prevent recurrence.
- Paediatric doses are often reconstituted either at the service delivery levels or patient home and must be discarded after a certain period. In other words, they invariably have a short shelf life. The volume of use within that period is unpredictable and can vary from site to site. This would make it difficult for the depot at Seshego to forecast demand consumption which could have been the case, another reason for the shortage of paediatric ARV drugs at Nkhensani.

Treatment failure is difficult to predict and to diagnose because both viral load and immunological response are important to consider in the case of each individual patient. This would be extremely difficult to do with so many patients at Nkhensani Hospital. Lakoloane et al., (2012) following a study at a resource poor hospital

argues that where possible data on virological failure collected randomly should always be used to monitor how the ART programme is performing at each level.

At Nkhensani Hospital, it would appear they used the morbidity method that relied on translating numbers of patients and services into quantities of drugs used. What was lacking was the understanding of the context in which ARVs were prescribed as was shown by the stock-outs. The initial use of the ARVs is usually influenced by patients' previous ARV drug history, other co-existing infections or conditions, provider prescribing patterns, drug supply and other factors. Indeed, it would appear drugs within a regimen as shown in Table 4.5 were adjusted over time to capture the changing needs of the patients. These were changes necessitated by, for example, side effects and toxicities to individual drugs, changing body weight brought about by the disease like tuberculosis or the use of ARVs, ARV stock out, pregnancy and treatment failure. Luckily, if the results of the study are anything to go by, ARV drug resistance was not a factor that brought about the changes. This, however, could change at Nkhensani Hospital as the number of HIV/AIDS patients on ART increases over time. Forecasts of ARV requirement also need to account for patients on nonstandard ARV drug regimen who may enter the treatment programme already on ART, as well as patients who may be on individual salvage therapy. ARV drugs have varying dosages and formulations to accommodate individual needs. Table 4.9 shows medication that was available in as many as 4 formulations for adults and paediatric patients.

The other challenges that could alter the level of ARV drug resistance found at Nkhensani Hospital - though a small number of patients were enrolled in the study, is related to human resource, specifically issuing of wrong medication to the patients due to lack of skills. Educating health care providers and policy makers on the mechanism and potential consequence of HIV-1 drug resistance is a need and requires training. Without this there is both short term risks for the individual patient and long term risk of losing the benefits of ARV therapy in a population (WHO, 2000). Another challenge was that the health-care providers were not involved in the introduction of new guidelines (example training).

5.6 Conclusion

The study revealed that they were several factors that could contribute to early-warning indicator of ARV drug resistance at the Nkhensani Hospital ARV treatment site that needed to be monitored. These included, firstly, poor adherence to the prescribed regimen that was observed with some patients who were either not properly taking medication or not complying with the prescribed regimen or forgot to take their medication. Some of these patients also lacked coping mechanisms. Secondly, the other factor that could have contributed was the interruption of ARV drug supply which led to stock out of some ARV drugs resulting in some patients supplied with different ARV strengths and formulations. Interruption of drug supply will inevitably lead to poor adherence to the prescribed treatment regimen which is yet another factor that could contribute to ARV drug resistance. Even though the present study demonstrated that these factors could be regarded as important to early-warning indicators of ARV drug resistance, the results show that there were low rates (11.76%) of virological failure and good immunological responses, and no ARV drug resistance were found at Nkhensani Hospital HIV/AIDS treatment site. The rate of patient retention on first-line ART was good and therefore prescribing practices were congruent with national treatment guidelines. Another possible conclusion is that during the study period the sample used was religiously following the instructions given to the letter. In other words it could be concluded that the patients in the sample were on time to pick their medication; on time to keep clinic appointments and adhered to the treatment instructions given.

5.7 Limitations and challenges encountered during the study

The limitations of the study were the sample population who took part in the study was small when compared to the number of HIV/AIDS patients who were on ART at the time. It would be difficult to generalize the finding to the majority who declined to take part in the study. It is always difficult to enrol patients for such studies, particularly if they do not see immediate benefit to them. Those who take part in such studies are expected to do so voluntarily with little expectations. The genotype testing was not done because the viral load of the participants was lower than 1000 cps/ml which is a requirement from genotype laboratory. A more sensitive method which requires much less copies would have been useful for the study. HIVDR could have been present in people who were carrying much less copies

The challenges of the study were the participants who met the criteria were few and not all participated to the study and it was difficult to recruit children to participate in study due to several factors. For example parents or guardians were not willing to release their children to participate in the study because it involved collecting of blood, financial problem (money for transport). Another challenge was shortage of health care providers, because it was only one professional nurse who was collecting the blood sample for the participants and even for the patients who was attending the HIV/AIDS treatment site and the professional nurse complained of work load. It was then agreed that patients will be send for blood collection with the completed NHLS form.

5.8 Relevance of the study

The results of the study demonstrate that HIVDR EWI monitoring can be used to assess the strength, weaknesses of ART programmes and guide managers and implementers on strategies to resolve and strengthen identified areas of weakness. This is so especially now that hospitals are expected to purchase their own medicines and treatment initiation has been recommended to start at CD_4 count level of 350 cell/mm^3.

Even though ARV-based products for HIV prevention are not in use yet, the study demonstrates the need for routine assessment of prescribing practices, availability of and access to appropriate regimens for adults and children, ARV supply continuity and measure to prevent HIV transmission.

5.9 Recommendations for future studies

It is recommended that the study be carried out with a bigger sample of a population that is inclusive of all age groups, patients on ARV treatment and CD4 count below 350 cell/mm^3 at Nkhensani Hospital and also at similar sites across the country to determine ARV drug resistance. In addition there should be an aggressive uptake of monitoring of HIVDR EWIs at all treatment sites, now that we know what they are. If possible, resistance testing should be performed on all infants failing PMTCT when the mother has been exposed to antiretroviral therapy. If affordable, it should be performed to all regimen failure and treatment naive patient. All health care providers

(including lower categories) should be trained when implementing new regimens or guidelines such as fixed dose combination tenofovir / emtricitabine / efavirenz. This will strengthen the knowledge of the health care provider. All patients should undergo adherence counselling before switching ARV drugs from single dose to FDC, in order to strengthen patient adherence to ARV treatment. The Department of Health should receive the results in order to improve the quality of service delivery.

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APPENDICES

Appendix A: Consent form

CONSENT FORM

Name of the study: THE LEVEL OF ARV DRUG RESISTANCE AT NKHENSANI HOSPITAL HIV/AIDS TREATMENT SITE

I have read the information/ heard the aim and objectives of the proposed study and was provided the opportunity to ask questions and given adequate time to re-think the issue. The aim and objectives of the study are sufficiently clear to me. I have not been pressurized to participate in any way. I understand that participation in this study is completely voluntary and that I may withdraw from it at any time without supplying reasons. This will have to influence the care that I receive from ARV treatment site. I know that this study has been approved by the research, ethics publication committee of the University of Limpopo (Turf loop campus), and lam fully aware that the results of this study will be used for scientific purpose and may be published. I agree to this, provide my privacy is guaranteed.

I hereby give written consent to participate in this study.

Name of the patient Signature of the patient

_____ _____

Place Date

_____ _____

Statement by the researchers

I provide verbal and written information regarding this study.

I agree to answer any future question concerning the study as best as I am able. I will adhere to the approved protocol.

Name of the researcher Signature

MACHETHE K.F _____

Place Date

_____ _____

Appendix B: Questionnaire

A. Demographic

1. Gender

Male ☐ Female ☐

2. Age

1-5 ☐ 5-10 ☐ 10-15 ☐

15-20 ☐ 20-25 ☐ 25-30 ☐

30-35 ☐ 35-40 ☐ 40-45 ☐

3. Level of education

Primary ☐ Secondary ☐ Tertiary ☐

Other (please specify) _____

4. Marital status

Single ☐ Married ☐ Divorce ☐

Separated ☐ Widowed ☐

5. Home language

Xitsonga ☐ Sepedi ☐ Venda ☐

Other (please specify) _____

6. Employment

Self employed ☐ Full-time ☐ Part-time ☐

Unemployed ☐ Pensioner (government grant) ☐

7. Are you a bread winner?

Yes ☐ No ☐

If yes, how many people do you support? _____

8. What is your income level? _____

B. interpersonal Management

Scale: 1 Strong disagree

2. Disagree.
3. Neutral.
4. Agree.
5. Strongly agree.

Attitude of health service providers was encouraging.	1	2	3	4	5
The health service providers were friendly.	1	2	3	4	5
The health service providers respected me.	1	2	3	4	5
The health service providers explained everything about my condition and management in language I understand.	1	2	3	4	5
My cultural values were respected.	1	2	3	4	5
I was given the opportunity to ask questions.	1	2	3	4	5
They made me aware of my rights, including the right to confidentiality.	1	2	3	4	5
My privacy was respected.	1	2	3	4	5

C. Accessibility/ convenience

It was easy to reschedule my visits after the first appointment.	1	2	3	4	5
The time taken to be seen was acceptable.	1	2	3	4	5
It is easy for me to reach the hospital.	1	2	3	4	5
I'm satisfied with the periodicity of my scheduled session.	1	2	3	4	5
The treatments sessions always start on time.	1	2	3	4	5

D. Financial aspects

1. Are you staying near the hospital?

Yes ☐ No ☐

2. How much do you pay for transport to visit the hospital?_____

3. How do you get the money? _____

E. Physical environment

I'm satisfied with the HIV/AIDS treatment site.	1	2	3	4	5
HIV/AIDS treatment site is neat and clean.	1	2	3	4	5
I am comfortable with the room attended my session.	1	2	3	4	5
The space was enough.	1	2	3	4	5

F. Treatment

1. What was your CD4 count, when you started taking the ARV treatment?

0-50cell/mm^3 ☐ 50-100cell/mm^3 ☐ 100-150cell/mm^3 ☐

150-200cell/mm^3 ☐

2. Which regimen are you on?

_____

3. Did the health care provider change your regimen?

Yes ☐ No ☐

If yes, why?

_____

4. What were you suffering from before starting ART?

_____

_____

5. Have you improved, remained the same or deteriorated since starting ART?

Improved ☐ Same ☐ Deteriorated ☐

6. Have you experienced side effects?

Yes ☐ No ☐

If yes, which ones?

_____

_____

7. Are you taking the ARV treatment, while you are on other treatment?

Yes ☐ No ☐

If yes, specify

_____

8. Are you also on anti-TB treatment?

Yes ☐ No ☐

9. Do you have any other disease except TB?

Yes ☐ No ☐

If yes, state them_____

_____

10. What helps you remember to take your medication?

Please explain_____

11. How often do you take your medication?

Once per day ☐ Twice per day ☐ Three times per day ☐

Four times per day ☐

12. Do you miss any drug doses?

Yes ☐ No ☐

If yes, why?

_____

13. Do you take your medications as instructed?

Yes ☐ No ☐

If no, what makes you not?

_____

_____

14. Do you benefit from ARV drugs?

Yes ☐ ☐

15. It is difficult for you to measure the correct dose for your child?

Yes ☐ No ☐

If yes, why?

_____

_____

16. How does the ART formulation taste?

Sour ☐ Bitter ☐ Sweet ☐

17. It is difficult for to store your ARV treatment?

Yes ☐ No ☐

If yes, why?

_____

_____

18. It is difficult for to take your ARV treatment when you're with your friends or family members?

Yes ☐ No ☐

If yes, why?

_____

Any other comments

_____

_____

_____

Appendix C: Request for permission to conduct research: Nkhensani Hospital.

LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

Department of Health and Social Development

NKHENSANI HOSPITAL
Tel: 015 812 3251/2
Fax: 015 812 2461

To: Chief Executive Officer
Date: 29/01/2007
From: Machethe K.F

RE: REQUESITION FOR USING HOSPITAL PATIENT RECORDS TO COLLECT DATA FOR MY RESEARCH.

I am hereby requesting to use the hospital information records department to collect data for my research. The topic is ARV drug resistance at Nkhensani hospital HIV/AIDS treatment site. The results of this research will be made available to the department of health and social development.

I hope my requisition will be considered.

Kind regard

Machethe K.F
.....

Appendix D: Request for permission to conduct research: Department of Health.

P.O. Box 481
Masingita
0832
03 January 2008

The Assistant Director
Department of Health
Private bag X9302
Polokwane
0700

Dear Sir/Madam

Re: APPLICATION FOR PERMISSION TO CONDUCT RESEARCH.

I hereby request for a permission to conduct research at Nkhensani hospital. The topic is "ARV level of drug resistance at Nkhensani hospital HIV/AIDS treatment site". I strongly believe that the results from the study will benefit the hospital, district and province as whole.

Attached, please find the research proposal for your information and clearing certificate from the research committee has not yet been released but will be provided to you as soon as is available.

Kind regard

.....
Machethe K.F (Miss)

Appendix E: Approval Certificate.

LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA
DEPARTMENT OF HEALTH AND SOCIAL DEVELOPMENT

ETHICS COMMITTEE
CLEARANCE CERTIFICATE
UNIVERSITY OF LIMPOPO
Polokwane/Mankweng Hospital Complex

PROJECT NUMBER: 053/2008

TITLE: ARV Level of drug Resistance at Nkhensani Hospital HIV/AIDS Treatment Site.

RESEARCHER: Kedibone Fortunate Machethe

ALL PARTICIPANTS: N/A

Department of Pharmacy
Faculty of Health Science, University of Limpopo

Supervisor: Prof N.Z. Nyazema
Co supervisor: Prof. Y.M. Dambisya

Date considered: 25.11.2008
Decision of Committee: Recommended for Approval

Date: 26.11.2008

Prof. A.J. Mbokazi

Chairman of Pietersburg Mankweng
Hospital Complex Ethics Committee

Note: The budget for research has to be considered separately. Ethics Committee is not providing any funds for projects.

1