#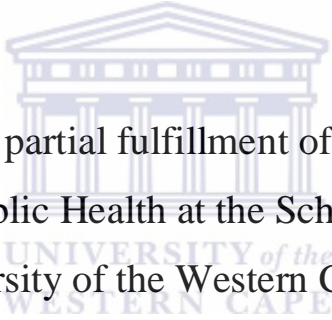 DRUG MUTATION PATTERNS AND RISK FACTORS ASSOCIATED WITH PATIENTS FAILING FIRST-LINE ANTIRETROVIRAL THERAPY REGIMEN IN OSHIKOTO AND OSHANA REGIONS, NAMIBIA.

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KEYWORDS

HIV

Drug resistance

Genotyping

HIV subtypes

Side effects

First-line regimen

Treatment failure

Risk factors

Namibia

LIST OF ABBREVIATIONS

ART   Antiretroviral Therapy

ARV   Antiretroviral

CD4   Cluster of Differentiation 4

PMTCT   Prevention of Mother-to-Child Transmission

TB   Tuberculosis

UNAIDS   United Nations Programme on HIV/AIDS

WHO   World Health Organization

DECLARATION

I declare that, the thesis on Drug mutation patterns and risk factors associated with patients failing first-line antiretroviral therapy regimen in Oshikoto and Oshana regions, Namibia, it has not been submitted before for any degree or examination in any other university, and that all the sources I have used have been indicated and acknowledged as complete references.

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Signed: _____   Date: 8 December 2015.

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ACKNOWLEDGEMENTS

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ABSTRACT

HIV/AIDS is a major health problem in Namibia with HIV prevalence estimated at 18.2% among pregnant women. Antiretroviral therapy (ART) was introduced in the public sector in 2003 and ART roll out was expanded throughout the country in the subsequent years. There are 221 ART sites in Namibia which include 34 district hospitals and 187 outreach service points. Currently there are 127,486 patients registered on ART in Namibia. However, there have been cases of patients experiencing treatment failure. The treatment failure can give rise to the emergence of HIV drug resistance. Genotyping information from patients with treatment failure can be valuable for tracking the dominant mutations conferring HIV drug resistance. However, HIV genotyping is not routinely available in Namibia due to cost. It is essential to determine the risk factors associated with development of HIV drug resistance so that these factors can be addressed. The aim of the current study was to describe HIV drug resistance mutations and the risk factors associated with HIV drug resistance among patients failing first- line ART regimen in Oshikoto and Oshana regions in Namibia.

The *case-control* study design was used to collect data from cases who were being suspected of treatment failure to the firstóline regimen in Oshikoto and Oshana regions in Namibia. The demographic, clinical and genotype information was collected from patient records.

Out of 168 cases, 97 cases were eligible for this study and were matched with 105 controls. The mean age was 44.8 (±13.2) years for controls and 43.3 (±13.3) years for cases. Cases from Oshana and Oshikoto regions harboured 63% and 71% respectively for nucleoside reverse transcriptase inhibitors mutations with the dominant mutation being M184V/I. Sixty-eight percent (68%) and 76% respectively harboured mutations for non-nucleoside reverse transcriptase inhibitors with dominant mutation being K103N. Missed appointments, initiating inappropriate first-line regimen and adverse events or side effects were identified as risk factors for virological failure with odd ratios (OR) of 21.58 (95% CI 6.50 -71.59); 11.70 (95% CI 1.69 - 80.99) and 7.17 (95% CI 1.89 -27.22) respectively.

Patients failing the first-line regimen need to be genotyped to assess the development of HIV drug resistance. The patients initiating ART should be educated on impacts of missing clinical

appointments and adverse events of the drugs in order to prevent the emergence of drug resistance.

CHAPTER 1: INTRODUCTION

1.1. Background

HIV/AIDS is a global health problem and all efforts are concentrating on how to combat it. The World Health Organization (WHO) and the Joint United Nations Programme on HIV/AIDS (UNAIDS) launched 3x5 initiative with the strategy to put 3 million eligible people on antiretroviral therapy (ART) by 2005 (WHO & UNAIDS, 2003). In another effort, the President's Emergency Plan for AIDS Relief was launched in 2003 to support countries which were heavily affected by epidemic (especially in sub-Saharan Africa, the Caribbean and Asia). The main strategy was to treat 2 million patients and prevent 7 million new HIV infections for the next five years. It was estimated that by 2010, 6.6 million adults and children in low and middle-income countries were put on ART (WHO, 2011).

The treatment scale up follows the public health approach of using the standardized and simplified treatment regimens that are consistent with international standards. The introduction of ART has decreased the morbidity/ mortality due to AIDS (Cavalcanti *et al.,* 2007; Hamers *et al.*, 2012). However, it has been observed that the treatment with antiretroviral drugs can be accompanied by emergence of HIV drug resistance (Bennett *et al.,* 2012; van Zyl *et al.,* 2011; Hamers *et al.*, 2012). This is due to the nature of the reverse transcriptase which is error-prone. The high rate of mutation can take place in the presence of drug's selective pressure even if the correct regimen is used. The clinical monitoring without genotyping for HIV treatment failure has led to the accumulation of HIV drug resistance in resource limiting settings (van Zyl *et al.,* 2011; Hamers *et al.*, 2012). This accumulation of drug resistance treatment has presented a challenge to the subsequent treatment for the second-line drugs that can be more toxic than first-line drugs (Bennett *et al.,* 2012).

Since each mutation is conferred by specific drug or by specific class of drugs, several studies were conducted to correlate between genotyping and clinical outcome to new treatment regimen being initiated. Studies were conducted to determine the impact of specific nucleoside reverse transcriptase inhibitor drugs in treatment experienced patients when certain mutations were present at baseline (Izopet *et al.,* 1999; Shulman *et al.*, 2001; Yerly *et al.*, 1996; Montaner *et al.,*

2000; Calvez *et al.*, 2002; Japour *et al.*, 1995). It was found that the combination of thymidine analogue mutations T215Y, M41L and 210W at baseline in patients who were exposed to zidovudine showed poor viral load suppression when they were treated with stavudine and didanosine or stavudine and lamivudine. These studies showed the cross-resistance among the drugs that confer thymidine analogue mutations. Thus, the patients who are failing first-line regimen should be genotyped before initiating the second-line regimen to determine the combination of mutations that are present. Genotyping the patients that are failing treatment can give guidance on making decisions about the combination of drugs that can be used in the subsequent treatment.

In other studies involving didanosine in nucleoside reverse transcriptase inhibitors experienced patients with or without the M184V mutation at baseline showed that there was good viral load suppression (Sproat *et al.,* 2005; Winters *et al*., 2003). The mutation, M184V, is conferred by nucleoside reverse transcriptase inhibitor lamivudine. These studies showed that didanosine continue suppressing the viruses with M184V mutation and presence of M184V does not preclude the use of didanosine in nucleoside reverse transcriptase inhibitor experienced patients. In a similar study conducted to determine virological response to etravirine in non-nucleoside reverse transcriptase inhibitor treatment experienced patients with K103N mutation at baseline. It was found that treatment with etravirine showed a good viral load suppression (Marcelin *et al.*, 2010). Thus, K103N did not have any effect on activity of etravirine. This study demonstrated that etravirine can be used in patients with mutation K103N which is conferred by nevirapine.

Further studies were conducted in non-nucleoside reverse transcriptase inhibitor experienced patients to determine the effects of pre-existing non-nucleoside reverse transcriptase inhibitor mutations in response to the first-line regimen (Kuritzkes *et al*., 2008; Lockman *et al*., 2010). It was found that the patients with pre-existing non-nucleoside reverse transcriptase inhibitor mutations experienced virological failure in comparison to patients without pre-existing mutations. These studies demonstrated that it is vital to perform genotyping testing before initiation of treatment if any patient has prior exposure to ARV drugs.

Summarily, the studies above have demonstrated that there is need of genotyping the patients who are being suspected of treatment failure of first-line regimen. This must be done before they are switched to the second-line regimen to avoid accumulation of HIV drug resistance. These

studies also prove that the selection of second-line drugs can be done based on mutation profile of patients to specific drugs on which they were initiated on first-line regimen. It was also shown that patients with prior known resistance to drugs need to be genotyped before continuing with subsequent treatment.

Most of the genotyping data available have been on subtype B and not much information on non-subtype Bs (Hamers *et al.*, 2012). Subtype B is commonly found in developed countries where ART first became available (Wainberg and Brenner, 2010). Due to genetic differences among the subtypes there are differential profiles of resistance being conferred upon exposure to the ARV drugs. This genetic diversity among subtypes dictates what type of resistance may arise and at what rate the resistance may develop or emerge. This may affect the degree of cross-resistance within a class of ARV that may affect the treatment outcome.

In order to tackle the challenges of development of HIV drug resistance, the WHO has developed a global strategy for prevention and assessment of HIV drug resistance that recommends the monitoring of early warning indicators (Sigaloff *et al.,* 2012; Jordan *et al.,* 2012). The early warning indicators can be used to assess factors that are associated with HIV drug resistance. The early warning indicators include: the ARV prescribing practices, patient's retention on first-line regimen at 12 months, patients lost to follow up at 12 months, on-time ARV drugs pick up, on-time for clinical appointment, ARV drugs supply continuity and viral load suppression (Jordan *et al.,* 2012).

HIV/AIDS is also a major health problem in Namibia. In 2012 HIV prevalence is estimated as 18.2% among pregnant women (MOHSS, 2012). Antiretroviral therapy was introduced in Namibia in the private sector in 1997 and in the public sector in 2003. In the public sector the ART is provided free of charge. Since 2003, the ART roll out was expanded throughout the country. There are 221 ART sites in Namibia which include 34 district hospitals and 187 outreach service points (MOHSS, 2010a). By the end of 2006 there were 33,591 individuals on treatment. In 2010, the Ministry of Health set the national target of achieving 95% ART coverage rates for eligible patients by 2015/16. Currently there are 127,486 patients registered on ART in Namibia (MOHSS, 2015).

The current Namibian ART guidelines recommends zidovudine + lamivudine+ nevirapine, or stavudine + lamivudine+ nevirapine, or zidovudine + lamivudine +efavirenz, or stavudine+ lamivudine+ efavirenz, or tenofovir + emtricitabine/ lamivudine+ efavirenz as the first-line ART regimen (MOHSS, 2010b; MOHSS, 2014). To be eligible for ART, HIV patients should have CD4 counts equal to or less than 350 cells/µl. After initiation on ART, patients are monitored by determining the viral load at six months and also in case of suspicion of treatment failure. In addition the CD4 is monitored every six months. In Namibia, treatment failure is defined as viral load more than 1000 copies/ml on two consecutive visits. The treatment failure may give rise to the emergence of HIV drug resistance (Bennett *et al.,* 2012).

1.2. Monitoring of early warning indicators in Namibia

In 2009 Ministry of Health and Social Services in Namibia piloted five WHO early warning indicators from nine selected ART sites in the public sector which had electronic dispensing pharmacy tools (Hong *et al.,* 2010). The early warning indicators identify the factors that affect the individual health facilities and programs that are potentially associated with emergence of HIV drug resistance. The early warning indicators abstracted were: ARV prescribing practices, patientøs retention on first-line regimen at 12 months, patients lost to follow up at 12 months, on-time ARV drugs pick up and ARV drugs supply continuity. These early warning indicators were abstracted from patient medical and pharmacy records.

The pilot study showed that all nine sites met the target of 100 % for prescribing practice of the appropriate first-line regimen. For patients lost to follow up at 12 months, 8 (89%) out 9 sites met the target of 20% or less that need to be achieved according to WHO. However it was found that 20.8% of patients had a period of absence without documented ART during their first year of treatment. For patientøs retention on first-line regimen at 12 months, it was found that 6 (67%) out 9 achieved a target of 0% of patients that switched to second-line regimen in 12 months period upon initiation. The data obtained from on-time ARV drugs pick up and drugs supply continuity was not satisfactory due to the patientsø medical and pharmacy records which were incomplete.

1.3. Problem statement

The risk factors associated with development of HIV drug resistance are required to be identified at patientsø individual level in Namibia. The individual early warning indicators would enable the health sector in Namibia to identify the factors that can lead to emergence of HIV drug resistance. Since the abstraction of early warning indicators did not involve the HIV genotyping, based on the information obtained from early warning indicators the individual patients from specific sites can be genotyped. This information can be used to determine the mutations that confer resistance to specific drugs and their respective subtypes. Currently no data is available on circulating subtypes in Namibia.

The emergence of HIV drug resistance presents a challenge to clinicians and ART programs as it requires the second-line therapy which could be costly and more toxic than first-line drugs. It is therefore critical to monitor patients on ART being suspected for developing drug resistance by genotyping (Bennett *et al.,* 2012). Routine genotyping of patients suspected of failing treatment can provide information to guide clinicians for better management of patients. The Namibian HIV drug resistance working group believes that genotyping of patients suspected of failing treatment will provide valuable information (MOHSS, 2013). This information on genotyping can be used in decision making on maintaining the effectiveness of the first-line ART. Genotyping information can also be valuable data for tracking the dominant mutations conferring HIV drug resistance and circulating virus subtypes at the population level. This data is valuable because it can guide policy-makers on the selection of ART regimen for second-line therapy and selection of prophylaxis. This can help to ascertain the change of treatment regimen to more effective and durable drugs, and consequently prevent the emerging of HIV drug resistance.

1.4. Outline of thesis

Chapter 2 covers the literature review to determine what is known about HIV drug resistance mutation pattern. This chapter also reviews the risk factors that contribute to emergence of HIV drug resistance and public health implications.

Chapter 3 covers the aim, objectives and methodology of the study. This chapter covers the study designs, the description of the population that was sampled, characteristics of the respondents, strategies for sampling and data collection tools. Data analysis, validity, reliability and ethics considerations are also covered in this chapter.

Chapter 4 covers the reporting of the obtained results from the study.

Chapter 5 covers the discussions of the significance of the obtained results.

Chapter 6 covers the main conclusions drawn from the study. Chapter 6 also outlines the main recommendations that were drawn from the study.

CHAPTER 2: LITERATURE REVIEW

2.1. Outline of literature review

This review will outline the pattern of specific HIV drug resistance mutations that are conferred by different classes of drugs which are used in the first-line regimen, subtype's distribution, factors that contribute to emergence of HIV drug resistance and WHO global strategy to reduce and prevent the emergence of HIV drug resistance.

Several studies that were conducted in different settings will be analysed to determine the frequency of mutations to the classes of antiretroviral that compose the first-line regimen. The prevalence of different mutations to specific drugs will be analysed to determine the dominant mutations. Dominant mutations will be analysed to determine their impacts on patients' management. This information can give guidance on the choice of drugs that can be used for the second-line regimens.

The studies from literature will be analysed to determine if the information on the circulating subtypes is required to make decision about the classes of drugs that can be used. The studies on subtypes from different settings will be analysed to determine the distribution of subtypes in different geographical areas. The mutation patterns from different subtypes will be analysed to determine if they have impacts on patient treatment.

The contributing factors that could lead to emergence of HIV drug resistance such as the ARV drugs and regimen factors, patient factors and health systems factors will be analyzed in this review. The analysis of these factors can provide information to policy makers about the necessary interventions that need to be implemented in order to reduce the emergence of HIV drug resistance. These factors will be analyzed to determine how they could lead to the emergence of drug resistance.

This review will analyze the WHO global strategy for prevention and minimizing the emergence of HIV drug resistance. The WHO strategy's three elements which are: the monitoring of early warning indicators, surveillance of acquired HIV drug resistance in population on ART and

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surveillance of transmitted HIV drug resistance in recently infected population will be critically reviewed. The strategy can be used for optimal selection of ARV drugs on population-basis and provides information for making program adjustments and optimize the patientsø quality care.

2.2. HIV drug resistance patterns

The firstóline regimen is consisted of two classes of drugs: the nucleoside reverse transcriptase inhibitors and non-nucleoside reverse transcriptase inhibitors. These drugs target the *POL* gene that codes for reverse transcriptase enzyme. Nucleoside reverse transcriptase inhibitors drugs are lamivudine, emtricitabine, abacavir, didanosine, tenofovir, stavudine and zidovudine. Abacavir, didanosine, tenofovir, stavudine and zidovudine are regarded as thymidine analogues. Non-nucleoside reverse transcriptase inhibitors drugs are nevirapine, efavirenz, etravirine and rilpivirine. The standard first-line regimen contains two nucleoside reverse transcriptase inhibitors and one non-nucleoside reverse transcriptase inhibitor backbone (Bennet *et al.*, 2008, Jordan *et al.*, 2012). Even though there is standardized firstóline regimen treatment with antiretroviral drugs it can be accompanied by emergence of HIV drug resistance (Bennett *et al.,* 2012; van Zyl *et al.,* 2011; Hamers *et al.*, 2012). The drugs become ineffective to suppress the HIV replication. This is due to the nature of the reverse transcriptase which is error-prone and a high rate of mutation can take place in presence of the drugs selective pressure even if the correct regimen is used. Mutations are errors that take place when reverse transcriptase is coping HIV genome during replication. Each mutation is conferred by specific drug.

Several studies have been conducted in different countries to determine the frequency or prevalence of mutations to specific antiretroviral drugs (Cavalcanti *et al.,* 2007; van Zyl *et al.,* 2011; Sigaloff *et al.,* 2012; Hamers *et al.*, 2012; Reynolds *et al.,* 2012). The retrospective description study conducted in Brazil found that nucleoside reverse transcriptase inhibitors mutations occurred at codon 184 (66%), while the thymidine analogue mutations such as M41L 67N, 70R, T210W, T215F/Y and 219Q/E/N were also observed. M184V/I is mainly conferred by lamivudine. Thymidine analogue mutations are mainly conferred by abacavir, didanosine, tenofovir, stavudine and zidovudine. In non-nucleoside reverse transcriptase inhibitors the mutations were observed at codon 103 (62%), 190(38.7%) and 181 (29.2%) (Cavalcanti *et al.,* 2007). The high resistance in codon 103 for non-nucleoside reverse transcriptase inhibitors

mutation was due to the use of, efavirenz and nevirapine and are usually used in the first line regime in Brazil, while 181 occurred due to the use of nevirapine.

Studies conducted in South Africa, (van Zyl *et al.,* 2011; Sigaloff *et al.,* 2012) and in 13 clinical sites in 6 sub-Saharan African countries (Hamers *et al.*, 2012) showed that for nucleoside reverse transcriptase inhibitors class, M184V was a dominant mutation followed by thymidine analogue mutations, K65R and Q151M. There are two types of thymidine analogue mutation pathways: thymidine analogue mutation-1 and thymidine analogue mutation-2. Thymidine analogue mutation-1 mutations included M41L and T215F/Y, while thymidine analogue mutation-2 mutations included D67N, K219E and K70R. The most frequent thymidine analogue mutation observed was D67N an indication that most patients favoured the thymidine analogue mutation -2 pathway (Hamers *et al.*, 2012). Overall these studies showed that patients receiving containing nucleoside reverse transcriptase inhibitors for extended period have a risk of developing K65R, thymidine analogue mutations and Q151M mutations that can increase the nucleoside reverse transcriptase inhibitors broad cross-resistance which present a challenge to the subsequent second-line treatment (Sigaloff *et al.,* 2012; Hamers *et al.*, 2012). It had been proven that the K65R and thymidine analogue mutations conferred a cross-resistance to all nucleoside reverse transcriptase inhibitors except AZT (Hamers *et al.*, 2012). These studies showed for non-nucleoside reverse transcriptase inhibitors class that K103N mutation was the most frequent followed by V106M, Y181C and G190A (van Zyl *et al.,* 2011; Sigaloff *et al.,* 2012; Hamers *et al.*, 2012). In addition it was found that patients who were on the regimen containing efavirenz were more likely to have K103N, V106M and P225H mutations than those on nevirapine containing regimen that harboured Y181C mutation (van Zyl *et al.,* 2011). These results showed the cross-resistance that occurred between nevirapine and efavirenz. Thus, the genotyping is required to confirm that the virological failure observed is due to drug resistance mutations.

A cohort study was conducted in Uganda to determine the pattern and frequency of nucleoside reverse transcriptase inhibitors resistance among patients who had showed the virological failure to the first-line regimen (Reynolds *et al.,* 2012). It was found that 29 (81%) of 36 patients had resistance to lamivudine, and one of the participants was resistant to lamivudine at the baseline (Reynolds *et al.,* 2012). This study had demonstrated that the detection of resistance among drug naïve patients could compromise the scaling up programs especially where the monitoring and

surveillance are not conducted routinely to detect the emergence of drug resistance (Reynolds *et al.,* 2012). The presence of resistance in drug naïve patients can lead to the accumulation of drug resistance at population level if it is not detected early.

2.3. Resistance and subtype diversity

HIV-1 is divided into three groups: M, N and O. Group M is further sub-divided into groups called subtypes. Available subtypes are: A, B, C, D, F, G, H, J, K and circulating recombinant forms (CRF) (Peeters *et al.,* 2013). HIV subtypes are genetically different from each other and can influence the drug resistance pattern (Rhee *et al.,* 2015). Thus, in order to have an effective therapy the information about the circulating subtypes is required to make decision about the classes of drugs that can be used.

Studies from different settings have shown that certain mutations are more dominant in certain subtypes that could have impacts on patient treatment outcome if treated with certain drugs (Cavalcanti *et al.,* 2007; van Zyl *et al.,* 2011; Hamers *et al.*, 2012). In the study conducted in sub-Saharan African countries had shown that there was more K65R (17.4%) in subtype C than in non-subtype C (6.9%) (Hamers *et al.*, 2012). Similar results were obtained from a study conducted in South Africa where it was found that patients with subtype C are more likely to develop mutation K65R upon exposure to stavudine or didanosine than the subtype B (van Zyl *et al.,* 2011). These studies have demonstrated that prolonging exposure of patients with subtype C to stavudine or didanosine may develop into treatment failure due to accumulation of K65R. It was also shown in these studies that K65R confers broad cross-resistance to nucleoside reverse transcriptase inhibitors class in subtype C which is dominant in sub-Saharan Africa than in subtype B. These differences could be attributed to the activities of reverse transcriptase from subtypes C and B (Wainberg and Brenner, 2010). The mechanism was proposed to be template dependent (Wainberg and Brenner, 2010). In subtype C during sequence synthesis there is a stop at codon 65 that give rise to K65R under the drug pressure. On the other hand, in subtype B during the sequence synthesis there is a stop at codon 67 that gives rise to D67N and thymidine analogue mutations instead of K65R pathways. The K65R pathway is selected in subtype C when exposed to didanosine and stavudine regimen as well as the presence of Y181C in the

sequence. The thymidine analogue mutations are favoured whenever there is a presence of zidovudine containing regimen (Wainberg and Brenner, 2010).

In a meta-analysis study it was showed that the T215 mutation for nucleoside reverse transcriptase inhibitors showed the highest proportion in subtype B (24%), while V75 mutation showed the highest proportion to subtype CRF01_AE (10%) (Rhee *et al.,* 2015). The analysis showed that Y181C, a nucleoside reverse transcriptase inhibitor mutation, showed the highest proportion to subtype CRF01_AE (33%), while K103N showed the highest proportion to subtype B (53%). P225H showed highest proportion to CRF02_AG (14%), while V106M showed the highest proportion to subtype C (5%). These studies have demonstrated that some mutations are more specific to certain subtypes. Thus, the information about circulating subtypes is important to have an effective treatment.

2.4. Factors contributing to HIV drug resistance and public health implications

The contributing factors to the emergence of HIV drug resistance can be divided into four groups: the antiretroviral therapy drugs and regimen factors; virus factors; patient factors and programme factors (Bertagnolio *et al.,* 2012). The analysis of these factors can provide information to policy makers about the necessary interventions that need to be implemented in order to reduce the emergence of HIV drug resistance.

2.4.1. Antiretroviral drugs and regimen-related factors

 WHO has recommended the use of two nucleoside reverse transcriptase inhibitors and one non-nucleoside reverse transcriptase inhibitor in the firstóline regimen (Bennett *et al.,* 2012). However, it has been found that the non-nucleoside reverse transcriptase inhibitors drugs possess the low genetic barrier that allows the HIV to acquire high level resistance (Bertagnolio *et al.,* 2012). It has also been found that the regimens containing nucleoside reverse transcriptase inhibitors backbone can cause cross-resistance across its class. In addition the use of single dose of nevirapine in prevention of mother to child transmission (PMTCT) can pose high risk for selection of HIV drug resistance.

It has been shown that some drugs give rise to adverse events for patients on ART. Several studies conducted in different settings to assess the impacts of adverse events on treatment of patients initiating the first-line regimen reported a wide range of adverse events such as: anaemia, diarrhoea, rashes, fatigue, nausea, taste disturbances, vomiting, headache, dizziness and insomnia (Lartey *et al*., 2014; Shet *et al.,* 2014; Marconi *et al*., 2013; Al-Dakkak *et al*., 2013). These studies showed that the adherence to ART was affected as a consequence of these adverse events. A study conducted in India on adherence of ART patients showed that the 15.5% of patients missed a dose due to adverse events of their ARV (Meena *et al*., 2014).

2.4.2. Virus subtype associated factors

It has been found that the mutation patterns may differ across different subtypes; for example the subtype D can develop more readily HIV drug resistance than other subtypes when it is exposed to single dose of nevirapine (Hauser *et al.,* 2011). A recent study conducted has revealed that HIV drug resistance was more common in subtype D than in subtype C among patients failing first-line regimen (Kyeyune *et al.,* 2013). This treatment failure was found to be associated with nucleoside reverse transcriptase inhibitors treatment. It is speculated that subtype D has high intrinsic fitness that lead to fast disease progression. Thus, from public health point of view the subtype D needs stringent monitoring. In other another study conducted in South Africa it was revealed that mutation K65R was dominant in subtype C among patients failing first-line regimen containing tenofovir (Sunpath *et al*., 2012). Another viral factor that contributes to rapid emergence of HIV drug resistance is the nature of HIV reverse transcriptase enzyme that has high replication rate and low fidelity (Kyeyune *et al.,* 2013).

2.4.3. Patient factors

Factors such as poor understanding about the disease, religious practices, higher pill burden, nondisclosure of HIV status and transport cost to and from the clinics, have known to result in poor adherence which eventually leads to the development of drug resistance (Bertagnolio *et al.,* 2012; Wasti *et al.,* 2012; Falang *et al*., 2012; do Prado *et al*., 2014).

The level of education and knowledge about the disease can influence the success or failure of HIV treatment. A study conducted in Nepal showed that the patients who could not read were five times more likely to be non-adherent to ARV (Wasti *et al.,* 2012). Patients may have a perception that ARV can only be used to treat symptoms rather than being a life-long treatment. Another study conducted in Nigeria showed that the patients with higher level of education had high adherence to ARV and showed a better knowledge of how it worked (Falang *et al*., 2012). Literate patients were able to communicate well with the health workers and remember the information that they were provided with.

The patientsø religious practices may also influence the adherence to ARV which can lead to the development of HIV drug resistance. Due to personal beliefs patients may question the effectiveness of ARV and prefer not to visit the ART clinics. Studies had shown that some patients missed their doses due to religious reasons (Falang *et al*., 2012; Wasti *et al.,* 2012). For example, for Muslims during Ramadan (the fasting festival) patients may change their routine of taking their drugs by missing the morning dose.

A study conducted in Nigeria had shown that the patients who take three dosages daily of ARV were more non-adherent than the patients who take two dosages daily (Falang *et al*., 2012). In addition, patients with TB/HIV co-infection who were found to have a high pill burden and were more likely to default due to combination of drugs side effects and drug toxicities (do Prado *et al*., 2014). Thus, less dosages per day are more durable than a large number of drugs per day which can have negative impact on adherence due to side effects.

The nondisclosure of HIV status to family members and friends has led to non-adherence of ARV since patients were subjected to discrimination. This discrimination discourages the patients to visit the ART clinics and only visit if nobody is seeing them. Patients develop fears of taking their medicines in the presence of others. Studies conducted in Nepal and Nigeria showed an association between disclosure and good adherence (Wasti *et al.,* 2012; Falang *et al*., 2012). Thus, patients were taking their drugs openly without any fear of stigmatization from family members and friends.

Studies have found that the cost of transport to the ART clinics was regarded also as a barrier to adherence (Falang *et al*., 2012; Wasti *et al.,* 2012; Kagee *et al.,* 2011). Unaffordability of

transport cost to the ART clinics discourages patients to go for drugs refill and this leads patients to miss dosages which can pose a risk for the development of drug resistance.

2.4.4. Health system factors

The health system factors such as the limited human resources, infrastructure, weak information system, weak monitoring and evaluation systems and a high cost for viral load monitoring can contribute to the emergence of HIV drug resistance (Bertagnolio *et al.,* 2012; Colvin *et al.,* 2014; Kagee *et al.,* 2011; Wasti *et al.,* 2012; Posse *et al.,* 2008; Cornell *et al.,* 2010).

The shortage of staff at ART clinics can affect the service delivery to patients (Colvin *et al.,* 2014; Kagee *et al.,* 2011). The task then shifts where HIV counsellors are conducting the work for nurses and vice versa. This is not part of their training and has generated challenges to the health system. As the consequence, patients may not get all the necessary attention. The staff shortage leads to long queues at the ART clinics and patients have to wake up early in the morning to queue for medication. This long waiting time at the ART clinics leads to overcrowding. Some patients may opt to take half doses so that the medication will last longer, then avoiding trips to the clinics. This situation discourages the patients to visit the clinics for much needed appointments.

The long distance and poor infrastructure of roads have also been found to discourage patients to visit the ART clinics (Kagee *et al.,* 2011; Wasti *et al.,* 2012; Posse *et al.,* 2008). If the roads are poor patients have to walk long distances to reach the clinics. A study conducted in Nepal showed that the patients who travelled a distance for more than one hour to the clinics were three times more likely to be non-adherent (Wasti *et al.,* 2012). This could cause the patients not pick up their refill drugs which could lead to the development of drug resistance.

Weak information system can hamper communication within and between ART clinics to determine patients who were still retained in care (Bertagnolio *et al.,* 2012; Colvin *et al.,* 2014). Overbooking of patients, cancelling of bookings and failure to book on time can be experienced if the information system for the programme is poor. These can lead patients to present to clinics late or miss the appointments; which eventually leads to non- adherence. When the patients do

not show up for the appointment there is no system to track them down and bring them back into care. In addition, if the ART program does not have access to death registers some deaths may go unreported and being documented as patients lost to follow up. The health facilities do not have the synchronized medical records as well as identifiers for patients so that they can be traced to any treatment site. The changing of treatment guidelines is also an important factor that can lead to ineffective treatment, especially if not communicated timeously (Colvin *et al.,* 2014). The HIV treatment guidelines are continuously changing and these changes should be available on information system. If health workers are unaware of changes in the treatment guidelines patients will end up receiving ineffective care.

The lack of monitoring and evaluation of system during the programme expansion had been found to lead to poor service delivery (Cornell *et al.,* 2010; Bertagnolio *et al.,* 2012). Unsustainability of current service care while decentralizing has been found to affect the performance of the health system. The demands for service care overtake the available resources capacity. The fund resources could be utilized for activities that were not part of the program priorities. A study was conducted in South Africa to determine the factors affecting program roll out (Cornell *et al.,* 2010). It was found that the scale up of the program put burden on ART services and health information system. Thus, during expansion the monitoring of the program needs to be continuously evaluated.

HIV viral load monitoring has been found to be a good measure for treatment failure (Hong *et al.*, 2011; Bertagnolio *et al.,* 2012). However, due to high cost, routine monitoring is limited which delays early detection of treatment failure. This eventually leads to the emergence of HIV drug resistance. Thus, viral load testing is required to monitor the progress of treatment.

2.5. WHO strategy for HIV drug resistance prevention and implications for public health

The scaling up of ART follows the public health approach of using the standardized and simplified treatment regimens that are consistent with international standards (Bennett *et al.,* 2012). However the treatment with ARV drugs can be accompanied by emergence and transmission of HIV drug resistance. The emergence of HIV drug resistance can limit the treatment options that will need switching to the second line regimens that is costly and can

produce long term toxicities (Bennett *et al.,* 2012). In order to counter the effects of HIV drug resistance, WHO developed a global strategy for the prevention and minimizing the emergence of HIV drug resistance (Bennett *et al.,* 2008; Bennett *et al.,* 2012; Jordan *et al.,* 2012). The WHO strategy involves three elements: the monitoring of early warning indicators, surveillance of acquired HIV drug resistance in population on ART and surveillance of transmitted HIV drug resistance in recently infected population. The results from these strategies can be used for optimal selection of ARV drugs on population-basis and provides information for making program adjustments and optimize the patientsø quality care.

2.5.1. Early Warning Indicators

The early warning indicators identify the factors that affect the individual health facilities to favor the emergence of HIV drug resistance. The timely identification of factors that negatively affect the health facilities helps to target the intervention that can reduce the emergence of HIV drug resistance (Hong *et al.*, 2010; Hong *et al.*, 2011; Bennett *et al.,* 2012). Early warning indicators are collected from patient medical records and pharmacy records to asses ART sites and program factors that are potentially associated with HIV drug resistance. The results obtained can be used to make adjustments in order to reduce the emergence of HIV drug resistance to individual sites and at patientsø individual level. WHO has outlined several early warning indicators that should be assessed such as: ARV prescribing practices, lost to follow up at 12 months, retention on first-line at 12 months, on time pill pick up, on-time clinic appointments, drug supply continuity, viral load suppression at 12 months (Bennett *et al.,* 2008; Jordan *et al.,* 2012).

ARV prescribing practices refers the percentage of patients initiating an appropriate first-line ART regimen which is listed in national guideline. The target should be 100%. Lost to follow up refers to percentage of patients who were lost to follow at 12 months upon initiation, while patients retention on first-line ART at 12 months refers to percentage of patients who initiated at ART site that are still on first-line ART regimen after 12 months and the target should be less than or equal to 20% and more than or equal to 70% respectively. On-time ARV drug pick up refers to percentage of patients picking up all prescribed ARV drugs on time during first 12 months and the target should be more than or equal to 90%. ARV drug ósupply continuity refers

to percentage of months in a year in which there was no ARV drug stock outs at the sites and the target should be 100%; while viral load suppression refers to percentage of patients with viral load less than 1000 copies/ml at 12 months and the target should be more than or equal to 70%.

2.5.2. HIV drug resistance surveillance

There are two surveys that are recommended by WHO: surveillance of acquired HIV drug resistance in population on ART and surveillance of transmitted HIV drug resistance in recently infected population (Bennett *et al.,* 2008; Jordan *et al.,* 2012).

Acquired HIV drug resistance is conducted at specific health facilities to assess the performance in achieving the prevention of HIV drug resistance for instance maintaining the suppressed viral load in patients at 12 months upon initiation of ART. This survey is also used to assess the HIV drug resistance in patients who failed the first-line therapy. The results obtained can be used for programmatic adjustment where necessary.

Transmitted HIV drug resistance survey assesses the recently infected individuals who become infected with a drug resistant HIV strain. This survey uses the sample size of 47 which is sampled from the first 47 patients who meet the criteria for the survey. The prevalence for HIV drug resistance is classified as low (<5%), moderate (5-15%) and high (>15%) in specific geographic area. The results from this survey give information about selection of future first-line regimens and about the performance of HIV drug resistance prevention programmes. Namibia was classified as low (<5%) based on 2006 HIV transmitted survey (MOHSS, 2007).

2.6. Summary

Several studies conducted in different settings were reviewed to determine the frequency of mutations to the classes of antiretroviral that are composed of the first-line regimen. Studies showed for nucleoside reverse transcriptase inhibitors class that M184V was the most frequent mutation, while for non-nucleoside reverse transcriptase inhibitors class, K103N was the most frequent mutation. Mutation M184V was conferred due to the use of lamivudine while K103N was conferred due to the use of nevirapine. The information about specific mutations that are

being conferred by drugs can give guidance on the choice of drugs that can be used for the second-line regimens.

Studies showed that in order to have an effective therapy the information about the circulating subtypes is required to make decision about the classes of drugs that can be used. HIV has subtypes which are genetically different from each other and can influence the drug resistance pattern. Studies from different settings have shown that certain mutations are more dominant in certain subtypes that could have impacts on patient treatment outcome.

Factors that are associated with emergence of HIV drug resistance such as the antiretroviral therapy drugs and regimen factors, patient factors and health systems factors were identified from the studies. The impact of each factor on emergence of drug resistance was analyzed. These factors can provide information to policy makers about the necessary interventions that need to be implemented in order to reduce the emergence of HIV drug resistance.

The studies outlined the WHO global strategy for prevention and minimizing the emergence of HIV drug resistance. The WHO strategyøs three elements such as: the monitoring of early warning indicators, surveillance of acquired HIV drug resistance in population on ART and surveillance of transmitted HIV drug resistance in recently infected population were identified. The strategy can be used for optimal selection of ARV drugs on population-basis and provides information for making program adjustments and optimize the patientsø quality care.

CHAPTER 3: METHODOLOGY

3.1. Aim and Objectives

The aim of the study was to determine the risk factors leading to the development of HIV drug resistance mutations and to describe the dominant HIV drug resistance patterns among patients failing first-line antiretroviral therapy regimen in Oshikoto and Oshana regions of Namibia.

The objectives of this study were as follow:

1) To describe the socio-demographic and clinical profiles of patients initiated with the appropriate prescribed first-line regimen.
2) To describe the profile of patients retained on first-line regimen after 6 and 12 months.
3) To describe the profile of patients who were compliant with ART (on-time for appointment and no treatment interruptions).
4) To describe HIV-1 drug resistance mutation patterns among patients failing the first-line antiretroviral therapy regimen.
5) To determine the risk-factors for HIV drug resistance among patients failing the first-line antiretroviral therapy regimen.

3.2. Study design

The *case-control* study design was used to review the existing laboratory sequencing result reports from the specimens received from private and public patients suspected of treatment failure to first-line ART regimens between January 2008 to December 2013 in Oshikoto and Oshana regions in Namibia. This design was regarded as suitable for this study since the cases were readily available in the Namibia Institute of Pathology laboratory database. Thus, the information on the prevalence of laboratory confirmed HIV drug resistance mutations could be collected. In addition, there was no cost to retrieve the information from Namibia Institute of Pathology laboratory database using this study design.

The cases in this study were patients with treatment failure with their HIV viral loads more than 1,000 RNA copies/ml. The controls were patients without treatment failure and their viral loads suppressed (less than 1,000 RNA copies/ml). The genotyped patients were regarded as cases in this study and their reports were available in Namibia Institute of Pathology laboratory database.

3.3. Study population and sample

Antiretroviral therapy was rolled out in Oshana and Oshokoto regions in the private sector in 1997 and in the public sector in 2003. Patients access treatment at public district hospitals and clinics, while some patients access the treatment at private practises where private clinicians are available. During the clinical visits the patients were assessed for ART response. A patient was considered to have a treatment failure if the result of a HIV viral load test was more than 1,000 RNA copies/ml on two consecutive visits despite undergoing intensive adherence counseling. The blood sample was collected from this patient and referred to Namibia Institute Pathology laboratory for HIV genotyping. Patients who were referred to Namibia Institute Pathology for genotyping between January 2008 to December 2013 from Oshana and Oshikoto regions were eligible for this study. Oshana and Oshikoto regions are the most densely populated and amongst the regions of Namibia with the highest burden on HIV infection with prevalence estimated at 22% among pregnant women in 2012 (MOHSS, 2012). Patients from these two regions, that had complete HIV genotyping results information within the designated study period, were included. Patients with unsuccessful genotyping results were excluded from the study.

By December 2013 Oshikoto region had 13,298 patients on ART, while Oshana had 13,700 patients on ART. When the Namibia Institute of Pathology database was searched for the requests of HIV-1 genotyping, 266 cases were obtained from January 2008 to December 2013 from Oshana region. While Oshikoto region only gave 28 cases. To determine odds ratio for developing resistance against the risk factors, a 95% confidence interval with 5% margin of error, a sample size of 140 patient records from Oshana region were randomly selected using STATA v.13 statistical analysis package (StataCorp, College Station, Texas) for review. For Oshikoto region all 28 patient records were selected for reviewing. This gave a total number of sample size of 168 cases for the study. A group of patients who initiated the treatment within the same period but with suppressed viral load were used as controls in this study. The controls were

matched with cases for sex and age which were easily available in their medical records. Out of 140 cases selected for Oshana region, 29 cases could not be amplified, 18 cases refused consent, 6 cases medical records were incomplete, 3 cases medical records were unavailable and in 4 cases could not find controls. For Oshikoto region, out of 28 selected cases one (1) sample could not be amplified, 4 cases medical records were incomplete, and in 6 cases medical records were unavailable (Fig.1). For Oshana region 80 cases were successfully matched with 88 controls and for Oshikoto region 17 cases with complete records were matched with 17 controls. The combined cases from both regions gave a total number of 97 and 105 for controls.

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│ 97 cases eligible for the study │
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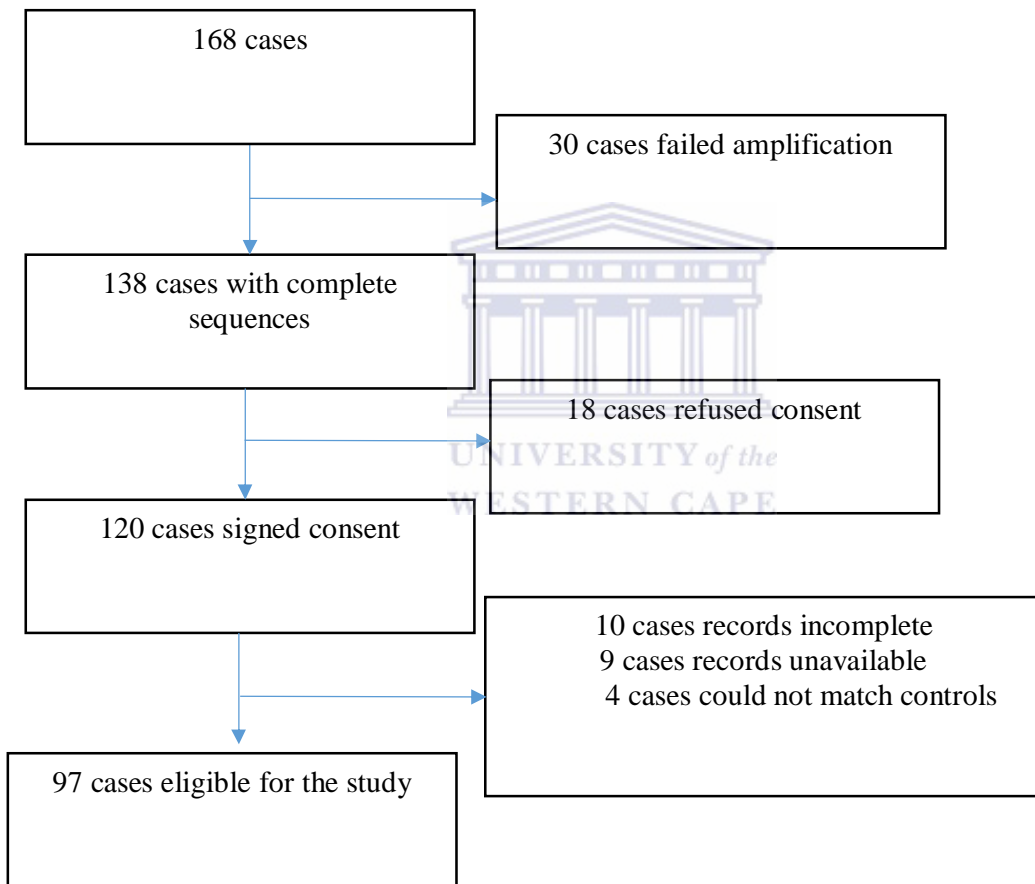
Figure 1. Flow diagram showing cases that were selected to meet the criteria

3.4. Data collection

3.4.1. Demographic characteristics

Once the informed consent was given the participant demographic variables such as gender, age, marital status, employment status and place of residence were abstracted from patientsø records and entered into a study specific data abstraction sheet (see Appendix A). For demographic information the controls and cases were matched for gender and age. Depending on the characteristics of the cases, controls with similar demographic characteristics were selected.

3.4.2. Clinical variables

Participants clinical variables such as ART initiation date, WHO clinical stage at initiation, baseline CD4, CD4 at 6 and 12 months, ART regimen at initiation, retention on first-line regimen for 6 and 12 months, viral loads at 6 months and genotyping were abstracted from patients records and entered into a study specific data abstraction sheet. In addition factors such as self-reported adverse events or side effects, TB infection, alcohol intake, missed appointments, number of treatment interruption and duration on ART prior to failure were also abstracted from patientø records and entered into a study specific data abstraction sheet. The cases and controls were matched for ART initiation date.

3.4.3. HIV resistance mutations and subtypes

The genotyping information for each case was entered into a study specific data abstraction sheet (see Appendix A). From each laboratory record the HIV resistance mutations for nucleoside reverse transcriptase inhibitors and non-nucleoside reverse transcriptase inhibitors were recorded. The corresponding information for the HIV-1 subtypes for protease and reverse transcriptase regions were also recorded on the study specific data abstraction sheet.

3.5. Data analysis

Data entered into the paper-based data abstraction sheets were transferred into an Excel database during the study. After data cleaning and coding the data was transferred into STATA v.13 statistical analysis package (StataCorp, College Station, Texas). For demographic variables, the proportion for cases and controls for each region, gender, age, marital status, employment status and place of residence as extracted from the statistical analysis package were calculated and tabulated. In addition for each comparison of cases and controls the p-values were calculated and P values <0.05 were considered as statistically significant.

In addition for clinical variables, the proportion for cases and controls for baseline CD4 (less than 200 or more than 200 cells/µl), CD4 at 6 and12 months were tabulated. The proportion for cases and controls for viral load at 6 months were recorded. The proportion of cases and controls for factors such as self-reported events, TB infection, alcohol intake, ART regimen at initiation, retention on first-line regimen at 6 and 12 months, missed appointments, and treatment interruptions were analyzed and reported descriptively.

For analysis of prevalence of HIV-1 resistance mutations and subtypes the information were interpreted according to the algorithm at Stanford Sequence Resistance Database (http://hivdb.stanford.edu). The proportion of HIV subtypes and HIV drug resistance were analyzed for Oshikoto and Oshana regions.

To estimate the association between different factors with HIV drug resistance the multivariate model including all factors with p-values less 0.232 was used to calculate unadjusted odds ratios (UOR) at 95% confidence interval (CI) with HIV drug resistance as the outcome variable. The odds ratios was adjusted (AOR) until P values <0.05 were obtained which were considered as statistically significant.

3.6. Validity and Reliability

The extraction tool used to collect data from patientsø records at the health facilities and also from Namibia Institute of Pathology database was standardised. The samples from both sources were selected by using the random simple method so that there would be no selection bias and measurement bias would be minimal. The extraction tool was piloted at ART facilities in Windhoek before used in Oshikoto and Oshana regions.

A different researcher collected 10% of the data from the patientsø records and laboratory records from Namibia Institute of Pathology database. A different researcher recorded the demographic, clinical, and HIV resistance mutations information from patientsø records and from Namibia Institute of Pathology database. Upon analysis of the collected data, results obtained were compared with the results that were reported in other similar studies which were done in different settings.

3.7. Ethics considerations
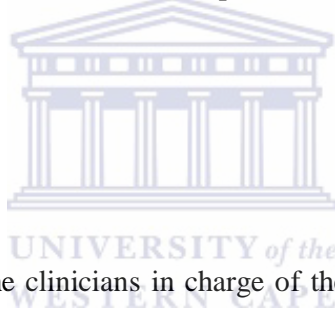
The consent was requested from the clinicians in charge of the health facilities to review their patient records. The clinicians were requested to read the information sheet that described the study and requested to sign the consent form. All the questions regarding the study were addressed accordingly. The study kept the patients personal information confidential by de-linking patientsø identification and assigned a study number for each patient. This study did not inflict any harm on the subjects. The abstraction sheets with the extracted data were locked up in safe place where no one had access except the principal investigator. In addition the laptop that was used to store electronic data was password protected. Ethics clearance for this study was obtained from University of the Western Cape, South Africa and Ministry of Health and Social Services, Namibia (see Appendices B and C).

CHAPTER 4: RESULTS

4.1. Demographic characteristics of respondents

The combined data for both regions, 47 (48%) of cases were females compared to 52 (50%) in the control group (Table1). The mean age was 44.8 (±13.2) years for controls and 43.3 (±13.3) years for cases. For marital status 50 (48%) of controls were single or windowed while 59 (61%) of cases were single or windowed which was statistical insignificant ($p<0.059$). Fifty-five (52%) controls were married while only 38 (39%) cases were married. In addition 75(71%) controls and 74 (76%) cases were employed. Forty (38%) controls and 60 (62%) cases lived in urban areas which was statistical significant ($p<0.003$).

4.2. Clinical characteristics of respondents

The patient records reviewed did not show the WHO clinical stage at initiation of ART by omission or as the result of incomplete records. The controls with of CD4 more than 200 cells/µl at baseline, 6 and 12 months showed a proportion of 65%, 90% and 91% respectively, while cases had a proportion of 54%, 69% and 78% respectively (Table1). Fifty-two (54%) cases failed treatment before reaching 12 months on treatment. Ninety-eight (98%) of controls had supressed viral load at 6 months compared to 13% of cases, with the difference being statistical significant ($p<0.001$).

It was found that 43 (44%) of cases had side effects or adverse events while only 26 (25%) of controls experienced side effects with the difference being statistical significant ($p<0.003$). Seven-teen 17 (18%) cases had TB infection while on ART and only 1 (1%) control had TB with the difference being statistical significant ($p<0.004$). Further analysis of cases with TB infection revealed that a proportion of 59% of males had TB infection. The results also showed that a proportion of 59% of cases with TB infection lived in urban areas. The results showed that 7 (7%) cases had used alcohol, while only 1 (1%) control had used alcohol with the difference not being statistical significant ($p<0.056$).

It was found that 99 (94%) and 78 (80%) had initiated first-line regimen for controls and cases respectively with the difference being statistical significant (p<0.009) (Table 1). The appropriate first-line regimens on which cases were initiated had two categories contained stavudine/ lamivudine/nevirapine (or efavirenz) and tenofovir/lamivudine/nevirapine (or efavirenz) with proportion of 13% and 3% respectively and other two categories contained zidovudine/lamivudine/nevirapine (or efavirenz) and tenofovir/emtricitabine/efavirenz (or nevirapine) with proportion of 32% each. The inappropriate first-line regimens on which cases were initiated had four categories contained zidovudine/lamivudine/lopinavir boosted with ritonavir, tenofovir/emtricitabine/aluvia, zidovudine/lamivudine/tenofovir/aluvia each with a proportion of 2% and zidovudine/lamivudine/aluvia with a proportion of 3%. Another ten categories contained lamivudine/aluvia, lamivudine/tenofovir/aluvia, aluvia/efavirenz, zidovudine/efavirenz, tenofovir/emtricitabine/zidovudine, zidovudine/prezista/raltegravir, stavudine/lamivudine/kaletra, stavudine/lamivudine, efavirenz, efavirenz/lamivudine with proportion of 1% each. It was found that 68 (70%) cases had missed one or more clinical appointments while only 11 (10%) controls had missed appointments with the difference being statistical significant (p<0.001).
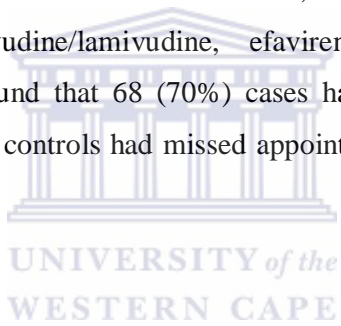
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Table 1 Patient characteristics by case-control status, N(%) or Mean(±SD)

	Controls	Cases	p-value
Region			0.800
Oshana	88 (84%)	80 (82%)	
Oshikoto	17 (16%)	17 (18%)	
Sex			0.879
Female	52 (50%)	47 (48%)	
Male	53 (50%)	50 (52%)	
Age(mean)(±SD)	44.8 (13.2)	43.3 (13.3)	0.071
Marital Status			0.059
Single or widowed	50 (48%)	59 (61%)	
Married	55 (52%)	38 (39%)	
Employment			0.232
Unemployed	30 (29%)	23 (24%)	
Employed	75 (71%)	74 (76%)	
Residence			0.003*
Urban	40 (38%)	60 (62%)	
Rural	65 (62%)	37 (38%)	
CD4 at Baseline			0.133
<200 cells/µl	37 (35%)	45 (46%)	
>200 cells/µl	68 (65%)	52 (54%)	
CD4 at 6 months			0.005*
<200 cells/µl	11 (11%)	25 (31%)	
>200 cells/µl	94 (90%)	56 (69%)	
CD4 at 12 months			0.069
<200 cells/µl	9 (9%)	10 (22%)	
>200 cells/µl	91 (91%)	35 (78%)	
Viral Load at 6 months			<0.001*
Suppressed	103 (98%)	13 (13%)	
Not suppressed	2 (2%)	68 (70%)	
Side effects			0.003*
No	79 (75%)	54 (56%)	

Yes	26 (25%)	43 (44%)	
TB infection			0.004*
No	104 (99%)	79 (82%)	
Yes	1 (1%)	17 (18%)	
Alcohol			0.056
No	104 (99%)	90 (93%)	
Yes	1 (1%)	7 (7%)	
ART regimen initiated			0.009*
First-line	99 (94%)	78 (80%)	
Not First-line	6 (6%)	19 (20%)	
Missed appointments			<0.001*
None	94 (90%)	29 (30%)	
Any	11 (10%)	68 (70%)	

*: indicates statistical significance.

4.3. Prevalence of HIV resistance mutations

For Oshana region 50 (63%) cases harboured nucleoside reverse transcriptase inhibitors mutations while 30 (38%) cases did not harbour any nucleoside reverse transcriptase inhibitor mutation (Table 2). The dominant mutations for nucleoside reverse transcriptase inhibitor with corresponding proportions were M184V/I (61%), T215Y/F (28%), K65R (16%), D67N (15%), M41L (15%) and K70R (11%). Fifty-four (68%) cases harboured non-nucleoside reverse transcriptase inhibitors mutations while 26 (33%) cases did not harbour any non-nucleoside reverse transcriptase inhibitor mutation (Table 3). The dominant mutations with their corresponding proportions were K103N and Y181C with 43% and 15% respectively. These were followed by G190A (11%), Y188C/L (11%), V106M (10%), K101E/P (10%) and P225H (9%).

For Oshikoto region 12 (71%) cases harboured nucleoside reverse transcriptase inhibitors mutations while 5 (29%) cases did not harbour any nucleoside reverse transcriptase inhibitor mutation (Table 2). The dominant mutations for nucleoside reverse transcriptase inhibitors with their corresponding proportions were M184V/I and T215Y/F with 47% each. These were followed by K70R (41%), D67N (35%), K219Q/E (29%), M41L (24%) and K65R (18%). Thirteen (76%) cases harboured non-nucleoside reverse transcriptase inhibitors mutations while 4 (24%) cases did not harbour any non-nucleoside reverse transcriptase inhibitor mutation (Table 3). The dominant mutations with corresponding proportions were K103N and V106M with 41% and 24% respectively. These were followed by G190A, Y188C/L, K101E/P and Y181C with 18 % each.

Overall a proportion of 23% cases did not show any mutation for nucleoside reverse transcriptase inhibitors and non-nucleoside reverse transcriptase inhibitors for both regions and these cases shared the same clinical risk factors with those that had mutations. For subtypes, Oshana region the dominant subtypes were subtypes C and 08_BC/C with proportion of 85% and 10% respectively. These were followed by C/31_BC, 31_BC/C, K/C and 02_AG/G with 1% each. For Oshikoto region the subtype C was the most dominant with proportion of 82%. This was followed by 08_BC/C, B/C and 01_AE/A1 with 6% each.

Table 2 Prevalence of nucleoside reverse transcriptase inhibitors (NRTI) HIV-1 drug resistance mutations among cases who were genotyped.

NRTI Mutations	Oshana N (%)	Oshikoto N(%)
Any NRTI mutation	50(63%)	12(71%)
M184V/I	49(61%)	8(47%)
D67N	12(15%)	6(35%)
T215Y/F	22(28%)	8(47%)
K70R	9(11%)	7(41%)
K219Q/E	3(4%)	5(29%)
M41L	12(15%)	4(24%)
A62V	1(1%)	-
V75I/M	3(4%)	1(6%)
K65R	13(16%)	3(18%)
L74I	2(3%)	-
L210W	1(1%)	1(6%)
E44D	3(4%)	-
T69D	5(6%)	1(6%)
F77L	1(1%)	
V118I	1(1%)	-
Without Mutations	30(38%)	5(29%)

Table 3 Prevalence of non-nucleoside reverse transcriptase inhibitors (NNRTI) HIV-1 drug resistance mutations among cases who were genotyped.

NNRTI Mutations	Oshana N(%)	Oshikoto N(%)
Any NNRTI mutation	54(68%)	13(76%)
K103N	34(43%)	7(41%)
V106M	8(10%)	4(24%)
G190A	9(11%)	3(18%)
Y188C/L	9(11%)	3(18%)
K101E/P	8(10%)	3(18%)
P225H	7(9%)	-
Y181C	12(15%)	3(18%)
L100I	1(1%)	1(6%)
V108I	6(8%)	1(6%)
K238K/T	1(1%)	-
M230L	5(6%)	1(6%)
A98G	5(6%)	1(6%)
F227L	3(4%)	1(6%)
V179D	2(3)	-
Without Mutations	26(33%)	4(24%)
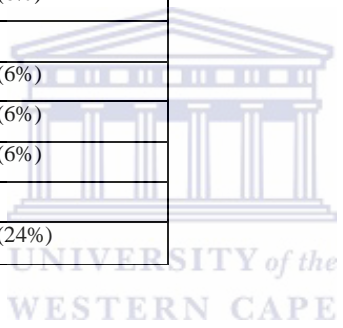
4.4. The risk factors associated with HIV drug resistance

Table 4 shows the association of demographic and clinical factors with HIV drug resistance as the outcome. Before the odd ratios were adjusted to remove the confounding factors, the results showed 12 potential risk factors could be associated with drug resistance from multivariate model. The patients who TB infection were 19 times more likely to develop HIV drug resistance and patients who had missed appointments (any) were 11 times more likely to develop HIV drug resistance. In addition the patients who had taken alcohol were 8 times more likely to develop drug resistance. The patients with CD4 at 6 and 12 months less than 200 cells/µl respectively were 5 and 7 times more likely to develop drug resistance. On the other hand, patients with CD4 at baseline less than 200 cells/µl were 2 times more likely to develop drug resistance. Patients initiated inappropriate regimen were 4 times more likely to develop HIV drug resistance, while patients who experiencing side effects were 3 times more likely to develop HIV drug resistance. Marital status of being single or widowed showed a risk of two. Patients residing in urban and being employed were 2 times more likely to develop drug resistance. The age did not show any association with drug resistance.

 As the study design is a matched case-control study, the conditional logistic regression was used for matched case-control. Multivariable conditional logistic regression models were then used to adjust for individual predictors or potential confounders. Variables that were pre-determined to be independent predictors of treatment failure were considered candidate variables for the multivariable logistic regression model. The backward elimination to determine the final multivariable model was used. Variables with p<0.05 were kept in the final model. After odd ratios adjustment of the model three risk factors such as side effects, initiating inappropriate first-line regimen and missed appointments showed an increase in odd ratios. The results showed that patients who missed appointments, initiating inappropriate first-line regimen and experienced side effects respectively were 22, 12 and 7 times more likely to develop HIV drug resistance.

Further multivariable conditional logistic regression models were analyzed against risk factors and prevalent mutations for cases (M184V/I and K103N) with drug resistance as outcome. The risk factors gender and side effects showed the association with prevalent mutations M184V/I and K103N. The results showed that males were 3 times more likely to develop resistance for lamivudine or nevirapine. In addition those patients who developed side effects were 2 times more likely to develop resistance to lamivudine or nevirapine.

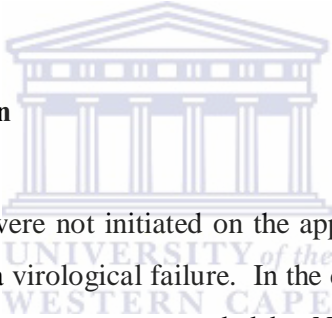**Table 4 Factors associated with HIV drug resistance**

Factor	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Age	0.94 (0.88 to 1.01)	
Marital status (single or widowed)	1.84 (0.98 to 3.48)	
Employment (employed)	1.65 (0.73 to 3.75)	
Residence (urban)	2.38 (1.33 to 4.17)	
CD4 at baseline <200 cells/µl	1.62 (0.86 to 3.05)	
CD4 at 6 months <200 cells/µl	4.63 (1.57 to 13.70)	
CD4 at 12 months <200 cells/µl	6.99 (0.86 to 56.89)	
Side effects (yes)	2.96 (1.44 to 6.08)	7.17 (1.89 to 27.22)
TB (yes)	18.81 (2.49 to 141.89)	
Alcohol (yes)	7.80 (0.95 to 63.91)	
Inappropriate first-line regimen	3.73 (1.38 to 10.07)	11.70 (1.69 to 80.99)
Missed appointments (any)	11.35 (4.90 to 26.27)	21.58 (6.50 to 71.59)

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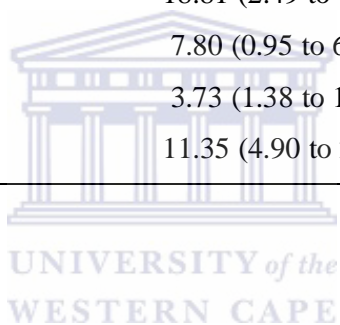
CHAPTER 5: DISCUSSION

To the best of my knowledge this is the first study investigating the individual factors that can contribute to the emergence of HIV drug resistance in Namibia. The discussion will outline how important it is to initiate the appropriate firstóline regimen for the ART. In addition the prevalence and profile of HIV drug resistance mutations obtained from the current study will be compared to the profiles obtained from other studies. The relationship between the treatment failure and the missing of clinical appointments will also be revealed. Adverse events that are experienced when the patients are subjected to ART will be outlined and how to overcome them in order to prevent treatment failure. The challenges that TB infection presents to the management of HIV will be analyzed. Finally the limitations in the current study that will pave the way for further research will be discussed.

5.1. Appropriate first–line regimen

In the current study patients who were not initiated on the appropriate firstóline regimen were 12 times more likely to experience a virological failure. In the current study, 20% of cases were not initiated on the firstóline regimen as recommended by Namibia national ART guideline. Some cases were initiated on mono or dual therapy. A similar study conducted in India showed that 58% of patients who failed the first-line regimen were not on the recommended combination of ARV drugs since some patients were on single and dual drugs (Gupta *et al*., 2010). Another study conducted in the United States to determine the predictors of virological failure among patients on ART showed that patients with prior history of ART, were two times more likely to experience the virological failure whenever initiated on the first-line regimen (Robbins *et al*., 2010). Ding *et al*. (2009) also demonstrated that patients who had prior history use of mono- or dual therapy were 3 times more likely to have a sub-optimal virological response than if they would have been drug naïve. Studies were conducted to determine the effectiveness of using a combination of three drugs in nucleoside reverse transcriptase inhibitors treatment experienced patients. When zidovudine exposed patients were treated with combination of drugs zidovudine/lamivudine/indinavir (Gulick *et al.,* 1997; Descamps *et al.,* 2002). In the group that was treated with combination of three drugs showed good viral load suppression in comparison

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to the group that was treated with zidovudine + lamivudine only. This is an indication how effective using the combination of three drugs. These studies reconfirmed what has been recommended by WHO. WHO guidelines recommended that 100% of patients should be initiated on an appropriate firstóline regimen that contains two nucleoside reverse transcriptase inhibitors and one non-nucleoside reverse transcriptase inhibitor (Bennet *et al*., 2008, Jordan *et al.*, 2012). Thus, the use of appropriate first-line regimen is necessary so that the long- term effectiveness of ART can be sustained and subsequent use of second line regimen cannot be compromised. Maintaining the appropriate first-line regimen can prevent the development of HIV drug resistance. Based on the results obtained from the current study there is a need for Namibia to reinforce the policy for patients to be initiated on appropriate first-line regimen in all settings by setting up an inspection team for each site initiating ART.

5.2. Prevalence of HIV resistance mutations

In the current study the most prevalent mutations for nucleoside reverse transcriptase inhibitors observed were M184V, T215 F/Y, K65R, D67N, M41L, K70R and K219Q/E. M184V/I is mainly caused by the use of lamivudine in the first-line regimen, while T215Y/F, K65R, D67N, M41L and K70R that are regarded as thymidine analogue mutations develop mainly due to the use of zidovudine, stavudine, tenofovir and abacavir which are standard first-line regimens (Whitcomb *et al.,* 2003). For non-nucleoside reverse transcriptase inhibitors the dominant mutations were K103N, Y181C, G190A, Y188C, V106M, K101E/P and P225H. Mutations K101E/P, K103N, G190A, Y188C, V106M, and P225H are mainly developed due to the use of nevirapine and efavirenz in the first-line regimen, while Y181C is mainly caused by nevirapine (Cavalcanti *et al.,* 2007; van Zyl *et al.,* 2011; Bacheler *et al.* 2000). The drug resistance patterns obtained from the current study were similar to the patterns obtained from other studies in different countries such as Kenya, South Africa and China (Hassan *et al.*, 2014; Manasa *et al.*, 2013; Hoffmann *et al.*, 2013; Xing *et al.*, 2013; Li *et al.*, 2013).

Several studies were conducted to determine the impact of specific nucleoside reverse transcriptase inhibitors drugs in treatment experienced patients when certain mutations were present at baseline (Izopet *et al.,* 1999; Shulman *et al.,* 2001; Yerly *et al.,* 1996; Montaner *et al.,* 2000; Calvez *et al.,* 2002; Japour *et al.,* 1995). It was found that the presence of combination of

thymidine analogue mutations T215Y, M41L and 210W at baseline in patients who were exposed to zidovudine showed poor viral load suppression when they were treated with stavudine + didanosine or stavudine + lamivudine. These studies showed the cross-resistance among the drugs that confer thymidine analogue mutations. Thus, the patients who are failing first-line regimen should be genotyped before initiating the second-line regimen to determine the combination of mutations that are present. Genotyping the patients that are failing treatment can give guidance on making decision about the alternative drugs that can be used in the subsequent treatment.

Currently, Namibia HIV treatment guideline recommends the use of zidovudine/tenofovir/ lamivudine/lopinavir boosted with ritonavir or zidovudine/tenofovir/lamivudine/atazanavir boosted with ritonavir as the second line regimen (MOHSS, 2014). Based on the data from the current study for M184V/I being a dominant nucleoside reverse transcriptase inhibitor mutation and also showed the association with gender and side effects, is an indication that lamivudine should not be used in the second-line regimen. Lamivudine should be replaced with didanosine in the second-line regimen. In addition it has been shown that the presence of M184V mutation can increase susceptibility to zidovudine and tenofovir (Boyer *et al.*, 2002). The use of didanosine instead of lamivudine will be effective since studies have also shown that M184V does not interfere with didanosine containing regimen when it is present at the baseline (Sproat *et al.*, 2005; Winters *et al.*, 2003). On the other hand for K103N being a dominant non-nucleoside reverse transcriptase inhibitor mutation, nevirapine should be removed from the first-line regimen since it has been widely used in PMTCT. Nevirapine should be replaced with etravirine since it has been shown that there was no cross-resistance between these drugs (Marcelin *et al.,* 2010).

The K65R mutation has been detected in the current study like in other studies conducted in HIV subtype C dominant areas such as; South Africa and India (Manasa *et al*., 2013, Hoffmann *et al*., 2013; Hammer *et al*., 2012). It has been shown in studies that some mutations are subtype specific. For example a study conducted in South Africa has revealed that mutation K65R was dominant in subtype C among patients failing-line containing tenofovir (Sunpath *et al*., 2012). In addition it had also been shown that, there were more K103N in subtype D and subtype A than in subtype C. In this study there was not much diversity of subtypes that could be linked to the specific HIV drug resistance since the study was dominated by subtype C. The HIV drug

resistance profile obtained in this study can give guidance to the clinicians when making decisions about what drugs to use for their patients who are found to have treatment failure.

5.3. Missing clinical appointments

Missing more than one clinical appointment was identified in current study as one of the risk factors for virological failure. The current study showed that the patients who missed any appointment were 22 times more likely to experience the virological failure. In the current study it was observed that only 30% cases were on-time for appointments. Several studies in different settings have shown that patients who missed clinical appointments or experienced treatment interruptions were 2-4 times likely to develop treatment failure that eventually lead to development of HIV drug resistance (Ncaca *et al.*, 2011; Kiwuwa-Muyingo *et al.*, 2013; Fox *et al.,* 2012; Robbins *et al.*, 2010; Ndiaye *et al.,* 2013; Arnedo *et al.*, 2012). These studies had reemphasized the recommendation of WHO guidelines that recommended more than 80% on-time for clinical appointments (Bennet *et al.*, 2008, Jordan *et al.*, 2012). Thus the missing clinical appointments should be avoided by all means to avoid treatment failure and potential resistance development. Based on the results obtained from the current study Namibia HIV treatment programs should focus on educating the patients on impact of missing appointments.

5.4. Adverse events

The results for adverse events obtained from the current study showed a wide range of adverse events which were experienced more frequently by cases than controls. There was an association between the presence of adverse events and virological failure. The current study showed that the cases with adverse events were 7 times more likely to experience the virological failure. Several studies were conducted in different settings to assess the impacts of adverse events on treatment of patients initiating the first-line regimen reported on a wide range of adverse events such as: anaemia, diarrhoea, rashes, fatigue, nausea, taste disturbances, vomiting, headache, dizziness and insomnia (Lartey *et al.*, 2014; Shet *et al.,* 2014; Marconi *et al.*, 2013; Al-Dakkak *et al.*, 2013).

The data from these studies showed that the adherence to ART was affected as consequences of adverse events. A study conducted in India on adherence of ART patients showed that the the 15.5% of patients missed a dose due to side-effects of their ARV (Meena *et al*., 2014). A study conducted in South Africa showed that there was an association with fatigue, rash and low baseline CD4 counts with virological failure (Marconi *et al*., 2013).

The current study and the other studies findings suggest that adverse events leading to decreased adherence may lead to the development of drug resistance. By knowing the adverse events can improve the care for patient management. This will beneficial to the programme management of HIV patients by educating them about the adverse events. Thus, the treatment programs need to put efforts on educating the patients about adverse events during counselling so that they will not develop poor adherence due to adverse events.

5.5. TB/HIV co-infection

Even after odd ratios adjustment of the model TB was excluded from the model as a confounding factor for virological failure, TB is a very important aspect whenever dealing with HIV infection. A study conducted in Israel showed that patients with TB/HIV co-infection were 2 times more likely to develop TB multidrug resistance. This study also revealed that there were more deaths among patients with TB/HIV co-infection compared to patients with TB infection only (Zohar *et al*., 2014). In another study it has been shown that patients returning to TB treatment after defaulting were 3 times likely to develop TB/HIV co-infection and patients with TB/HIV co-infection were less likely to get the positive smear for TB diagnosis (do Prado *et al*., 2014). For TB/HIV patients being less likely to get a positive smear warrants the use of sensitive screening tools for HIV positive patients for TB. In addition treating TB/HIV patients could pose a challenge of being a risk factor due to burden of drugs side effects and drug toxicities that could result in defaulting. Thus, there is a need for collaborative effort for the treatment of TB/HIV co-infection by evaluating the performance of TB and HIV clinics to decrease the co-infection.

Further analysis of the results for cases with TB infection revealed that males were vulnerable for TB/HIV co-infection. The current study also showed that there were more TB/HIV co-infection cases in urban areas than in the rural areas. The current study results agreed with

results from other studies that showed that males and people living in urban areas were being at risk to experience TB/HIV co-infection (do Prado *et al.,* 2014; Zohar *et al*., 2014; Agbor *et al.,* 2015). These studies also showed that low level of education can also a risk factor for developing TB/HIV co-infection. However, in the current study the information on education level was not available in the patient records.

5.6. Study Limitations

Since this study concentrated on patientsø records, some selected records were not available for reviewing due to patients who were transferred out of health facilities or deceased. In addition some patients were lost to follow up after initiation of ART that led to incomplete records that were not usable. Due to the small size of health facilities some cases could not be matched to the controls. The samples that had failed amplification had affected the sample size dramatically. This might have been caused by transport conditions since the samples were transported to a South African laboratory for genotyping. Due to a large number of samples that were lost from the study that might have introduced bias since the sample size was reduced. In addition since there was no baseline genotyping information available, no evidence that the mutations detected in this study were acquired instead of being transmitted. In addition there was no genotyping information available for the controls that could shed more light about presence of mutations and circulation subtypes among the controls.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

This is the first study being conducted in Namibia that investigate the risk factors that are associated with emergence of HIV drug resistance. The current study revealed that there are some ART sites that are not complying with the Namibian ART guidelines. The use appropriate first-line regimen is necessary so that the long- term effectiveness of ART can be sustained and subsequent use of second line regimen cannot be compromised. Maintaining the appropriate first-line regimen prevents the development of HIV drug resistance.

It was deduced from this study that the missing of clinical appointments was strongly associated with emergence of drug resistance. The patients who missed any appointment were found to be 22 times more likely to experience the virological failure. The missing of clinical appointments should be avoided by all means to avoid treatment failure and potential resistance development. Thus, Namibia HIV treatment programs should focus on educating the patients on impact of missing appointments.

 The adverse events were found to be strongly associated with development of HIV drug resistance. This study revealed that cases with adverse events were 7 times more likely to experience the virological failure. By knowing the adverse events can improve the care for patient management. Thus, the treatment programs need to put efforts on educating the patients about adverse events during counselling so that they cannot develop poor adherence due to adverse events. Patients being initiated on the ART and TB treatment need to be monitored very closely because of the high burden of drugs that can lead them to missing doses due to adverse events. The patients should be informed about the adverse events due to interaction of ARV and TB drugs. During clinical visits patients should be encouraged to report any adverse event so that alternative drugs can be arranged.

The drug resistance patterns of mutations observed in this study were similar to patterns observed in other studies in patients failing the first-line regimens. The current study showed that the dominant mutations were M184V/I and K103N for nucleoside reverse transcriptase

inhibitor and non-nucleoside reverse transcriptase inhibitor respectively. In addition the dominant mutations M184V/I and K103N showed an association against gender and side effects. Since M184V/I is conferred by lamivudine which is currently used in the first-line regimen and can also be used in the second-line regimen, this drug should not be used in the second-line regimen without being confirmed by genotyping. Lamivudine should be replaced with didanosine in the second-line regimen. Didanosine has been shown to be effective in patients with lamivudine resistance since does not cause any cross-resistance. On the other hand for K103N which is conferred by nevirapine and is widely used in PMTCT, this drug should be removed from the first-line regimen and be replaced with etravirine. Thus, the patients being initiated on the first-line regimen need to be monitored closely for development of HIV drug resistance that may arise. The HIV drug resistance profile obtained from monitoring can give guidance to the clinicians when making decisions about what drugs to use in the second line regimen.

This study has demonstrated the importance of genotyping the patients being suspected of failing treatment. There were 23% of cases who did not harbor any mutation for nucleoside reverse transcriptase inhibitors and for non-nucleoside reverse transcriptase inhibitors for both regions and these cases shared the same clinical risk factors with those had mutations. If these cases were not genotyped and switched to second-line regimen, this would have been an unnecessary switch to second-line regimen. Thus, genotyping is necessary to confirm the presence of mutations that confer resistance before changing the regimen. In addition this study revealed the importance of adherence counselling, since these cases did not harbor mutation there is a possibility they were not adhering to treatment.

6.2. Recommendations

Based on the results from this study there is a need for Namibia Ministry of Health to reinforce the policy for patients to be initiated on appropriate first-line regimen by educating the health workers about importance of use the correct regimen. The ART sites should be monitored more closely to inspect if the patients are being initiated on the correct first-line regimen. The health workers should abide by Namibia HIV treatment guidelines for treatment and monitoring HIV patients. Currently, Namibia HIV treatment guideline recommends the use of

zidovudine/tenofovir/lamivudine/lopinavir boosted with ritonavir or zidovudine/tenofovir/lamivudine/atazanavir boosted with ritonavir as the second line regimen. Based on the data from the current study I recommend zidovudine/tenofovir/didanosine /lopinavir boosted with ritonavir or zidovudine/tenofovir/ didanosine /atazanavir boosted with ritonavir to be used as the second-line regimens. Lamivudine should be removed from the second-line regimen. In addition I recommend that nevirapine should be removed from the first-line regimen and be replaced with etravirine.

In addition Namibia HIV treatment programs should focus on educating the patients on impact of missing appointments. The Ministry of Health should introduce a policy on default tracing of patients who are missing the clinical appointments. There should be a database with patients being initiated on ART and each patient should provide a telephone number/physical address where he/she can be contacted in case of missing any clinical appointment. The Ministry should introduce the strategies of reminding patients for their upcoming appointments. The Ministry should also involve more community health workers to visit patients and remind them how important to keep clinical appointments.

The HIV patients should be educated on adverse events during counselling so that they cannot develop poor adherence. In addition patients should be assessed for adherence at the baseline and at each clinical visit. Patients should be encouraged to report adverse events so that alternative treatment can be arranged. HIV viral load monitoring should be used more routinely to monitor the patientsø adherence to treatment.

Since TB is a threat to HIV patients there should be a collaborative effort for the treatment of TB/HIV co-infection by evaluating the performance of TB and HIV clinics. The HIV and TB programs need to coordinate to provide ART to TB/HIV co-infected patients and intensifying the TB case finding among people infected with HIV. The Ministry of Health should make it compulsory for HIV patients who are experiencing TB symptoms to be screened with Gene-Xpert for TB that is widely known to be sensitive for early detection of TB. The Ministry of Health should make it compulsory for patients with TB/HIV co-infection to undergo direct observed treatment.

Since there were many incomplete and unavailable records in this study the ART sites need to be monitored regularly to assess the completeness of patientsø records. The Ministry of Health should reinforce the use of electronic record keeping so that information can be retrieved readily.

In this study the contributing factors to the development of HIV drug resistance and dominant HIV drug mutations among patients failing first-line ART regimen in Oshikoto and Oshana regions were identified. The results from this study can give guidance on decision making about factors that need to be addressed for prevention of treatment failure for the patients being on the first-line regimen.

6.3. Recommendations for further research

This study has opened up questions that can be addressed through further studies. Further studies are required to determine the profiles of different adverse events due to the use ARV. Studies need to link the specific adverse events to the initiated regimen. Further studies are required to quantify patientsø adherence to ARV. Studies also need to be conducted to determine the number of missed clinical appointments that can lead to development of HIV drug resistance.
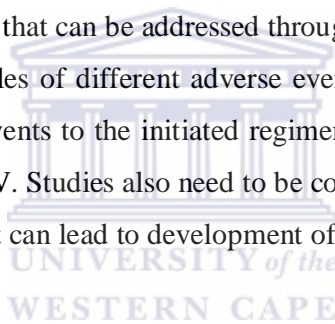
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APPENDIX A

Data abstraction sheet

A. Demographic information

1. Case or Control _____

2. NIP requisition number_____

3. Study identification number_____

4. Initiation date_____

5. Region_____

6. Facility_____

7. Date of birth_____

8. Gender_____

9. Marital status

   a) Single

   b) Married

   c) Divorce

   d) Widow

10. Employment status

   a) unemployed

   b) employed

11. Place of residence

   a) urban

   b) rural

B. Clinical information

   1. WHO clinical stage at initiation_____

   2. Baseline CD4_____

   3.   CD4 at 6 months_____

   4. CD4 at 12 months_____

   5. Self-reported adverse event/side effect prior to failure_____

   6. Any TB infection _____

   7. Alcohol/drug abuse recorded_____

C. Factors

1. First line regimen at initiation_____

2. Retention on first-line regimen at 6 months_____

3. Retention on first-line regimen at 12 months_____

4. Missed clinical appointments prior to failure_____

5. Number of treatment interruptions prior to failure _____

6. Viral load at 6 months_____

7. Viral load at genotyping _____

8. Duration on ARV prior to failure_____

D. Genotyping information

1. Genotyping year_____

2. Was amplification successful?_____

3. Were mutations detected? _____

4. Subtype_____

5. List of NNRTI mutations detected

6. List of NRTI mutations detected
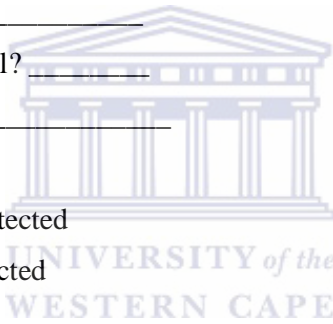
APPENDIX B

Ethics Clearance

OFFICE OF THE DEAN
DEPARTMENT OF RESEARCH DEVELOPMENT

UNIVERSITY of the
WESTERN CAPE

11 June 2014

To Whom It May Concern

I hereby certify that the Senate Research Committee of the University of the Western Cape approved the methodology and ethics of the following research project by:
Dr A Shiningavamwe (School of Public Health)

Research Project:	Contributing factors for patients failing first-line regimen and HIV-1 Drug Mutation Patterns in Oshikoto and Oshana regions, Namibia.
Registration no:	14/5/26

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

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

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

Private Bag X17, Bellville 7535, South Africa
T: +27 21 959 2988/2948 . F: +27 21 959 3170
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APPENDIX C

Ethics Clearance

REPUBLIC OF NAMIBIA

Ministry of Health and Social Services

Private Bag 13198 Windhoek Namibia	Ministerial Building Harvey Street Windhoek	Tel: 061 – 203 2560 Fax: 061 – 222558 E-mail: tkakili@@mhss.gov.na

OFFICE OF THE PERMANENT SECRETARY

Ref: 17/3/3
Enquiries: Ms. T. Kakili
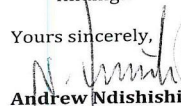
Date: 03 June 2014

Dr. Andreas Shiningavamwe
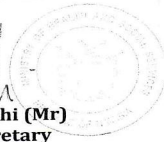**P.O. Box 98276**
Pelican Square
Windhoek

Dear Dr Shiningavamwe

Re: Contributing factors for patients failing first-line regimen and HIV-1 drug mutation patterns in Oshikoto and Oshana regions, Namibia.

1. Reference is made to your application to conduct the above-mentioned study.

2. The proposal has been evaluated and found to have merit.

3. **Kindly be informed that permission to conduct the study has been granted under the following conditions:**

3.1 The data to be collected must only be used for completion of your Master of Public Health degree;

3.2 No other data should be collected other than the data stated in the proposal;

3.3 A quarterly report to be submitted to the Ministry's Research Unit;

3.4 Preliminary findings to be submitted upon completion of the study;

3.5 Final report to be submitted upon completion of the study;

3.6 Separate permission should be sought from the Ministry for the publication of the findings.

Yours sincerely,

Andrew Ndishishi (Mr)
Permanent Secretary

"Health for All"