**Acquired HIV drug resistance mutations on first-line antiretroviral therapy in Southern
Africa: Bayesian evidence synthesis**

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Abstract

Background: Until 2019, first-line antiretroviral therapy (ART) in Southern Africa consisted of one non-nucleoside reverse transcriptase inhibitor (NNRTI) and two nucleoside reverse transcriptase inhibitors (NRTI). As a response to the increasing level of NNRTI resistance, these drugs are being replaced by dolutegravir (DTG), an integrase inhibitor with a high barrier to resistance. Patients failing an NNRTI-based regimen might therefore start DTG-based therapy with preexisting NRTI resistance, potentially jeopardizing the long-term success of DTG-based ART. We performed a systematic review and meta-analysis to quantify the prevalence of NRTI drug resistance mutations (DRMs) in patients failing NNRTI-based ART in Southern Africa.

Methods: We searched several bibliographic databases, including Embase and Medline, from inception to May 2019 to identify studies reporting NRTI DRMs observed among adult HIV-positive patients experiencing virological failure on first-line NNRTI-based regimens in countries of Southern Africa. After screening titles and abstracts, two independent reviewers assessed full manuscripts of potentially eligible studies and extracted data. We developed a hierarchical logistic meta-regression model to synthesize the effect of different ART regimen on the emergence of NRTI and NNRTI DRMs across studies, accounting for ART duration and study-specific effects. Analyses were performed in a Bayesian framework using the *rstan* package in R.

Results: Of 7,579 studies, 3,247 were duplicates and 4,135 were excluded after initial screening. After assessing 194 full-texts, we included 15 studies with 17 study samples and 2,432 individuals from South Africa (13 studies), Mozambique (1), Botswana (1), Lesotho (1) and Zambia (1). We analyzed the dynamics of nine NRTI DRMs by ART regimen. Baseline levels of DRMs were low, ranging from 0.2% to 7.8%. The use of emtricitabine/lamivudine was associated with development of high levels of the M184V/I mutation (1.2% at baseline vs. 64% after 3 years on treatment). When emtricitabine/lamivudine was combined with tenofovir disoproxil fumarate, a substantial increase in the K65R mutation (0.8% at baseline vs. 69.5% after 3 years) was observed. We also analyzed the dynamics of seven NNRTI DRMs after 3 years. With a prevalence of 45.6% after 3 years of efavirenz, K103 was the most prevalent NNRTI resistance mutation, followed by V106 (35.5% after 3 years of efavirenz) and Y181 (14.7% after 3 years of nevirapine).

Interpretation: In patients failing first-line ART in Southern Africa, the prevalence of NRTI DRM is high, suggesting that a substantial proportion of patients failing NNRTI-based regimen will switch to DTG-based regimen with non-working NRTIs. This could potentially impair the long-term efficacy of DTG-introduction in Southern Africa.

Introduction

UNAIDS released the 90-90-90 targets in 2014 with the aim to end the AIDS epidemic by 2030 by ensuring that 90% of people living with HIV know their HIV status, 90% of people with diagnosed HIV infection are on antiretroviral therapy (ART) and 90% of patients on ART have undetectable viral load [1]. In 2020 two third of the estimated 38 million people living with HIV knew their HIV status and were on ART [2]. Meeting the third of the 90-90-90 targets depends on patients' adherence to treatment and the development, or avoidance, of drug resistance [3]. Drug resistance mutations may already be present at the start of treatment (pre-treatment or transmitted drug resistance), or may develop during ART (acquired drug resistance) and consequently lead to treatment failure. Also, the patterns of drug resistance mutations may be HIV subtype specific [4].

In the last two decades, first-line treatment implemented in most low- and middle-income countries (LMIC) consisted of a non-nucleoside reverse-transcriptase inhibitor (NNRTI) combined with two nucleos(t)ide reverse transcriptase inhibitors (NRTI). For many NNRTIs, high level resistance can be reached by acquiring a single mutation, for example K103N [5]. Pre-treatment resistance to NNRTIs has exceeded the WHO threshold of 10% in the Southern African nations of Botswana and Namibia [6], which, according to WHO guidelines, calls for a change in regimen. Several countries in this region are therefore transitioning from the NNRTI based first-line triple therapy towards an integrase-strand-transfer-inhibitor (InSTI) based first-line triple therapy, namely dolutegravir (DTG). DTG has a high genetic barrier to resistance, but levels of acquired NRTI drug resistance may also be important to the success of first-line DTG [7]. A recent cross-sectional survey in South Africa showed that 83% of patients failing NNRTI-based treatment had the M184V/I mutation and over half the patients developed K65R [8]. Such results highly depends on the study characteristics, for instance the regimen used and treatment duration. In contrast to transmitted drug resistance, only few studies have assessed acquired drug resistance in patients failing first-line ART in Southern Africa [6].

We performed a systematic review and meta-analysis of studies reporting frequencies of drug resistance mutations in patients failing first-line ART in Southern Africa, a region heavily affected by HIV and where subtype C causes most of infections [9–11].

Methods

Literature search

We searched several bibliographic databases, including Embase and Medline, using terms for “HIV” AND “anti-retroviral therapy” AND “drug-resistance mutations” AND “Southern Africa”. For each term, we used both index terms (Emtree or MeSH) and free text words (see S1 File). We did not include any time limit but the subject effectively limited the search to articles from 2004 onwards, when combination ART began to be introduced in Southern Africa. We performed the final search on 16 May 2020 and imported the bibliographic references into an EndNote database (version 18.0.0) where we de-duplicated the references with the aid of the built-in deduplication algorithm. We registered the systematic review in the International Prospective Register of Systematic Reviews (PROSPERO) under number CRD42017076406 [12].

Inclusion criteria

We searched for studies from Southern Africa in adult (15 years or older) HIV-1 infected patients on NNRTI-based first-line antiretroviral combination therapy who were at least three months on treatment and experienced virologic failure. Reverse transcriptase genotypes had to be obtained using standard RT-PCR and at least ten genotypes had to be reported. To prevent the selective reporting of individual mutations, studies had to report the mutation frequencies of all major mutations that appear in the Stanford HIV drug resistance database [13].

In the first round of article selection, FG assessed articles based on their title and abstract. In a second round, AH and FG assessed potentially eligible studies based on the full text and additional material online. If the full text was inconclusive, we searched for additional data from related publications from the same study. We compared the results of full text screening and reached consensus by revisiting discrepant studies. If any two publications were based on overlapping datasets, we included the article with the larger sample size, or the most recent publication.

Data extraction

From each publication, we extracted details on the study population and the setting, study year, the number of patients tested for viral load, the number with virologic failure, the definition of failure used in the study (threshold viral load, number of measurements above threshold) and the most common first-line ART regimen. We also recorded the number of patients with an HIV genotype and extracted the number of individuals with any drug resistance mutation, any NRTI mutation, and any NNRTI resistance mutation. We grouped resistance mutations by locus (for example M184I/V). Some studies provided only relative frequencies; we back calculated these values to absolute frequencies

using the number of individuals genotyped. We defined year of study as the year the study was performed, the year enrollment ended or using the 'sampling date' field from the nucleotide sequences of that study deposited in GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>). If studies reported the statistics for multiple populations (for example by country or level of urbanization), we extracted and analyzed the data separately.

Bayesian analysis of the NRTI DRMs

We developed a hierarchical logistic meta-regression model that estimates the prevalence of different NRTI mutations (M41L, K65N/R, D67N/G/E, K70E/G/R, M184I/V, L210W, T215_F/I/N/S/Y, K219D/E/N/Q/R) at baseline (i.e. before treatment) and after virological failure under a given regimen ([Figure 1](#) and [S2 File Figure S1](#)). We used a Bayesian framework, which has the advantage to fully propagating parameter uncertainty. To synthesize the effect of different NRTI drug combinations on NRTI DRM emergence across studies, we integrate a logistic regression into the model adjusting for the use of each NRTI drug and the time spent on ART.

We considered five different NRTI drugs: didanosine (ddI), emtricitabine/lamivudine (FTC/3TC), tenofovir disoproxil fumarate (TDF), stavudine (d4T) and zidovudine (ZDV). As the two prescribed NRTI backbones mostly comprise either ddI or FTC/3TC and either TDF, d4T or ZDV, we observe high correlation between the five NRTI drugs (see [S2 File Figure S2](#)). This multicollinearity of the covariates undermine the ability of the model to retrieve the effect of a single NRTI drug on a given NRTI DRM. Therefore, we tested different models that deal with correlated covariates in a simulation study and selected the ones with the best performance (see [S2 File Figure S3](#)). More information about the different models tested and the hypotheses done in the simulation study can be found in [S2 File Section 2](#) and in <https://github.com/anthonyhauser/ADR-meta-analysis>.

The hierarchical structure of the model allows it to distinguish between the two main sources of heterogeneity: 1) the study-level heterogeneity that will influence the prevalence of any ADR mutation in a given study, and 2) the mutation-level heterogeneity that remains after removing the study-level heterogeneity. To do so, the model includes a main random effect for the study-level heterogeneity and an additional random effect at the mutation level. To identify these two sources of heterogeneity, we estimated the prevalence of the eight single NRTI DRMs simultaneously.

Estimating the prevalence of having at least one TAM mutation

We also estimated the prevalence of any thymidine-analog mutation (TAM) after failing a given regimen. As only few studies report the prevalence of any TAM, we adopted a different method from the one used for estimating the prevalence of single NRTI DRMs. We inferred the correlation between the 6 TAMs from the studies reporting both the prevalence of the single TAM mutations

and the prevalence of any TAM, assuming a multivariate Bernoulli distribution. We then used this estimate on the correlation to calculate the prevalence of any TAM from the prevalence of the six single TAM mutations that were previously estimated by the model.

Bayesian analysis of the NNRTI DRMs

We applied the same model to estimate the prevalence of the different NNRTI mutations (K101E/H/P/Q, K103N/R/S, V106A/I/M, V108I, Y181C/I/V, Y188C/H/L, G190A/E/R/S) after virological failure under either an efavirenz (EFV)- or a nevirapine (NVP)-based regimen. Unlike the NRTI DRMs, it was not possible to estimate the baseline prevalence of NNRTI DRMs, as both EFV and NVP are assumed to have an effect on the occurrence of the different NNRTI DRMs.

Risk of bias and sensitivity analysis

Two of us (AH, ME) independently assessed study designs to gauge the degree of representativeness of the patients for whom HIV genotypes were available. Specifically, we calculated the percentage among patients with virologic failure who had HIV genotypes. We considered studies at high risk of selection bias if i) genotyping rates were low (<50% of patients with failure were successfully genotyped); ii) there were relevant differences between the characteristics of genotyped and non-genotyped patient groups or iii) there was no clearly defined source population for patients to be genotyped. In the sensitivity analysis, we removed studies fulfilling one or more of these criteria and reran the analyses to examine the effect these studies had on the results.

Results

Selection and characteristics of studies

Our initial search produced 7579 articles of which 3247 were duplicates ([Figure 2](#)). We excluded 4138 papers based on title and abstract. We read the full texts of the remaining 194 articles. Of these, 15 studies were eligible, with 17 unique study samples and 2,432 individuals with genotyping information from South Africa (13 studies), Mozambique (1), Botswana (1), Lesotho (1) and Zambia (1) [8,14–30]. Most of the studies were performed in an urban setting. [Table 1](#) summarizes the study samples, the numbers of patients included and their characteristics. Most studies had used a definition of viral failure as a viral load >1000 cp/ml, either as a single measurement or confirmed by a second measure, as recommended by the WHO. The most commonly used NRTI combinations were either FTC/3TC with TDF (38% of patients), d4T (38%) or ZDV (19%). These NRTI drugs were mostly combined with either EFV (74%) or NVP (26%). [Table 2](#) provides a detailed description of each study population. Of 3592 patients with virological failure, 2432 (68%) had HIV genotype data available. The gap between the two numbers was driven by three large studies: a national survey in South Africa [8], which included 1033 patients but obtained genotypes only for 788, and three studies in South Africa [14,26,27], where only a subsample of the patients failing first-line ART had genotyping of HIV performed. Across all study populations, the median percentage of patients with virologic failure who had information on the HIV genotype was high (92%, [Table 1](#)).

Prevalence of acquired NRTI resistance mutations

The model assessed both the baseline (before treatment) prevalence of eight NRTI DRMs and the prevalence after 3 years, by first-line regimen ([Figure 3A](#)). The prevalence of any TAM mutation (i.e. M41L, D67E/G/N, K70E/G/R, L210W, T215F/I/N/S/Y, K219Q/E) at baseline and after 3 years of ART is also displayed. We observe low prevalence at baseline for all eight single NRTI DRMs, ranging from 0.2% to 7.8%. The use of FTC/3TC was associated with development of high levels of the M184V/I mutation (1.2%, credibility interval (CrI): 0%-31.5%, at baseline vs. 64%, CrI: 43.1%-80.4%, after 3 years on treatment). When FTC/3TC was combined with TDF, a substantial increase in the K65N/R mutation (0.8%, CrI: 0.1%-4.5%, at baseline vs. 69.5%, CrI: 37.3%-91.6%, after 3 years) was observed. The prevalences of each of the six TAM mutations after 3 years of FTC/3TC combined with either TDF or ZDV were low, ranging from 0.8% to 13.2%. Finally, the model estimated that around 21% patients harbored at least one of the six TAM mutations after 3 years of ART, which indicates some level of correlation between the different TAMs.

Prevalence of acquired NNRTI resistance mutations

Figure 4 shows the estimated prevalence of the seven NNRTI DRMs after 3 years of either EFV- or NVP-based regimens. K103N/R/S was the most frequent NNRTI DRM, with similar prevalences after 3 years of EFV-based (45.6%, CrI: 33%-59.7%) or NVP-based regimens (42.5%, CrI: 34.4%-50.7%). Other NNRTI DRMs with point estimates over 10% included V106A/I/M, Y181C/I/V and G190A/E/R/S. We did not observe clear differences in prevalences of NNRTI DRMs between the two NNRTI drugs EFV and NVP. Of note, the model estimated a slightly higher prevalence of the Y181 mutations after the use of NVP (14.7%, CrI 9.7%-20.9%) than with EFV (7.9%, CrI 3.7%-15.1%), which reflects the higher impact of Y181 on NVP, as observed in [31].

Risk of bias and sensitivity analysis

We identified four studies at high risk of selection bias [14,18,26,27]. Three had rates of genotyping below 50% [14,26,27]. Two of these by design only genotyped a subsample of infections [26,27]. One study [26] reported that every third patient was systematically sampled for genotyping, however, the number of infections genotyped was substantially lower (10.1% rather than 33.3%, Table 2). Genotyping in the other study [27] depended on the availability of residual plasma. The last study lacked a clearly defined source population. It was based on referrals of patients with suspected first-line treatment failure at a University Teaching Hospital [18]. Estimating the prevalence of the eight NRTI DRMs and seven NNRTI DRMs excluding the high-risk studies showed that most estimates were very similar to the main analysis, but with larger uncertainty (S2 File Figure S4, Table S5).

Discussion

This study provides an overview of all clinically relevant drug resistance mutation prevalent in patients failing first-line treatment in Southern Africa. We extracted mutation frequencies from 15 different studies and although the majority of studies were from the Republic of South Africa, our systematic review covers most of the region. The K65, M184 and K103 mutations were the three most frequent mutations among patients with virological failure, with estimated prevalences of 69.5%, 64% and 45.6% after 3 years on treatment, respectively. K103 is readily transmitted and thus poses a threat in newly infected individuals starting ART [32]. K65 and M184 confer high level of resistance to 3TC/FTC and TDF, the two NRTI backbones that are usually combined in the new DTG-based regimens. The model also estimated that around 21% of patients failing NNRTI-based regimen had at least one TAM. By using a hierarchical structure and by adjusting for the use of different drugs, the model provides reliable estimates of the major NRTI/NNRTI mutations, even if some heterogeneity subsists.

We observe a high prevalence of the K103 mutation, irrespective of the NNRTI drug used (45.6% for EFV, 42.5% for NVP). As K103 alone confers high level of resistance to both drugs, this emphasizes the need of a second-line regimen for these people. Unfortunately, the high cost of PI-based regimen prevents its wide use. In South Africa for instance, it was estimated that only 4% of people were treated with second-line PI-based regimen in 2016 [33]. Long duration on a failing first-line ART facilitates the spread of NNRTI resistance, as shown by the increasing prevalence of NNRTI PDR in Southern Africa, which had led many countries to transition to DTG-based first-line therapy [34,35]. Among the other NNRTI drug resistance mutations, the Y181C/I/V mutation is of particular concern. The average prevalence of this mutation was 9.7% and 14.7% after 3 years of EFV and NVP, respectively. This mutation also confers resistance to the newer generation of NNRTIs (such as etravirine and rilpivirine), which generally can be used in the presence other NNRTI mutations.

In the context of DTG introduction, our results regarding NRTI resistance mutations are highly relevant. In countries where viral load monitoring is not routinely available, many patients on first-line ART will be switched to DTG-based ART despite detectable viral load. This could increase the risk of selection of resistance to DTG as many patients with virologic failure on first-line NNRTI-based ART will switch with pre-existing NRTI resistance.

The DAWNING trial [36] showed that DTG-based ART is effective in second-line ART provided it is combined with at least one fully active NRTI, and there were no primary INSTI resistance mutations in the DTG group. However, it is unknown whether DTG will be effective with no fully active NRTIs. Wandeler and colleagues showed the increase in risk of treatment failure when DTG is used as a

monotherapy compared with when combined with a NRTI backbone (resp. 8.9% vs 0.7% after 48 weeks of ART). The efficacy of DTG in patients with two non-working NRTI backbones might actually be still higher than DTG-monotherapy, as some NRTI activity may subsist even in case of full resistance. This is suggested by the EARNEST study, where the presence of NRTI resistance did not impair virological response to second-line regimen [37].

Combining the high frequencies of K65N/R (69.5%) and M184I/V (64%) suggests that between 44.5% ($69.5\% \cdot 64\%$) and 64% of the patients failing first-line ART will have both mutations, depending on the correlation between the mutations. In its new guidelines, the South African Department of Health recommends to switch patients failing first-line regimen that includes 3TC/FTC and TDF to DTG combined with 3TC/FTC and ZDV, in order to have at least one working NRTI [38]. However, in view of the toxicity and side effects associated to ZDV and because there does not exist a single pill combining DTG and ZDV, TDF might be preferred over ZDV. In this context, our results show that nearly half of these patients will the start a DTG-based regimen without working NRTI backbone, which will impair the long-term efficacy and sustainability of such regimen.

Our meta-analysis also show that TAM mutations are present at a moderate level among people failing NNRTI-based first-line regimen. Interestingly, in several studies the detection of NRTI resistance and particularly of TAMs prior to starting second-line ART was associated with better virological suppression, possibly because patients who develop resistance may on average have higher levels of adherence [39,40]. Clearly, the large-scale switch to DTG-based ART should be accompanied by longitudinal, real-world studies of virologic failure and drug resistance.

Our study has several strengths. Most previous reviews of HIV drug resistance focused on transmitted drug resistance, rather than resistance that was likely acquired during ART[41]. Previous reviews on acquired resistance tended to focus on failure rates, CD4-positive lymphocyte counts and the prevalence of drug resistance mutations overall [42,43], or on a subset of mutations [6,44,45]. Our meta-analysis provides estimates of all relevant acquired drug resistance mutations according to the drugs used, for the whole region of Southern Africa, the region carrying the burden of the HIV epidemic. To our knowledge, this is the first model that uses a hierarchical structure and estimates the DRMs simultaneously in order to identify and adjust for the study effect.

Our review also has limitations. Many of the included studies were based on patients attending one or few outpatient clinics. We carefully assessed the likely representativeness of the patients undergoing HIV genotyping but even if results reflect the situation in these clinics, they may not be representative of all patients who fail first-line ART in the region. Indeed, the between-study heterogeneity was large, and decision makers should consider the studies most relevant to their

settings, as well as the regional data. Many factors may have introduced heterogeneity, including differences between patient populations, their levels of adherence, the first-line regimen and the time spent on a failing regimen. The hierarchical structure of the model adjusted for regimen and time spent on a failing regimen, but some heterogeneity remains. Of note, in our sensitivity analyses, the likely risk of selection bias did not appear to influence estimates. [6]. Finally, the data analyzed in this systematic review and meta-analysis are strictly speaking frequencies of resistance mutations in patients failing treatment. The studies attempted to assess pre-treatment NRTI mutations and but it was not possible to disentangle pre-treatment from acquired drug NNRTI resistance.

Although we searched for studies from the whole of Southern Africa, the majority of data included in our analysis were from the Republic of South Africa. South Africa is one of few countries in the regions that have implemented routine viral load monitoring in patients on ART, and routine viral load monitoring is associated with a reduced probability of drug resistance [45]. Therefore, the overrepresentation of South Africa studies in our meta-analysis might underestimate the different mutations frequencies in the rest of Southern Africa.

In conclusion, our analysis demonstrates that throughout Southern Africa, patients failing first-line ART exhibit high frequencies of drug resistance mutations, with important implications for the likely future transmission of drug resistance, the choice of second-line therapy, and the large-scale transition to DTG-based first-line ART in settings where viral load monitoring and drug resistance testing are not routinely available.

303 **Conflict of Interest Statement**

304 The authors have no conflict of interest to declare.

305 **Authorship**

306 ME, RK and FG conceived the study. FG and AH searched and collected the literature. FG, AH and MR
307 performed the screening of publications. AH and JR performed the statistical analysis. NL, ME and FG
308 analyzed the risk-of-bias. All authors interpreted the results. AH drafted the manuscript. All authors
309 provided comments on the manuscript.

310 **Acknowledgements**

311 We are grateful to Doris Kopp and Beatrice Minder for their support with searching and evaluating
312 the literature. This study was funded by the National Institute of Health under award number
313 U01AI069924. RK is supported by Starting Grant No. 155851 and ME by special project funding (No.
314 174281) from the Swiss National Science Foundation.

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518 **Table 1. Characteristics of the 17 study samples included in the systematic review.**

Total No. of patients with virologic failure	3592
Total No. of patients with genotype data	2432
Median (range) number of patients with virological failure	103 (31-950)
Median (range) number of patients with HIV genotype	68 (19-788)
Median (range) percent patients with HIV genotype among patients with virologic failure	92% (10-100%)
Definition of virologic failure (copies/mL)	
Confirmed >1000	6
Single value >1000	6
Value >5000	3
Single value >400	1
Confirmed >80	1
Median (range) study year	2012 (2004-2014)
Most common first-line regimen	
d4T + 3TC + EFV	6
TDF + 3TC + EFV	2
ZDV + 3TC + NVP	2
ZDV + 3TC + EFV	3
Other	4
Country	
Botswana	1
Lesotho	1
Mozambique	1
South Africa	13
Zambia	1
Urbanization	
Urban	8
Rural	5
Both	4

519 The number of study populations is shown unless otherwise specified. Further details are given in Table 2.

520

521 **Table 2. Characteristics of 17 study populations included in the systematic review and meta-analysis.**

No.	Publication	Study population	Setting	Urban / rural	Study period	No. of patients (% female)			Definition of VF		Most common first-line regimen (%)	Use of NNRTI and NRTI reported	At high risk of bias
						No. tested for VL at least once	No. with VF*	No. with genotype#	Threshold (cps/ml)	No. of measures			
1	Barth et al. 2012 [14]	Cohort of patients on first-line ART ≥2 years	Ndlovu Medical Centre, Elandsdoorn, South Africa	Rural	n.r.	562 (72%)	47	19	>1000	Repeated measure	d4T+3TC+EFV (58%)	NRTI: yes NNRTI: yes	Yes
2	Brehm et al. 2012 [24]	Patients participating in CIPRA-SA trial [46] of nurse versus physician led care (NCT00080522)	Primary care clinics in Masiphumelele, Cape Town and Soweto, Johannesburg, South Africa	Urban	2007	812 (71%)	63	63	>1000 after 24 weeks	Single measure	d4T+3TC+EFV (65%)	NRTI: yes NNRTI: yes	No
3	El-Khatib et al. 2010 [25]	Cross-sectional study of patients on first-line ART >12 months	Outpatient clinics at Chris Hani Baragwanath Hospital, Soweto, South Africa	Urban	2008	883 (75%)	102	94	>400	Single measure	d4T+3TC (100%)	NRTI: yes NNRTI: no	No
4	Hoffmann et al. 2009 [26]	Cohort of HIV-positive miners on first-line ART	Multi-site workplace HIV program in mining regions, South Africa	Both	2007	3727 (7%)	676	68	>1000	Mostly repeated measure	ZDV+3TC+EFV (95%)	NRTI: yes NNRTI: yes	Yes
5	Hoffmann et al. 2013 [27]	Cohort of patients on first-line ART switching to TDF	Multisite workplace and community HIV program, South Africa	Both	2011	1682 (50%)	270	40	>1000, with prior RNA <400	Repeated measure	TDF+3TC+EFV/NVP (100%)	NRTI: yes NNRTI: no	Yes
6	Hunt et al. 2017 REF	Cross-sectional study of patients on first-line ART >12 months	15 ART-providing primary healthcare facilities in	Rural	2017	1299	123	123	>1000	Single	TDF+3TC (85%)	NRTI: yes NNRTI: no	No

			KwaZulu-Natal, South Africa										
7	Labhardt et al. 2016 [28]	Cohort of patients on first-line ART ≥ 6 months	Ten nurse-led clinics in rural Lesotho	Rural	2014	1563	80	74	>80	Repeated measure	ZDV/TDF+3TC+EFV (65%)	NRTI: yes NNRTI: yes	No
8	Marconi et al. 2008 [29]	Cross-sectional study of patients on first-line ART >6 months	Outpatient clinic in Durban in Durban and Mariannhill, South Africa	Both	2006	n.r.	124	115 (52%)	>1000	Single measure	d4T+3TC+EFV (49%)	NRTI: yes NNRTI: yes	No
9	Novitsky et al. 2007 [15]	Participants in one arm of Tshepo trial [47,48] (NCT00197613)	Princess Marina Hospital, Gabarone, Botswana	Urban	2004	108 (69%)	31	23	>5000 after 16 weeks	Repeated measure	ZDV+ddI+EFV/NVP (100%)	NRTI: yes NNRTI: no	No
10	Rossouw et al. 2017 [16]	Cohort of patients on first-line ART ≥ 6 months	Tshwane District Hospital in Pretoria, South Africa	Urban	2012	n.r.	103 (73%)	102	>1000	Repeated measure	ZDV+3TC+EFV (23%)	NRTI: yes NNRTI: yes	No
11	Rossouw et al. 2017 [16]	Cohort of patients on first-line ART ≥ 6 months	Clinic in Hlabisa subdistrict, KwaZulu-Natal, South Africa	Rural	2013	n.r.	492 (72%)	492	>1000	Repeated measure	d4T+3TC+EFV (44%)	NRTI: yes NNRTI: yes	No
12	Ruperez et al. 2015 [17]	Cross-sectional study of patients on first-line ART ≥ 1 year.	District Hospital in Manhica, Mozambique	Rural	2013	332 (70%)	81	61	>1000	Single measure	ZDV+3TC+NVP (90%)	NRTI: yes NNRTI: yes	No
13	Seu et al. 2015 [18]	Patients referred after failing first-line ART after >6 months	University Teaching Hospital, Lusaka, Zambia	Urban	2012	n.r.	68 (54%)	68 (54%)	>1000	Single measure	ZDV+3TC+NVP (38%)	NRTI: yes NNRTI: yes	Yes
14	Singh et al. 2011 [19]	Patients failing first-line ART after ≥ 6 months	McCord Hospital, Durban, South Africa	Urban	2009	n.r.	45 (65%)	43	>5000	Single measure	d4T+3TC+EFV (51%)	NRTI: yes NNRTI: yes	No
15	Steege et al. 2017 [8]	National survey of patients failing first-line ART after ≥ 6 months	91 healthcare facilities in nine provinces in South Africa	Both	2014	n.r.	1033	788 (65%)	>1000	Repeated measure	TDF+3TC (52%)+EFV (82%)	NRTI: yes NNRTI: yes	No
16	Sunpath et al. 2012 [20]	Cross-sectional study of patients on TDF-containing	McCord Hospital, Durban, South Africa	Urban	2011	585	35 (46%)	33	>1000	Single measure	TDF+3TC+EFV (89%)	NRTI: yes NNRTI: yes	No

first-line ART >5 months													
17	Wallis et al. 2010 REF	Patients with virologic failure on first-line regimens	Charlotte Maxele Academic Hospital and Helen Joseph Hospital, Johannesburg, South Africa	Urban	2007	n.r.	226	226	>5000 or >1000, resp.	Repeated measure and single measure, resp.	d4T+3TC+EFV (65%)	NRTI: yes NNRTI: yes	No

ART, antiretroviral therapy; VL, viral load; VF, virologic failure; n.r.; not reported; d4T, stavudine; 3TC, lamivudine; TDF, tenofovir; NVP, nevirapine; EFV, efavirenz; LPV/r, lopinavir/ritonavir; ACTG, AIDS Clinical Trials Group; NCT,

* As defined in the corresponding study.

Reasons for lack of genotyping in patients with virologic failure:

1: >12 months between the two failure time points, no blood sample available at both time points.

2: Not applicable.

3: Unsuccessful amplification.

4: Genotyping in subsample of patients only. "Individuals with genotype results were similar in terms of sex, age, weight at cART initiation, median CD4 count at cART initiation, median HIV RNA level at cART initiation, WHO stage, and type of NNRTI received to those viremic individuals who did not undergo genotyping (all, $P>0.10$)." [26]

5: No stored plasma from the time of first detection of virologic failure. "Time on TDF at failure was longer for those with stored samples (261 days versus 140 days, $p<0.001$); whereas CD4 count and HIV RNA at failure were similar between patients with and without stored serum ($p>0.1$)." [27]

6: Not applicable.

7: Unsuccessful amplification.

8: Unsuccessful amplification; data incomplete.

9: Missed confirmatory visit, unsuccessful amplification, or technical error.

10: Subtype B.

11: Not applicable.

12: Unclear

13: Not applicable

14: Unsuccessful amplification.

15: "The main reason for exclusion was the inability of obtaining a PCR product ($n=151$, 14.6%). Other reasons for exclusion from the study were: volunteers <18 years of age ($n=35$, 3.4%); <6 months of ART exposure ($n=16$, 1.5%); treatment with a non-standard regimen at time of failure ($n=11$, 1.1%); inadequate completion of informed consent ($n=10$, 1.0%); poor sample integrity ($n=8$, 0.8%); <6months of NNRTI exposure ($n=6$, 0.6%); or failure to obtain a sequence ($n=3$, 0.3%). In addition, five sequences (0.5%) were shown to be duplicate samples as per phylogenetic analysis." [8]

16: Unsuccessful amplification.

17: Not applicable.

549 **Figure 1. Bayesian hierarchical model adjusting for the different levels of heterogeneity.**

550

551 **Figure 2. PRISMA flow-chart of inclusion of studies and populations in the systematic review.**

552

553

Figure 3. Prevalence of nine NRTI drug resistance mutations by first-line regimen. Panel A: baseline prevalences and prevalences after 3 years of treatment according to NRTI use. Panel B: Prevalence of the K65 mutation over time. Panel C: Prevalence of the M184 mutation over time. Points and vertical lines: median and 95% credibility intervals of baseline prevalence (black), prevalence after 3 years on 3TC/FTC + TDF (red) or 3TC/FTC + ZDV (blue). Shaded area: 95% credibility interval over time.

561 **Figure 4. Prevalence of seven NNRTI drug resistance mutations.** Points and vertical lines: median
562 and 95% credibility intervals of prevalence after 3 years on EFV (red) or NVP (blue).