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Rates of virological suppression and drug resistance in adult HIV-1-positive patients attending primary healthcare facilities in KwaZulu-Natal, South Africa

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Background: KwaZulu-Natal (KZN) Province in South Africa has the highest HIV disease burden in the country, with an estimated population prevalence of 24.7%. A pilot sentinel surveillance project was undertaken in KZN to classify the proportion of adult patients failing first-line ART and to describe the patterns of drug resistance mutations (DRMs) in patients with virological failure (VF).

Methods: Cross-sectional surveillance of acquired HIV drug resistance was conducted in 15 sentinel ART clinics between August and November 2013. Two population groups were surveyed: on ART for 12–15 months (Cohort A) or 24–36 months (Cohort B). Plasma specimens with viral load $\geq$1000 copies/mL were defined as VF and genotyped for DRMs.

Results: A total of 1299 adults were included in the analysis. The prevalence of VF was 4.0% (95% CI 1.8–8.8) among 540 adults in Cohort A and 7.7% (95% CI 4.4–13.0) of 759 adults in Cohort B. Treatment with efavirenz was more likely to suppress viral load in Cohort A ($P = 0.005$). Independent predictors of VF for Cohort B included male gender, advanced WHO stage at ART initiation and treatment with stavudine or zidovudine compared with tenofovir. DRMs were detected in 89% of 123 specimens with VF, including M184I/V, K103N/S, K65N/R, V106A/M and Y181C.

Conclusions: VF in adults in KZN was <8% up to 3 years post-ART initiation but was associated with a high frequency of DRMs. These data identify key groups for intensified adherence counselling and highlight the need to optimize first-line regimens to maintain viral suppression.

Introduction

The public health approach of using a standardized first-line combination of antiretrovirals has facilitated the rapid scale-up of ART provision, with ~15 million persons in sub-Saharan African countries receiving ART by 2014.[1] Coupled to this, the WHO has devised a global strategy for the prevention and assessment of HIV drug resistance (HIVDR), particularly in resource-limited settings where routine resistance testing for individual patients failing ART is not standard practice.[2] This includes surveys of HIVDR among treatment-naive persons initiating ART, the routine monitoring of HIVDR early warning indicators at representative ART sites, and assessing the emergence of HIVDR among treated individuals. Surveys of acquired HIVDR are designed to determine the proportion of patients on ART who fail to achieve viral suppression and to identify specific HIVDR mutations in patients failing therapy. These surveys also provide information as to the appropriateness of empirical second-line ART in the country. Previously recommended prospective monitoring surveys of persons on ART have provided valuable information on HIVDR; however, the logistics of conducting these cohort studies in resource-limited settings limited their application. The US CDC, in collaboration with the WHO and the HIV Resistance Network (HIVResNet), have developed a pilot cross-sectional survey methodology to assess acquired HIVDR at ART sites in resource-limited settings. Pilot surveys were implemented

at the same time in Kenya and Tanzania, and have informed an updated methodology for assessing acquired HIVDR.[3]

South Africa has an estimated 6.4 million HIV-infected persons,[4] with >2 million receiving ART.[5] KwaZulu-Natal (KZN) Province has the highest HIV prevalence in South Africa, with 37.4% of pregnant women aged 15–49 years reported to be HIV-1-positive in 2012.[6] By the end of 2014, ~850000 persons were on ART within the province.[7] ART services were offered through 615 public health facilities, and ~180000 persons were initiated on ART that year. Monitoring and prevention of HIVDR and related programmatic factors is critical to ensure the continued efficacy of a standardized regimen approach for ART provision in this province.

Surveys of transmitted HIVDR in KZN suggest that low to moderate levels (<15%) of NNRTI HIVDR may be circulating.[8–10] This is further supported by data from two studies showing that transmitted drug resistance is around 7% in KZN: the first is a study showing that 7.4% of women screened for inclusion in a prevention trial between 2010 and 2011 harboured at least one drug resistance mutation (DRM);[11] and the second is a population-based HIV surveillance study at a rural site which showed 7.1% of transmitted resistance in 2012.[12] Studies on acquired HIVDR have reported that >80% of patients failing a HAART regimen in KZN had at least one DRM.[13–15] The expansion of the treatment programme and reports of transmitted and acquired drug resistance underscore the need for continued monitoring of HIVDR in this province. A pilot cross-sectional study was therefore conducted in KZN to estimate the proportion of adult patients retained on first-line ART at public-sector primary healthcare facilities for 1 year and 2–3 years who show virological failure (VF), and to describe the patterns of HIV DRMs in patients with non-suppressed viral load (VL).

Methods

Site selection and inclusion criteria

Fifteen public-sector ART-providing primary healthcare (PHC) facilities in KZN were selected using a purposive, non-probability algorithm to ensure representation of all sites in the province, and based on geographic location and facility size. The study population consisted of adult patients (≥18 years) who were retained on first-line ART for at least 12 months but not longer than 15 months (Cohort A) as well as those retained on ART for at least 24 months but not longer than 36 months (Cohort B). The study was conducted between August and November 2013. Patients receiving any standard first-line ART (stavudine/tenofovir/zidovudine or lamivudine/emtricitabine or efavirenz/nevirapine) regimen were recruited to the study, including those with first-line drug substitutions and those with prior treatment interruption but who were still receiving first-line ART at time of enrolment. Following informed consent, data were extracted from the medical records of each patient and recorded on the study data collection form. Patients were identified by clinic healthcare workers as fitting eligibility criteria and recruited into the study by dedicated surveillance officers (SOs). Data were collected from medical records by the SOs. SOs and healthcare workers were trained to include all eligible patients irrespective of their adherence and/or virological outcome.

HIV VL testing and HIVDR genotyping

Whole blood in EDTA was collected from all participants at the time of interview. Specimens that were haemolysed, lipaemic or failing assay internal quality assessments were considered unsuitable for use and rejected. VL testing was performed on plasma using the Abbott® SP/RT System at the Department of Virology, University of KwaZulu-Natal (UKZN), and VF was defined as a single VL ≥1000 copies/mL. Stored plasma specimens with HIV VL ≥1000 copies/mL were transported by air on dry ice to the National Institute for Communicable Diseases (NICD) laboratory in Johannesburg for genotypic analysis. Genotyping of the *protease* and *reverse transcriptase* regions of the HIV-1 *pol* gene was performed using a validated in-house genotyping assay.[16] Sequence alignments were performed using Sequencher® v. 5.0 sequence analysis software (Gene Codes Corporation, Ann Arbor, MI, USA). Drug-associated resistance mutations were classified using the Stanford HIV Drug Resistance Database algorithm and clinical resistance data were recorded on RegaDB, a database with software tools used to store clinical data related to HIV (https://rega.kuleuven.be/cev/regadb).[17] VL data and drug resistance test results were returned to the clinical sites after study completion.

Statistical analysis

Sample sizes were estimated using Power Analysis and Sample Size (PASS) 11, Confidence Intervals for One Proportion, with 95% CI half-widths of 10% for acquired HIVDR. Sample sizes were adjusted for design effects of 2 in order to account for the increased variation due to clustering within sites. Assuming a VF rate of 20%, a sample size of 520 participants per group, adjusted to 1040 after accounting for design effects, was required to estimate viral suppression rates and prevalence of acquired HIVDR in each population. All analyses accounted for the sample structure by stratifying by site size (large and small) and clustering within clinic. This was accounted for using the svyset function and svy prefix in STATA and indicating the clinic as the primary sampling unit. This provided estimation of both the within- and between-clinic variability, which provided appropriate standard errors for 95% CIs. Modified Poisson regression with robust error estimation was used to identify independent predictors of VF. For inclusion into initial multivariate models, we considered covariates that were significant at the 20% level in bivariate analysis as well as those deemed relevant to VF *a priori*. Variables were removed manually one by one, starting with those with the highest *P*-values so as, finally, to construct a model with those variables that did have a statistically significant effect. All variables available are reported in the crude estimates, whereas only the final significant variables are reported for the adjusted estimates. Significance was set at $P < 0.05$. All analyses were conducted using STATA v. 13 (STATA Corp., College Station, TX, USA).

Ethics review and participant confidentiality

The protocol and informed consent were reviewed and approved by the Institutional Review Boards responsible for oversight of the study, including the University of the Witwatersrand (M121017), KwaZulu-Natal Department of Health and the CDC. All laboratory specimens, data collection forms and other records were identified by a coded participant identification (PID) number to maintain subject confidentiality. All records were kept in a secured area and computer entry and networking programs were done with coded PID numbers only. A master list linking study PID numbers to patient ART clinic number was maintained by the site coordinator.

Results

Participants

A total of 1863 whole-blood specimens were collected for the study with details of eligibility described in Figure 1. At completion of study enrolment, 540 participants were eligible for analysis in Cohort A and 759 participants in Cohort B. Sixty-five specimens were rejected for poor quality; a further 106 specimens were rejected as no regimen start date was recorded for 47 participants and current regimen was not recorded for 59 participants. A total of 349 participants did not fit study inclusion criteria in terms of

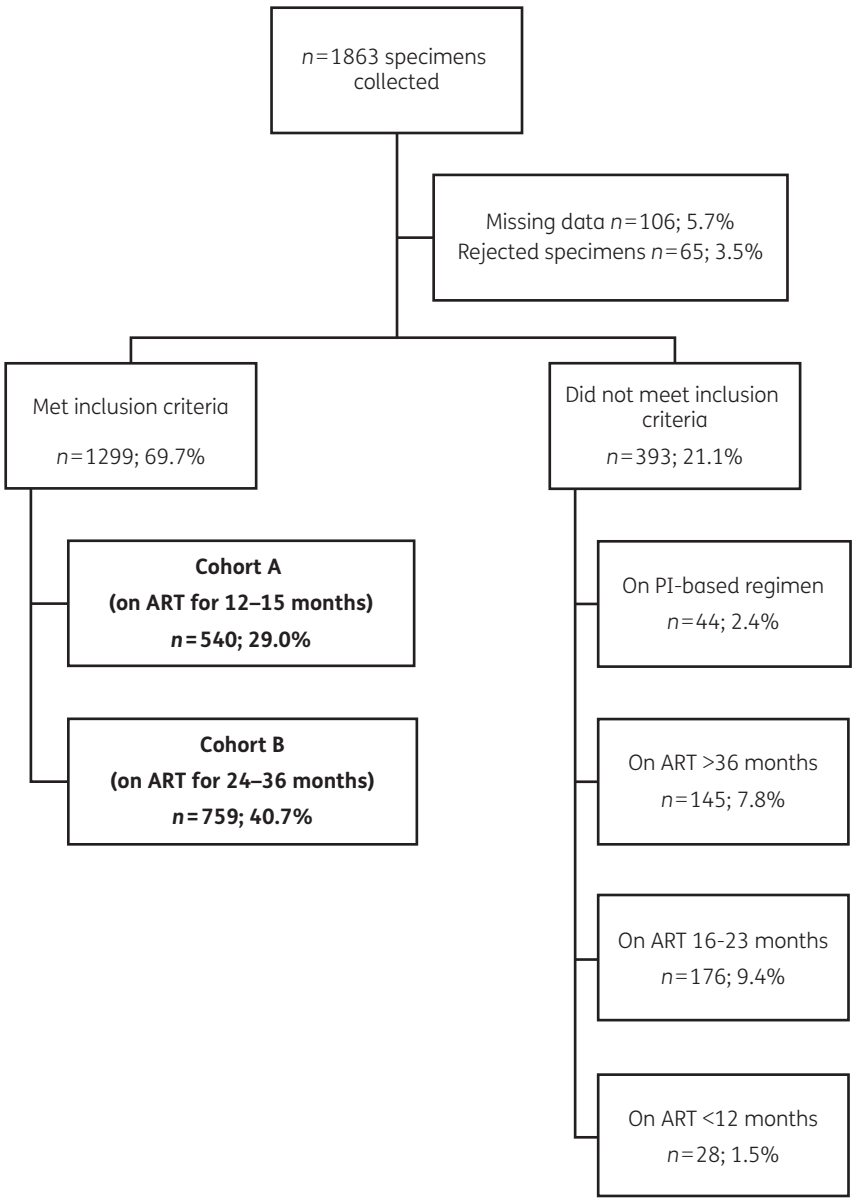

Figure 1. Total study adult patient enrolment.

time on treatment: 145 had been on a first-line regimen for >36 months, 176 for 16–23 months, and 28 for <12 months. A further 44 participants were excluded from analysis as they were receiving lopinavir/ritonavir-based (non-standard first-line) regimens.

The adjusted baseline characteristics of the study participants are shown in Table 1. Females accounted for 81.3% of participants in Cohort A and 86.6% of participants in Cohort B. Most participants enrolled in the study were in the 26–49 year age group (86.8% in Cohort A and 81.2% in Cohort B), with a mean age of 36.7 (95% CI 34.6–38.8) and 38.9 (95% CI 37.8–39.9) years, respectively. The mean CD4 count at ART initiation was 230 (95% CI 192–269) cells/mm^3 and 212 (95% CI 162–262) cells/mm^3 in Cohorts A and B, respectively. The large majority of participants had been initiated on efavirenz, tenofovir and lamivudine, in accordance with 2010 national treatment guidelines.[18,19] However, 37.4% (95% CI 25.6%–50.8%) in Cohort B were on a nevirapine-based regimen which was standard practice at the time of therapy initiation in this group.

HIV VL measurements

Using the WHO-recommended threshold to define VF[20] as a VL assessment of ≥1000 copies/mL and weighting for proportional contribution from the different clinics, 4.0% (95% CI 1.8%–8.8%) were failing at 12–15 months post ART initiation, and 7.7% (95% CI 4.4%–13.0%) at 24–36 months (Table 1).

Table 1. Demographic and clinical characteristics of the adult participants on ART enrolled in the survey

	12–15 month cohort ($n = 540$)			24–36 month cohort ($n = 759$)		
Characteristic	*N*	%	95% CI	*N*	%	95 CI
Age category (years)						
50+	48	6.4	3.1–13.0	101	15.8	9.4–25.3
26–49	369	86.8	75.7–93.3	544	81.2	71.6–88.1
18–25	47	6.8	3.3–13.5	29	3.0	1.4–6.3
Gender						
female	390	81.3	68.0–89.9	570	86.6	77.7–92.2
male	142	18.7	10.1–32.0	182	13.4	7.8–22.1
CD4 at ART initiation (cells/mm^3)						
350+	38	6.3	3.1–12.4	46	8.8	3.3–21.2
200–350	254	49.9	26.7–73.2	191	38.2	26.8–51.0
<200	209	43.8	22.0–68.2	478	53.0	40.5–65.2
WHO stage						
1	146	35.4	13.9–65.0	175	34.5	16.7–58.0
2	160	22.2	10.8–40.1	183	29.3	18.2–43.5
3	108	39.7	16.6–68.5	162	29.9	14.3–52.0
4	11	2.8	0.6–11.4	44	6.4	3.1–12.8
NNRTI						
nevirapine	47	6.9	3.2–14.1	287	37.4	25.6–50.8
efavirenz	486	93.1	85.9–96.8	472	62.6	49.2–74.4
NRTI						
tenofovir	524	85.6	37.5–98.3	694	84.4	60.1–95.1
stavudine/zidovudine	11	14.1	1.6–62.9	40	10.1	1.8–40.5
abacavir/didanosine	5	0.3	0.0–2.2	26	5.4	0.9–25.5
Virological failure						
VL ≥1000 copies/mL	38	4.0	1.8–8.8	85	7.7	4.4–13.0

Predictors of virological failure

Univariate analysis of adult participants on ART for 12–15 months (Cohort A) showed that sex, age, baseline CD4, WHO stage, type of NNRTI or NRTI in ART regimen, or clinic size were not significantly associated with VF (see Table 2). However, on multivariate analysis, efavirenz was associated with reduced risk of VF, compared with nevirapine-based ART [adjusted prevalence ratio (PR) 0.31, 95% CI 0.14–0.70]. Univariate analysis of participants on ART for 24–36 months (Cohort B) showed that being male, of younger age (<50 years), having advanced WHO staging at ART initiation and the use of stavudine as part of the NRTI backbone was a significant predictor for VF ($P < 0.05$, Table 3). On multivariate analysis, only male sex (adjusted PR 1.75, 95% CI 1.06–2.88), advanced WHO stage (adjusted PR 2.71, 95% CI 1.28–5.78; adjusted PR 2.94, 95% CI 1.40–6.17 for WHO stages 2 and 3, respectively, versus WHO stage 1) and type of NRTI in ART regimen (adjusted PR 3.02, 95% CI 1.41–6.49 for use of stavudine versus tenofovir, and adjusted PR 4.10, 95% CI 1.60–10.49 for use of zidovudine versus tenofovir) were independent predictors for VF.

HIVDR genotyping

A total of 123 specimens had a VL ≥1000 copies/mL and HIVDR genotyping was successful. DRMs were detected in 32 of 38 (84%) specimens from Cohort A, 27 (71%) of which displayed dual NRTI and NNRTI class resistance. DRMs were detected in 76 of 85 (89%) Cohort B specimens, of which 66 (78%) showed dual-class resistance patterns. The predominant NRTI mutations detected were: M184I/V (57.9%; 72.9%) and K65R (44.7%; 48.2%) in Cohorts A and B, respectively (Table 4). NNRTI resistance was prevalent in 81.6% and 87.1% of participants with viraemia in Cohorts A and B, respectively. The NNRTI mutations detected most frequently were K103N/S (55.3%; 50.6%), V106A/M (44.7%; 27.1%) and Y181C (26.3%; 23.5%). All sequences clustered with subtype C (data not shown).

At a cohort level, the unweighted prevalence of drug resistance among participants on therapy for 12–15 months was 5.9% (32/540) and 10.0% (76/759) amongst participants on treatment for 24–36 months. NNRTI resistance was prevalent in 5.7% and 9.7% of viraemic participants in Cohorts A and B, respectively. Amongst those receiving a tenofovir-based regimen, failure rates were 7.3% and 10.0% in Cohorts A and B, respectively, and within failure sequences, the K65R mutation was present in 42.9% and 54.5%.

Discussion

This pilot survey was conducted in 15 clinics in KZN to assess levels of acquired HIVDR among participants receiving standard first-line

Table 2. Predictors of virological failure (VL ≥1000 copies/mL) among adult participants on ART for 12–15 months

Characteristic	Crude PR	95% CI	*P* value	Adjusted PR	95% CI	*P* value
Gender						
female	1.00	1.00				
male	1.49	0.78–2.84	0.229			
Age category (years)						
50+	1.00	1.00				
26–49	0.95	0.30–3.07	0.937			
18–25	1.02	0.22–4.81	0.979			
CD4 at ART initiation (cells/mm^3)						
350+	1.00	1.00				
200–350	1.05	0.13–8.29	0.965			
<200	4.73	0.66–33.87	0.122			
WHO stage						
1	1.00	1.00		1.00	1.00	
2	1.17	0.45–3.07	0.745	1.25	0.48–3.26	0.642
3	2.32	0.94–5.70	0.067	**2.45**	**1.01–5.94**	**0.047**
4	0.00	0.00–0.00	0.000	—		
NNRTI						
nevirapine	1.00	1.00		1.00	1.00	
efavirenz	0.52	0.23–1.17	0.113	**0.31**	**0.14–0.70**	**0.005**
NRTI						
tenofovir	1.00	1.00				
stavudine	—					
zidovudine	—					
abacavir/didanosine	—					
Clinic size						
small	1.00	1.00				
large	1.65	0.77–3.52	0.196			

Significant associations are highlighted in bold text.

ART regimens in the public healthcare sector. Viral suppression rates were high overall but, as expected, were higher in the 12–15 month cohort compared with the 24–36 month cohort. However, DRMs were common amongst those who were failing therapy, with 84% shown to have NNRTI mutations and ∼75% having NRTI mutations. Overall, this pilot survey has provided valuable information to the ART programme in KZN, showing that current first-line regimens remain effective in achieving good levels of virological suppression, but that drug resistance is common in patients failing these regimens.

Assessment of predictors of VF at a crude analysis level showed that participants who were younger and of male gender were at higher risk of failing ART at 12–15 months and 24–36 months post therapy initiation, highlighting a need to intensify adherence counselling and care and support for these patient groups. At a programmatic level, treating patients with nevirapine as opposed to efavirenz, and stavudine/zidovudine as opposed to tenofovir was associated with a higher prevalence of VF, indicating a need for enhanced care and support for patients that receive these regimens. As the use of stavudine is being phased out in the country, the risk factors associated with this regimen use may no longer be relevant. Alternatively, patients maintaining viral suppression could be switched to regimens containing efavirenz and tenofovir wherever possible.

The high proportion of VFs that harboured DRMs supports the timely switching of viraemic patients to PI-based regimens, as per treatment guidelines. However, ∼10% of participants with viraemia were failing first-line ART in the absence of detectable DRMs. Whilst Sanger sequencing technologies do not detect minority quasispecies which may include resistant variants, a more likely explanation is that levels of adherence vary within these participants. Intensified adherence counselling before repeat VL testing and regimen switch may achieve virological suppression in these patients.

Of note was the high number of samples with the tenofovir-selected K65R mutation, detected in ∼45% of sequences. The detection of high rates of K65R mutation amongst failing patients has also been noted by others,[21–24] and is consistent with the current high usage of tenofovir in standard first-line regimens. Although this mutation has been shown to be more rapidly selected in subtype C viruses,[24,25] the impact of this mutation on overall phenotypic response to WHO-recommended first-line regimens warrants further research, given that a study showed that it potentially reduces viral fitness[26] and is antagonistic with thymidine analogue mutations.[27] While the presence of K65R is unlikely to impact response to standard second-line regimens, there is concern about the potential impact of transmission of resistant virus to naive patients and on treatment efficacy for both adults and children infected perinatally.

Table 3. Predictors of virological failure (VL ≥1000 copies/mL) among adult participants on ART for 24–36 months

Characteristic	Crude PR	95% CI	*P* value	Adjusted PR	95% CI	*P* value
Gender						
female	1.00	1.00		1.00	1.00	
male	**1.74**	**1.15–2.63**	**0.009**	**1.75**	**1.06–2.88**	**0.028**
Age category (years)						
50+	1.00	1.00		—		
26–49	**4.53**	**1.45–14.09**	**0.009**			
18–25	3.48	0.74–16.36	0.114			
CD4 at ART initiation (cells/mm^3)						
350+	1.00	1.00				
200–350	0.53	0.19–1.45	0.217			
<200	1.27	0.54–3.00	0.581			
WHO stage						
1	1.00	1.00		1.00	1.00	
2	**2.43**	**1.16–5.11**	**0.019**	**2.71**	**1.28–5.78**	**0.009**
3	**2.98**	**1.43–6.20**	**0.003**	**2.94**	**1.40–6.17**	**0.005**
4	1.76	0.57–5.49	0.329	1.15	0.33–3.99	0.823
NNRTI						
nevirapine	1.00	1.00				
efavirenz	0.94	0.62–1.42	0.776			
NRTI						
tenofovir	1.00	1.00		1.00	1.00	
stavudine	**3.01**	**1.78–5.09**	**<0.001**	**3.02**	**1.41–6.49**	**0.005**
zidovudine	1.31	0.45–3.86	0.624	**4.10**	**1.60–10.49**	**0.003**
abacavir/didanosine	3.34	0.66–16.87	0.143	—		
Clinic size						
small	1.00	1.00				
large	1.49	0.92–2.43	0.107			

Significant associations are highlighted in bold text.

VF rates calculated in this study were slightly lower than those reported in other recent analyses, including global systematic reports from low- and middle-income countries primarily using data collected from observational cohorts.[28–30] Differences in reported VF rates may be complicated by a lack of consistency in the definition of VF across these studies, but may also reflect a higher degree of adherence and treatment success amongst patients on ART in KZN or by the use of fixed dose combinations. Routine data can be confounded by the inclusion of data from non-routine specimens, such as additional VL tests being done for clinical management reasons. However, this survey has shown that amongst patients on ART at PHCs in KZN, current standardized first-line regimens are very effective. Furthermore, these findings indicate that currently available second-line regimens remain suitable for most patients failing NNRTI-based regimens.

Challenges were experienced in conducting this pilot study in terms of identifying participants who met the inclusion criteria and proper consenting procedures. In many clinics, suitability for inclusion could only be confirmed at the end of the day after the SOs were able to check through paper-based medical records. To overcome these obstacles, better training of clinic coordinators and more immediate access to medical records is required, or alternative enrolment procedures should also be considered in future survey design. In many cases, relevant data were absent from the patients' files, including cases where patients were transferred in from other facilities. Many of these challenges will be overcome when integrated electronic data capture using standardized unique patient identifiers becomes the norm. However surveys performed in PHC settings may need to utilize more simplified inclusion/exclusion criteria.

A total of 145 participants were not eligible for study inclusion as they had been on a first-line regimen for >36 months. As these did not conform to study inclusion criteria, they were not included in the statistical analyses. However, the crude VF rate amongst these participants was 15.2%, and DRMs were detected in 19/21 sequences (90%) from viraemic participants. The patterns of DRMs in this group did not differ significantly from those in Cohorts A and B, other than a lower proportion of sequences with the K65R mutation (data not shown) probably due to lower exposure to tenofovir, as this was not widely used in earlier regimens. A further 176 participants were similarly not included as they had been on first-line ART for between 16 and 23 months. Within this group, 19 (10.8%) participants had a VL ≥1000 copies/mL and DRMs were present in 58% of these sequences.

The results of the study described herein are susceptible to bias due to misclassification of eligible participants and possible selection bias during the enrolment processes. These misclassifications resulted in suboptimal sample sizes being reached, and could be avoided in future surveys through access to improved electronic record keeping. However, this cross-sectional survey of acquired

Table 4. Resistance mutations detected by drug class in patients failing ART following 12–15 months and 24–36 months of treatment

Mutation	12–15 months, Cohort A ($n = 38$)			24–36 months, Cohort B ($n = 85$)		
	N	%	95% CI	*N*	%	95% CI
NRTI						
any	28	73.7	59.5–87.9	68	80.0	71.4–88.6
M41L	0			3	3.5	0–7.5
A62V	2	5.3	0–12.5	9	10.6	4.0–17.2
K65N/R	17	44.7	28.7–60.8	41	48.2	37.5–58.9
D67N	3	7.9	0–16.6	6	7.1	1.6–12.5
69ins	0			1	1.2	0–3.5
K70E/R	3	7.9	0–16.6	9	10.6	4.0–17.2
L74V	0			1	1.2	0–3.5
V75I	3	7.9	0–16.6	3	3.5	0–7.5
M184I/V	22	57.9	42.0–73.8	62	72.9	62.4–83.5
T215F	0			1	1.2	0–3.5
K219E/Q	2	5.3	0–12.5	10	11.8	4.9–18.7
NNRTI						
any	31	81.6	69.1–94.1	74	87.1	79.9–94.2
A98G	4	10.5	0.6–20.4	4	4.7	0.2–9.2
L100I	1	2.6	0–7.8	6	7.1	1.6–12.5
K101E/P	2	5.3	0–12.5	5	5.9	0.9–10.9
K103N/S	21	55.3	39.2–71.3	43	50.6	39.4–61.8
V106A/M	17	44.7	28.7–60.8	23	27.1	17.6–36.6
V108I	3	7.9	0–16.6	6	7.1	1.6–12.5
Y181C	10	26.3	12.1–40.5	20	23.5	14.5–32.6
Y188C/H/L	4	10.5	0.6–20.4	18	21.2	12.4–29.9
G190A/S	2	5.3	0–12.5	10	11.8	4.9–18.7
P225H	2	5.3	0–12.5	7	8.2	2.4–14.1
M230I/L	4	10.5	0.6–20.4	6	7.1	1.6–12.5

Please note that negative bounds have been adjusted to zero.

HIVDR provides data on the prevalence of VF and patterns of drug resistance among patients retained in care on first-line ART in the KZN Province of South Africa. This pilot study has also provided important information to guide the implementation of HIVDR surveillance in other provinces in South Africa, which would ideally inform future selection of population-based ART regimens and modification of treatment-failure algorithms.

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Representatives of the study sponsor did not participate in the collection of data but did participate in the study design, analysis and interpretation of data, the writing of this report and the decision to submit this paper for publication.

Transparency declarations

None to declare.

Author contributions

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Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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