SURVEILLANCE OF HIV DRUG RESISTANCE IN ADULT PATIENTS THROUGH ROUTINE ART PROGRAMME MONITORING IN SOUTH AFRICA

2019

PEPFAR
CDC CENTERS FOR DISEASE
NATIONAL HEALTH LABORATORY SERVICE
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1. LIST OF ACRONYMS

ABC	Abacavir
ADR	Acquired HIV Drug Resistance
ART	Antiretroviral therapy
ARV	Antiretroviral
AZT	Azidothymidine / Zidovudine
CDC	Centers for Disease Control and Prevention
CGH	Center for Global Health
CI	Confidence Interval
d4T	Stavudine
DCF	Data Collection Form
DGHA	Division of Global HIV and Tuberculosis
EFV	Efavirenz
HCW	Health Care Worker
HIV	Human immunodeficiency virus
HIVDR	HIV drug resistance
ID	identification number
LPV/r	Lopinavir/ritonavir
NICD	National Institutes of Communicable Diseases
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor
NVP	Nevirapine
PI	Protease inhibitor
PCR	Polymerase chain reaction
PMTCT	Prevention of mother to child transmission of HIV
SOP	Standard operating procedure
VF	Virological failure
VL	Viral load
WHO	World Health Organisation
3TC	Lamivudine

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4. INTRODUCTION

4.1 Background

Countries have designed and implemented antiretroviral treatment (ART) programs to control the human immunodeficiency virus (HIV) epidemic and contain disease progression into acquired immunodeficiency syndrome (AIDS). ART programmes in resource-limited settings are characterized by the use of standardized antiretroviral (ARV) regimens. To maximize the long-term effectiveness of first-line ART and ensure sustainability of ART programmes, it is essential to monitor and minimize the further spread of HIV drug resistance (HIVDR). HIVDR can affect the efficacy to subsequent ART regimens, as well as be a source of HIVDR transmission.[1]

In South Africa, it is estimated that 7.9 million people contracted HIV by 2017.[2] Scale-up of ART has been ongoing since 2004. The introduction of a universal test and treat strategy in 2016, wherein all HIV-infected individuals are eligible for ART, led to 4.4 million HIV-infected adults and children receiving ART at ~4,000 clinical sites nationally in 2017. The standard first-line ART for adults in South Africa is efavirenz (EFV)/emtricitabine (FTC)/tenofovir (TDF) [TEE] and the standard second-line ART is ritonavir-boosted lopinavir (LPV/r)/lamivudine (3TC)/zidovudine (AZT).[3] Towards the end of 2019, South Africa released updated treatment guidelines for expected implementation in 2020,[4] wherein first-line regimens for adults and adolescents will consist of dolutegravir (DTG)/emtricitabine (FTC)/tenofovir (TDF) [TLD], whereas efavirenz-based first-line ART will be available for women peri-conception.

As part of a coordinated approach to prevent, monitor, and respond to the emergence of HIVDR, the World Health Organization (WHO) recommends surveillance on acquired HIVDR (ADR, HIVDR in adult populations receiving ART).[5] The results obtained from these surveillance data are used for assessing the effectiveness of the ART programmes in terms of suppressing the virus, informing the optimal selection and management of second-line therapies, and providing insight on the extent to which patients are switching therapies unnecessarily. Included in the WHO Global Action Plan on HIV Drug Resistance is a series of recommendations aimed at preventing HIVDR from undermining efforts to achieve global targets on management of HIV,[6] given that steady increases in HIVDR prevalence has been demonstrated, particularly in Southern and Eastern African countries.[1] These include efforts to prevent and respond to HIVDR, monitor HIVDR levels through surveillance, conduct research and innovation, improve laboratory capacity, and develop governance structures.

4.2 Rationale for programmatic monitoring of HIVDR prevalence

In many low- to middle-income countries (LMIC), HIVDR testing is not offered at treatment initiation nor at first-line regimen failure, primarily due to cost and limited capacity. Treatment failure is defined as two consecutive VL tests performed 2 months apart that are ≥1,000 copies/ml. First-line regimen failure is managed by switching to standardised second-line treatment regimens. In these settings, continued and regular surveillance of transmitted and ADR is critical for the management of ART programs. Nationally representative surveillance of HIVDR is necessary to assess the quality of ART programmes and inform the selection of first- and second-line ART regimens. Suboptimal VL suppression (VS) and the detection of HIVDR in populations receiving ART may reflect gaps in ART program quality, including inadequate adherence assessment and counselling, interruptions in drug supply and low retention in care.[7]

The WHO has previously recommended nationally representative surveys be implemented in LMIC to assess levels of pre-treatment and ADR. However, uptake of these surveys in countries with high HIV burden has been slow and complex. Recently, it has been proposed to use programmatic VS data to estimate the consequence of increasing HIVDR levels on first-line treatment outcomes and to monitor and evaluate the ART program.[5] Additionally, countries can use convenience cohorts and/or laboratory-based sampling of treatment failures to facilitate surveillance outcomes and generate more-timely data.

In South Africa, HIV VL testing is recommended at six months after treatment initiation, then again at 12 months. Samples collected from public health facilities through routine programme monitoring were used for the survey. This strategy is feasible in South Africa because there is strong network of 16 HIV VL laboratories that contribute programmatically to VL testing with coverage rates of >80% across all nine provinces. Estimates from the National Health Laboratory Service (NHLS) showed that 13% of 3.3 million people with a VL test performed during 2018 had VL ≥1,000 copies/ml (source: NHLS HIVVL dashboard, accessed September 2018).

5. STUDY OBJECTIVES

The objective of the study was to estimate the prevalence of HIVDR among adult patients receiving ART who present for routine monitoring with a VL ≥1,000 copies/ml during 2019, using remnant plasma specimens.

6. METHODS

6.1 Sampling Strategy

This study used a two-stage sampling approach. For the first stage, a systematic random sample of remnant VL test samples were selected at each of the 16 national VL laboratories over a five-day period. The NHLS LIMS (TRAKCare) database was then used to identify each sample and retain only those samples that were taken from adults and that had an unsuppressed VL. In the second stage, a random sample of unsuppressed VL tests were selected and stratified by VL laboratory from those retained from Stage 1.

6.2 Inclusion and exclusion criteria

6.2.1 Inclusion criteria

To be included in this study, samples were enrolled if all of the following criteria were met:

- Leftover sample was from an adult male or female aged ≥18 years or older
- Blood specimens were sent for routine VL testing
- HIV VL results were already available and authorised (released) in the NHLS laboratory information management system
- Leftover sample was available and not older than 96 hours from time of collection/venepuncture
- HIV VL result was ≥1,000 copies/ml

6.2.2 Exclusion criteria:

- Sample was older than 96 hours from time of collection
- Minimal data fields were not available in the laboratory information system, including age, sex, facility, and clinic or hospital record number.
- Under the age of 18 years
- HIV VL was <1,000 copies/ml

6.3 Sample Size calculations

This study estimated an effective sample size of 700 specimens, after adjusting for a 10% specimen rejection rate, 15% genotyping failure rate, and 6% specimen exclusion rate due to age. This would require us to sample 973 total specimens with VL ≥1,000cpm. Therefore, in order to select 973 unsuppressed VL tests, a minimum required sample a total of 7,485 VL tests were collected and stored during Stage 1.

Table 6.1: Number of samples necessary to estimate the proportion of HIV drug resistance in the cross sectional surveillance study to assess levels of HIV drug resistance (HIVDR) in adults with viraemia, June - July 2019, South Africa

Statistical Precision				Sample size adjustments		
Proportion Estimated (P)	Error size (e)	95% CI confidence interval	Effective Sample Size	Genotyping failure (15%)	Unusable sample (10%)	Underage sample (6%)
0.5	0.037	1.96	700	824	915	973

Sample sizes was also influenced by feasibility. In this study, we had the capacity to test 700 HIVDR samples. The effective sample size of 700 would require the collection of 973 blood samples. In this case, our error size would be approximately 3.7%.

6.4 Specimen collection and randomization

Specimens were selected at each of the 16 NHLS VL laboratories between June and July 2019, by selecting every 11th specimen once the VL result was authorised on the laboratory information system (TrakCare). Remnant plasma was decanted into a separate tube and allocated a study ID. Once decanted, the NHLS episode number and corresponding study ID was captured in a RedCap (https://redcap.core.wits.ac.za/) database. The decanted specimen was labelled with the Study ID only. The PI and data manager had access to the linkage component of the database. Specimens were shipped to the NHLS HIV Genotyping Laboratory at Charlotte Maxeke Johannesburg Academic Hospital for storage at -80ºC.

6.5 HIV drug level testing (DLT)

All specimens were tested for antiretroviral drugs used in the public sector (3TC, FTC, NVP, EFV, LPV, atazanavir (ATV), darunavir (DRV), DTG and raltegravir (RAL)) using liquid chromatography mass spectrometry (LC/MS) in a multiplex testing approach. Results were reported at limit of quantitative (LOD) detection. This analysis was performed at the NHLS Chemical Pathology Laboratory at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), and this information was used as a proxy for current treatment regimen.

6.6 HIVDR genotyping

Remnant specimens from adult patients and with a VL ≥1,000 copies/ml were selected for HIVDR genotyping using next generation sequencing-based in-house genotyping procedure.

Total nucleic acid was extracted from 500µl plasma using the MagNA Pure 96 Instrument and the MagNA Pure LC Total Nucleic Acid Isolation Kit Large Volume (Roche). PCR amplification of the PR and RT genes was performed using the HIV-1 Genotyping Kit Amplification Module (Thermo Scientific). PCR amplicons were purified using AMPure XP beads (Agencourt) and quantified using Quant-iT™ PicoGreen™ dsDNA Assay Kit (Thermo Scientific). Quantified amplicons were diluted and pooled in equimolar concentrations to achieve a library, which was sequenced using MiSeq V3 Sequencing Kit (Illumina, San Diego, CA, USA). FastQ sequences were submitted to PASeq (paseq.org) for NGS HIV Drug Resistance analysis, and consensus (20%) sequences were submitted to Stanford University HIV Drug Resistance Database (hivdb.stanford.edu).

6.7 Statistical Analysis

Proportions of HIVDR were presented for categorical variables and medians with corresponding interquartile ranges (IQR) for continuous variables. All analyses were weighted by proportional contribution to national testing volumes and survey design. Log binomial regression was performed to model associations between region and province and having detectable levels of resistance. Significance was set at p-value of less than 0.05. All analyses were conducted using STATA version 13 (STATA Corp., College Station, TX, USA).

7. OUTCOMES

7.1 Specimen collection

A total of 8,202 remnant VL specimens were collected and shipped to NHLS Genotyping lab over the collection period (May – July 2019), spanning a 9-week period (Table 8.1). During this period, 1,410,096 VL tests were performed at the NHLS nationwide, of which 198,034 had VL ≥1,000 copies/ml (14.0%). Of the 8,202 specimens collected, 7,609 met inclusion criteria; of these 1,052 had VL ≥1,000 copies/ml and 779 were randomly selected for further testing (Table 8.1). The median VL of included specimens is 19,300 (IQR 4,630 – 84,700) copies/ml.

7.2 Laboratory testing

Drug level testing (DLT) was successful for all 779 specimens. ART drugs were detected in 434 specimens (55.71%). The most frequently detected drugs were EFV (42.49%), FTC (30.10%) and 3TC (12.55%) (Figure 8.1).

Table 7.1. Number of remnant VL specimens collected and tested in the cross sectional surveillance study to assess levels of HIV drug resistance (HIVDR) in adults with viraemia, June - July 2019, South Africa

Site Code	Total Number VL Tests Performed	Number with VL ≥1,000 c/ml	Proportion of VL ≥1,000 c/ml Nationally	Number of Samples Collected	Number that meet inclusion criteria	Number with VL ≥1,000 c/ml	Final Number Tested	Final Number required	Number Successfully Genotyped
1	112,472	10,729	5.4%	548	513	53	42	38	40
2	264,868	33,318	16.8%	1371	1277	168	131	118	128
3	109,745	13,927	7.0%	547	492	57	55	50	53
4	103,849	13,077	6.6%	477	442	51	51	46	51
5	30,430	6,229	3.1%	145	134	30	25	23	24
6	49,489	5,946	3.0%	360	334	37	23	21	22
7	48,144	5,652	2.9%	347	306	28	22	20	20
8	38,140	3,754	1.9%	337	320	35	15	14	15
9	104,706	18,561	9.4%	550	505	88	73	66	73
10	82,117	14,261	7.2%	635	598	94	56	50	50
11	128,826	18,109	9.1%	898	821	122	71	64	70
12	108,500	12,947	6.5%	591	564	59	51	46	51
13	38,353	10,605	5.4%	228	209	52	42	38	40
14	38,345	8,572	4.3%	197	187	47	34	31	31
15	45,790	6,058	3.1%	275	254	36	24	22	21
16	106,322	16,289	8.2%	696	653	96	64	58	64
	1,410,096	*198,034*	*100.0%*	*8202*	*7609*	*1053*	*779*	*701*	*753*

VL: Viral Load. c/ml: copies/millilitre.

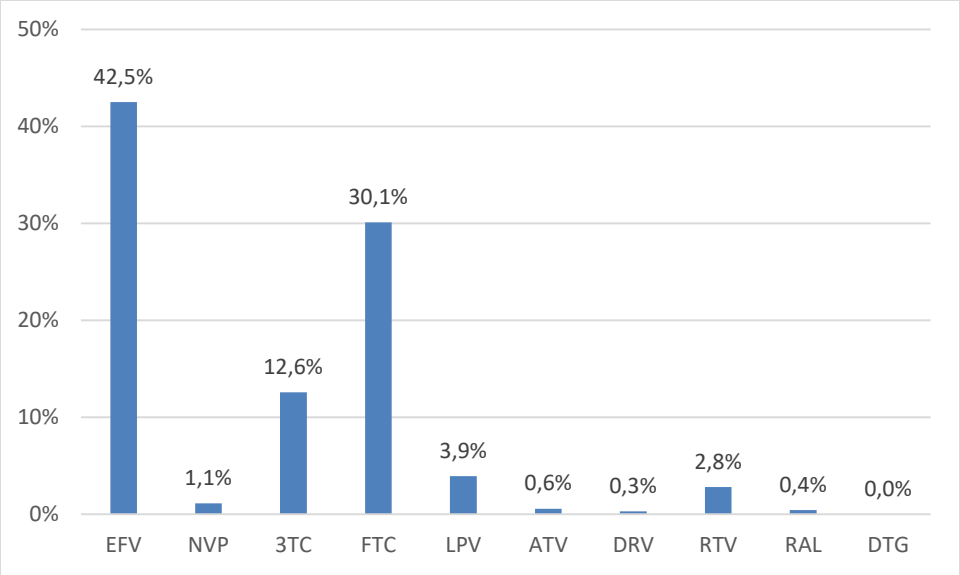

Figure 7.1. Proportions of specimens with detectable levels of EFV, NVP, 3TC, FTC, LPV, ATV, DRV, RTV, RAL and DTG in the cross sectional surveillance study to assess levels of HIV drug resistance (HIVDR) in adults with viraemia, June - July 2019, South Africa. EFV: efavirenz. NVP: nevirapine. 3TC: lamivudine. FTC; emtricitabine. LPV: lopinavir. ATV: atazanavir. DRV: darunavir. RTV: ritonavir. RAL: raltegravir. DTG; dolutegravir.

Of the 779 samples selected for further testing, HIVDR genotyping was successful for 753 (96.7%). HIVDR was detected in 72.1% (95% CI 66.8%–76.9% of specimens, with resistance to NNRTI in 70.5% (64.7%–75.7%, resistance to NRTI in 49.0% (44.7%–53.3%) and resistance to PI in 2.2% (95% CI 1.3%–3.5% (Table 8.2).

When analyzed according to drug level detection (any ART detected vs not detected), resistance levels were higher in specimens that had detectable ART levels (86.6% vs 55.6%, p=0.000).

Table 7.2 Proportions of specimens with detectable HIV drug resistance in the cross sectional surveillance study to assess levels of HIV drug resistance (HIVDR) in adults with viraemia, June - July 2019, South Africa

	n/N	%	95% CI
ALL SPECIMENS			
RESISTANCE ANY CLASS	542/753	72.1%	66.8% - 76.9%
RESISTANCE TO PI	16/753	2.2%	1.3% - 3.5%
RESISTANCE TO NRTI	363/753	49.0%	44.7% - 53.3%
RESISTANCE TO NNRTI	528/753	70.5%	64.7% - 75.7%
ART DETECTED			
RESISTANCE ANY CLASS	356/414	85.6%	79.7% - 89.9%
RESISTANCE TO PI	13/414	3.1%	1.9% - 5.1%
RESISTANCE TO NRTI	299/414	72.7%	66.4% - 78.2%
RESISTANCE TO NNRTI	347/414	83.7%	77.7% - 88.4%
ART NOT DETECTED			
RESISTANCE ANY CLASS	183/333	55.6%	46.6% - 64.2%
RESISTANCE TO PI	3/333	1.0%	0.3% - 3.0%
RESISTANCE TO NRTI	63/333	19.9%	15.6% - 25.1%
RESISTANCE TO NNRTI	178/333	54.3%	45.0% - 63.3%
NNRTI-BASED REGIMENS			
RESISTANCE ANY CLASS	294/335	87.3%	82.2% - 91.0%
RESISTANCE TO PI	0/335		
RESISTANCE TO NRTI	256/335	75.8%	69.5% - 81.1%
RESISTANCE TO NNRTI	291/335	86.6%	81.7% - 90.4%
PI-BASED REGIMENS			
RESISTANCE ANY CLASS	30/36	82.3%	62.1% - 93.0%
RESISTANCE TO PI	11/36	32.3%	17.5% - 51.6%
RESISTANCE TO NRTI	30/36	82.3%	62.1% - 93.0%
RESISTANCE TO NNRTI	26/36	70.6%	47.1% - 86.7%

PI: Protease Inhibitors. NNRTI: Non-nucleoside reverse transcriptase inhibitors. NRTI: nucleoside reverse transcriptase inhibitors.
CI: Confidence Interval. Note: all analyses were weighted by proportional contribution to national testing volumes and survey design

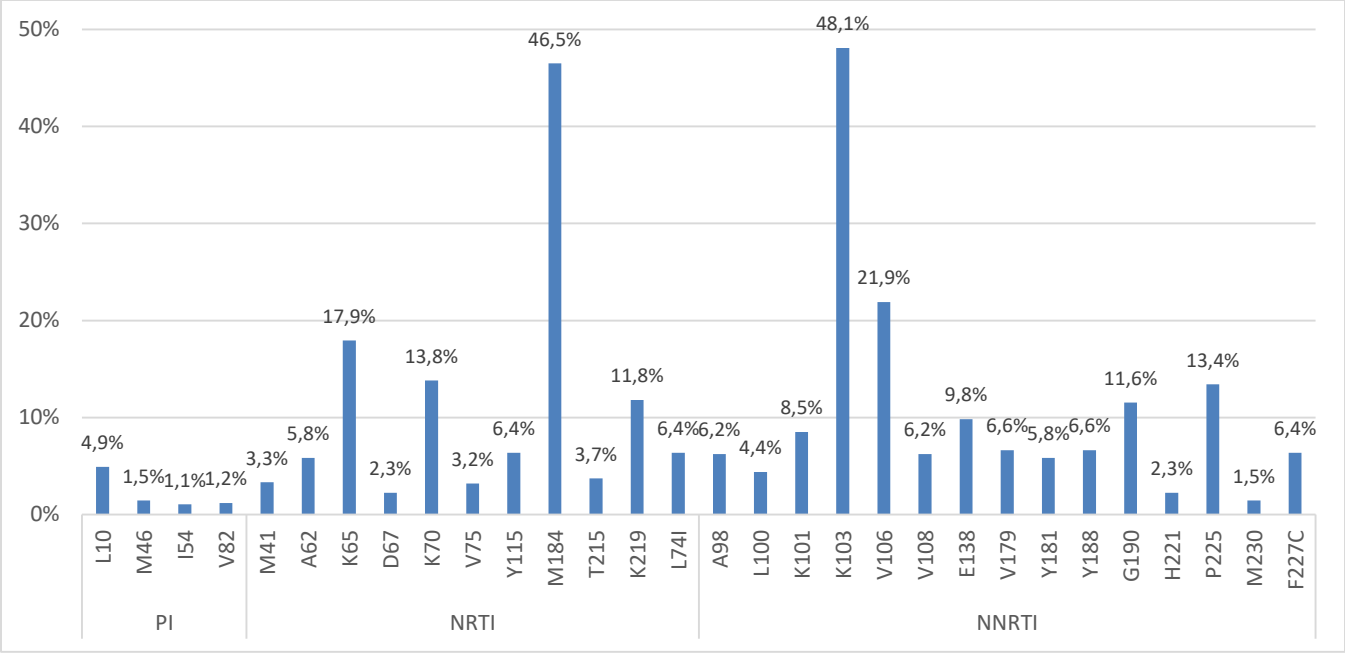

Figure 7.2 HIVDR mutations detected in 753 specimens successfully genotyped in the cross sectional surveillance study to assess levels of HIV drug resistance (HIVDR) in adults with viraemia, June - July 2019, South Africa. PI = Protease Inhibitors; NRTI = nucleoside reverse transcriptase inhibitors; NNRTI = non-nucleoside reverse transcriptase inhibitors

7.3 Resistance patterns by age group

Median age at time of enrollment was 36 years (IQR 30 – 44 years). Whilst a trend was evident towards higher levels of resistance amongst age groups 35 – 54 years, this was not statistically significant (p=0.942. Figure 8.3). Similarly, no significant differences were noted for drug class resistance.

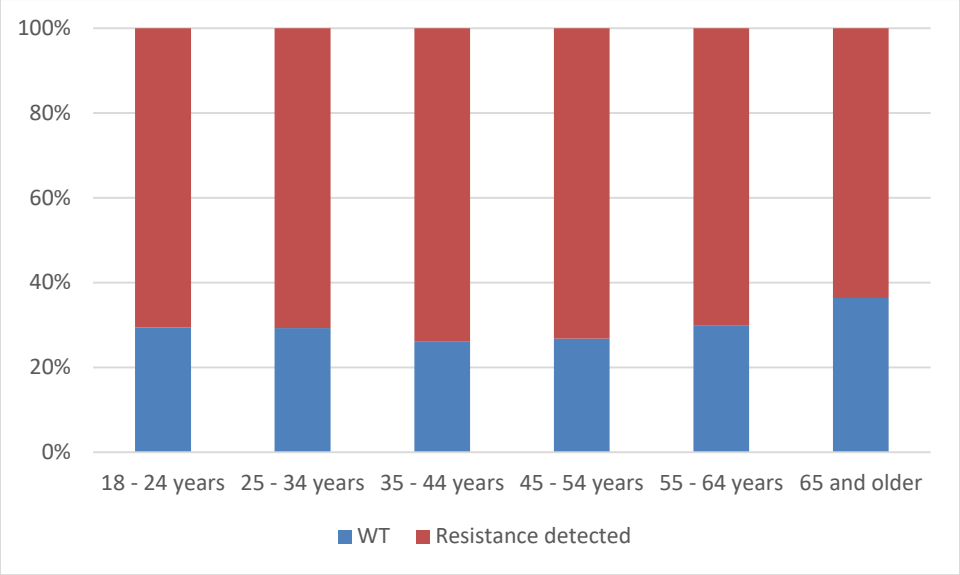

Figure 7.3 Proportions of specimens with resistance detected by age group in the cross sectional surveillance study to assess levels of HIV drug resistance (HIVDR) in adults with viraemia, June - July 2019, South Africa.

WT: Wild type (no resistance detected)

7.4 Prevalence ratios by testing site and province

Risk for having HIVDR was assessed relative to province (Table 8.3) and site (Table 8.4) to assess possibility of clustering. These results suggest that most provinces have higher HIVDR in comparison to Gauteng Province, with PR directionality >1.0 in 5 provinces. However, as these differences are marginal, clustering within provinces is not evident. Analysis at the site level suggests that most labs have higher HIVDR in comparison to CM, with PR directionality >1.0 in 14 of 15 sites. However, these differences are less than 30% and therefore not large in magnitude. In addition, it should be noted that the study was not significantly powered to assess these levels.

Table 7.3 Risk of having HIV drug resistance by province in the cross sectional surveillance study to assess levels of HIV drug resistance (HIVDR) in adults with viraemia, June - July 2019, South Africa

PROVINCE	PR	95% CI			P-VALUE
EC	1.1	1.0	-	1.3	0.149
FS	1.1	0.9	-	1.3	0.601
GP	(ref)				
KZ	1.1	1.0	-	1.3	0.065
LP	1.2	1.0	-	1.4	0.056
MP	1.0	0.8	-	1.2	0.792
NW	1.0	0.8	-	1.3	0.922
WC	1.1	0.9	-	1.3	0.486

PR: Prevalence Ratio. EC: Eastern Cape; FS: Free State; GP: Gauteng; KZ: KwaZulu-Natal; LP; Limpopo; MP: Mpumalanga; NW: North West Province; WC

Table 7.4 Risk of having HIV drug resistance by VL testing site in the cross sectional surveillance study to assess levels of HIV drug resistance (HIVDR) in adults with viraemia, June - July 2019, South Africa

PROVINCE	SITE	PR	95% CI	P-VALUE
EC	FR	1.3	1.0 - 1.6	0.048
EC	MT	1.2	0.9 - 1.5	0.170
EC	PE	1.2	1.0 - 1.5	0.090
FS	UN	1.1	0.9 - 1.4	0.223
GP	CM	(ref)		
GP	DG	1.3	1.1 - 1.6	0.012
KZ	**AD**	**1.3**	**1.0 - 1.6**	**0.038**
KZ	**ED**	**1.3**	**1.0 - 1.5**	**0.018**
KZ	IA	1.0	0.7 - 1.4	0.885
KZ	**MD**	**1.4**	**1.1 - 1.8**	**0.006**
KZ	NG	1.2	1.0 - 1.5	0.080
LP	**MK**	**1.3**	**1.1 - 1.5**	**0.012**
MP	NE	1.1	0.9 - 1.4	0.324
NW	TS	1.1	0.8 - 1.5	0.512
WC	GS	1.3	1.0 - 1.6	0.096
WC	TY	1.1	0.8 - 1.5	0.649

PR: Prevalence Ratio. EC: Eastern Cape; FS: Free State; GP: Gauteng; KZ: KwaZulu-Natal; LP; Limpopo; MP: Mpumalanga; NW: North West Province; WC: Western Cape

8. CONCLUSION

Our survey showed that 72% of HIV positive patients on ART with unsuppressed VL in the public sector harbour resistance to ART. The most common resistance found was to NNRTI, with 71% of specimens harbouring resistance to NNRTI, 49% of specimens harbouring resistance to NRTI and 2% of specimen exhibiting resistance to PI. The most frequently detected mutations were K103NS, M184IV, V106M, and K65R.

HIVDR was lower in patients that had undetectable levels of ART, presumably due to lack of drug selection pressure ($p<0.0000$). Notably, 45% of patients on ART and presenting for routine VL testing had undetectable levels of ART.

The use of leftover specimens proved advantageous in that it allowed for proportion to size sampling, and reduced data collection time and cost for data collection. However, demographic and clinical data was not available through the laboratory information systems.

The survey will be repeated in 2021 – 2023 and will include laboratory testing for integrase inhibitors.

9. REFERENCES

1. World Health Organization. WHO | HIV drug resistance report 2019. *WHO* (2019).

2. Human Sciences Research Council (HSRC). *The Fifth South African National HIV Prevalence, Incidence, Behaviour and Communication Survey, 2017: HIV Impact Assessment Summary Report*. http://www.hsrc.ac.za/uploads/pageContent/9234/SABSSMV_Impact_Assessment_Summary_ZA_ADS_cleared_PDFA4.pdf (2018).

3. National Department of Health. *National Consolidated Guidelines for the prevention of mother to child transmission of HIV and the management of HIV in children, adolescents and adults*. (2015).

4. National Department of Health. 2019 ART Clinical Guidelines for the Management of HIV in Adults, Pregnancy, Adolescents, Children, Infants and Neonates | Department of Health Knowledge Hub. https://www.knowledgehub.org.za/elibrary/2019-art-clinical-guidelines-management-hiv-adults-pregnancy-adolescents-children-infants (2019).

5. Raizes, E., Hader, S. & Birx, D. The US President's Emergency Plan for AIDS Relief (PEPFAR) and HIV Drug Resistance: Mitigating Risk, Monitoring Impact. *J Infect Dis* **216**, S805–S807 (2017).

6. World Health Organization. World Health Organization Global Action Plan on HIV Drug Resistance 2017-2021. (2017).

7. World Health Organization. WHO global strategy for the surveillance and monitoring of HIV drug resistance 2012. *World Health Organization, Geneva Switzerland* (2012).