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

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PEPFAR

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NATIONAL HEALTH LABORATORY SERVICE

NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES
Division of the National Health Laboratory Service

Study Contacts:

Name	Affiliation	Role	E-mail
Kim Steegen	National Health Laboratory Service	Principal investigator	kim.steegen@nhls.ac.za
Gillian Hunt	National Institute for Communicable Diseases (NICD)	Co- Investigator	gillianh@nicd.ac.za
Lucia Hans	National Health Laboratory Service	Co-investigator	Lucia.hans@nhls.ac.za
William MacLeod	HE^{2}RO Boston University	Co-investigator	wmacleod@bu.edu
Naseem Cassim	National Health Laboratory Service	Co-investigator	Naseem.cassim@nhls.ac.za
Jaya George	National Health Laboratory Service	Co-investigator	Jaya.george@nhls.ac.za
Elliot Raizes CITI ID: 47856822; Expiration March 9, 2025	Centers for Diseases Control and Prevention (CDC Atlanta)*	Co-Investigator	gwq0@cdc.gov
Kassahun Ayalew CITI ID: 45007574 Expiration date: 8 Dec 2024	CDC South Africa *	Co-investigator - statistician	ylo8@cdc.gov
Karidia Diallo CITI ID: 28180472 Expiration date: 26 Oct 2024	CDC South Africa *	Co-investigator	edu9@cdc.gov
Melissa Briggs-Hagen CITI ID: 37627755 Expiration date: February 14, 2024	CDC South Africa *	Co-investigator	vka5@cdc.gov
* *CDC investigators are not considered "engaged" and did not intervene nor interact with participants or have access to identifiable information*			

CONTENTS:

1. ROLES OF INVESTIGATORS

The study is a collaboration of investigators from the National Health Laboratory Service (NHLS), National Institute for Communicable Diseases (NICD), and the Centre for Disease Control (CDC).

1.1. NHLS/NICD

Dr. Kim Steegen served as principal investigator for this study. She provided leadership, study implementation, specimen processing, data analysis, reporting of study findings.
Dr Gillian Hunt served as a co-investigator for this study. She provided leadership in design of the protocol and data analysis
Dr. Lucia Hans served as a co-investigator for this study. She provided technical assistance in protocol development, data analysis, and reporting of results.
Prof. Bill Macleod served as a co-investigator for this study. He provided technical assistance in protocol development, especially on sample size determination, sampling methodology, data management, data analysis, and reporting of results.
Dr Naseem Cassim served as a co-investigator for this study. He provided technical assistance in protocol development, database design, data management, data analysis, and reporting of results.
Prof Jaya George served as a co-investigator for this study. She provided technical assistance in protocol development, data analysis, and reporting of results for drug level testing.

1.2. U.S. Centers for Disease Control and Prevention

Dr. Elliot Raizes* served as a co-investigator for this study. He provided technical assistance in protocol development, data analysis, and reporting of results.
Dr. Karidia Diallo* was involved in protocol development monitored the laboratory components of the study in collaboration with NHLS/NICD co-investigators
Mr. Kassahun Ayalew* served as a statistician during protocol development and was involved in data analysis.
Dr. Melissa Briggs-Hagen* was involved in protocol development and provided technical assistance in data analysis and interpretation and reporting of results.

* CDC investigators are not considered “engaged” and will not intervene nor interact with participants or have access to identifiable information

Disclaimer
"The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the funding agencies.”

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2. 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
DTG	Dolutegravir
EFV	Efavirenz
FTC	Emtricitabine
HCW	Health Care Worker
HIV	Human immunodeficiency virus
HIVDR	HIV drug resistance
ID	identification number
INSTI	Integrase strand transfer inhibitor
3TC	Lamivudine
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
TDF	Tenofovir
TLD	Tenofovir Lamivudine Dolutegravir
VF	Virological failure
VL	Viral load
WHO	World Health Organisation
3TC	Lamivudine

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5. INTRODUCTION

5.1. 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 ART 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 there were 7.8 million people living with HIV in 2020. [2] The scale-up of ART has been ongoing since April 2004, based on the latest figures 5.6 million people living with HIV in South Africa receive ART. [2] The standard first-line ART for adults in South Africa was efavirenz (EFV)/emtricitabine (FTC)/tenofovir (TDF) [TEE] and the standard second-line ART was ritonavir-boosted lopinavir (LPV/r)/lamivudine (3TC)/zidovudine (AZT). [3] Towards the end of 2019, South Africa released updated national treatment guidelines which were implemented from 2020 onwards, wherein first-line regimens for adults and adolescents consist of dolutegravir (DTG)/lamivudine (3TC))/tenofovir (TDF) [TLD]. 4 The roll-out of TLD in South Africa was delayed as there were safety concerns regarding the development of neural tube defects.5 Therefore, men, adolescent boys, women on reliable contraception and older women were initially prioritized. Subsequent studies showed that the risk of neural tube defects was significantly lower than initially feared.6,7 Based on this additional information, all women, regardless of age, were included in the second phase of the roll out, which started in 2021. According to the National Department of Health, close to 3.2 million people living with HIV in South Africa had been initiated or switched to DTG by March 2022, which is approximately 57% of those on treatment (unpublished).
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). [8] 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, [9] given that steady increases in HIVDR prevalence have 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.

5.2. 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 viral load (VL) tests performed two months apart with ≥1,000 copies/ml of the virus present. First-line regimen failure is managed by switching to standardized second-line treatment regimens. In these settings, continued and regular surveillance of transmitted and ADR is critical for the management of ART programmes. 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. [8]

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. [10] 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 and annually thereafter. 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.

6. 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 2021, using remnant plasma specimens in South Africa.

7. METHODS

7.1. Sampling Strategy

This study used a two-stage sampling approach. For the first stage, a systematic random sample of remnant VL test samples from public health facilities were selected at each of the 16 national VL laboratories over a five-day period. The NHLS laboratory information system (LIS) (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 specimens with a VL>1,000 copies/mL were selected proportionately to VL failure rate at each VL laboratory from those retained from Stage 1.

7.2. Inclusion and exclusion criteria

7.2.1. Inclusion criteria

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

- Remnant plasma specimen 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 authorized (released) in the NHLS laboratory information management system
- Leftover sample was available in sufficient amount (>500 ul) and not older than 96 hours from time of collection/venipuncture
- HIV VL result was ≥1,000 copies/ml

7.2.2. 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, facility, and clinic or hospital record number.
- Under the age of 18 years
- HIV VL was <1,000 copies/ml

7.3. Sample size calculations

This study estimated an effective sample size of 385 specimens, after adjusting for a 10% specimen rejection rate, 5% genotyping failure rate, and 6% specimen exclusion rate due to age and a design effect of 1.5 (Table 6.1). This would require us to sample 660 total specimens with VL ≥1,000 copies/ml. Therefore, to select 660 unsuppressed VL tests, a minimum required sample total of 5,081 VL tests, assuming 87% of patients with available VL tests were virologically suppressed, had to be collected and stored during Stage 1.

Table 7.3.1: Sample size calculation
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, August – September 2021, South Africa

Statistical Precision					Sample size adjustments		
Proportion Estimated (P)	Error size (e)	95% CI (Z0.05/2)	Effective Sample Size	Design Effect 1.5	Genotyping failure (2%)	Unusable sample (5%)	Underage sample (6%)
0.5	0.05	1.96	385	578	590	621	660

7.4. Specimen collection and randomization

Specimens were selected at each of the 16 NHLS VL laboratories between August and September 2021, by selecting every 11th specimen once the VL result was authorised on the LIS. 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 the RedCap electronic database hosted at the University of the Witwatersrand [11,12] The decanted specimen was labelled with the Study ID only. The principal investigator and data manager had access to the linkage component of the database. Specimens were shipped to the NHLS HIV Genotyping Laboratory at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) for storage at -80^{o}C.

7.5. HIV drug level testing (DLT)

All specimens were tested for antiretroviral drugs used in the public sector (3TC, FTC, Nevirapine (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 detection (LOD). This analysis was performed at the NHLS Chemical Pathology Laboratory at CMJAH, and this information was used as a proxy for current treatment regimen.

7.6. 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 Nuclisens EasyMag (BioMérieux). PCR amplification of the protease and reverse transcriptase (PR/RT) regions of the HIV-1 pol gene was performed using the HIV-1 Genotyping Kit (Thermo Scientific, Waltham, MA, USA). PCR amplicons were purified using AMPure XP beads (Beckman Coulter, Indianapolis, IN, USA) and quantified using Quant-iT™ PicoGreen™ dsDNA Assay Kit (Thermo Scientific, Waltham, MA, USA). Quantified amplicons were diluted and pooled in equimolar concentrations to obtain 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). Resistance was defined as at least low-level resistance for PIs and INSTIs and at least intermediate resistance for NRTIs and NNRTIs, as predicted by the Stanford HIVdb.

7.7. Statistical Analysis

Proportions of HIVDR were presented for categorical variables. Medians with corresponding interquartile ranges (IQR) were used for continuous variables. All analyses were weighted by proportional contribution to national testing volumes and survey design. 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).

8. DISSEMINATION OF RESULTS

This survey report will be used to disseminate findings to key stakeholders on the prevalence of HIVDR among patients receiving ART in South Africa, once CDC approval has been obtained. Individual genotyping results were returned to the corresponding HAST (HIV/AIDS, STI's and Tuberculosis) programme managers. Conference abstracts and manuscripts will be developed for dissemination as deemed appropriate by the investigators, the NHLS and the NICD.
The final evaluation report will be uploaded to the respective agency website within 90 days after vetting by the relevant authorities.

9. ETHICAL CONSIDERATIONS

Ethical approval was obtained from the Human Research and Ethics Committee at the University of the Witwatersrand (M181067) and the US CDC's Division of Global HIV and TB, and Centre for Global Health for ethical review. The requirement for individual informed consent was waived as only remnant viral load specimens were used from patients undergoing routine ART and all samples were delinked.
The protocol was conducted according to the principles of Good Clinical Practice as established by the International Conference on Harmonisation.
All samples were delinked and confidentiality was maintained in the collection, storage, entry, and analysis of data. The laboratory episode number of the collected specimens were captured in a secure database (RedCap) where only the PI had access to the linked data, which was required to return the genotyping results to the corresponding HAST (HIV/AIDS, STI's and Tuberculosis) programme managers. Electronic data files, computers and other storage devices that contain data are password protected. All NHLS and NICD staff complied with institutional confidentiality policies and agreements, as stated in NHLS Standard Operating Procedure GPQ0061.
Institutional approvals were obtained from CDC and NHLS.

10. CONFLICT OF INTEREST

The investigators have no conflicts of interest to declare.

11. BUDGET

The total budget and annual expenditures related to the evaluation will be included in the evaluation report. The amount will be shared with the activity manager/project office for entry into the DATIM evaluation inventory.

12. OUTCOMES

12.1. Specimen collection

During the study period, a total of 976,696 VL tests were performed at the NHLS nationwide, of which 105,551 had VL ≥1,000 copies/ml (10.8%). Remnant VL specimens were collected and shipped to the NHLS Genotyping laboratory over the collection period (August - September 2021), spanning a 5-week period. Due to incorrect sampling at one site, sampling was repeated for 5 days in October 2021 for site TY (Table 7.1). For this site, the originally collected specimens were discarded and replaced by those that were sampled in October. A total of 7,008 specimens were collected, of which 621 were randomly selected for further testing (Table 7.1). The median VL of included specimens was 15,839 copies/ml (IQR 3,043 – 95,700).

Table 12.1.1. Number of remnant viral load specimens collected and tested in the cross-sectional surveillance study to assess levels of HIV drug resistance (HIVDR) in adults with viraemia, August to September 2021, South Africa

Lab	Total Tested Nationally	Total Unsuppressed Nationally	Proportion Unsuppressed Nationally	Sampling Proportion Nationally	Total to be Sampled	Unsuppressed to be sampled	Samples Collected	Unsuppressed Samples Collected	Proportion Unsuppressed Collected
AD	87,499	7,403	8.5%	7%	368	31	492	41	8.3%
CM	174,203	14,387	8.3%	14%	735	60	822	71	8.6%
DG	75,585	8,753	11.6%	8%	420	48	540	64	11.9%
FR	39,167	5,858	15.0%	6%	315	47	379	71	18.7%
ED	61,407	5,156	8.4%	5%	263	22	468	40	8.5%
GS	28,066	3,780	13.5%	4%	210	28	275	38	13.8%
IA	39,591	3,281	8.3%	3%	158	13	273	35	12.8%
MD	30,949	2,370	7.7%	2%	105	8	174	15	8.6%
MK	70,897	10,232	14.4%	10%	525	75	565	88	15.6%
MT	41,841	4,423	10.6%	4%	210	22	296	36	12.2%
NG	77,048	6,216	8.1%	6%	315	25	546	51	9.3%
PE	21,244	5,245	24.7%	5%	263	64	312	85	27.2%
NE	82,234	8,133	9.9%	8%	420	41	562	56	10.0%
TS	57,050	8,686	15.2%	8%	420	63	462	64	13.9%
TY	27,947	3,744	13.4%	4%	210	28	273	38	13.9%
UN	61,968	7,884	12.7%	7%	368	46	569	66	11.6%
	976,696	*105,551*	*10.8%*	*100.0%*	*5,250*	*621*	*7,008*	*859*	*12.3%*

VL: Viral Load. copies/ml: copies/millilitre. AD Addington Hospital, CM Charlotte Mexeke Hospital, DG Dr George Mukhari Hospital, Fr Frere Hospital, ED Edendale Hospital, GS Groote Schuur Hospital, IA Inkosi Albert Luthuli Hospital, MD Madedeni Hospital, MK Mankweng Hospital, MT Mtatha Hospital; NG Ngwelezane Hospital, PE Port Elizabeth Hospital; NE Rob Ferreira Hospital, TS Tshepong Hospital, TY Tygerberg Hospital, UN Universitas Hospital

12.2. Laboratory testing – drug level testing

Drug level testing (DLT) was successful for all 621 specimens. ART drugs were detected in 323 specimens (52.0%, 95% Confidence Interval (CI) 48.7% - 55.3%). The most frequently detected drugs were EFV (35.8%, 95% CI 32.1% - 39.6%), FTC (23.5%, 95% CI 20.3% - 27.0%) and 3TC (9.6%, 95% CI 7.6% - 12.3%) (Figure 7.1). Dolutegravir was only detected in 7.2% (95% CI 5.3% - 9.6%) of specimens.

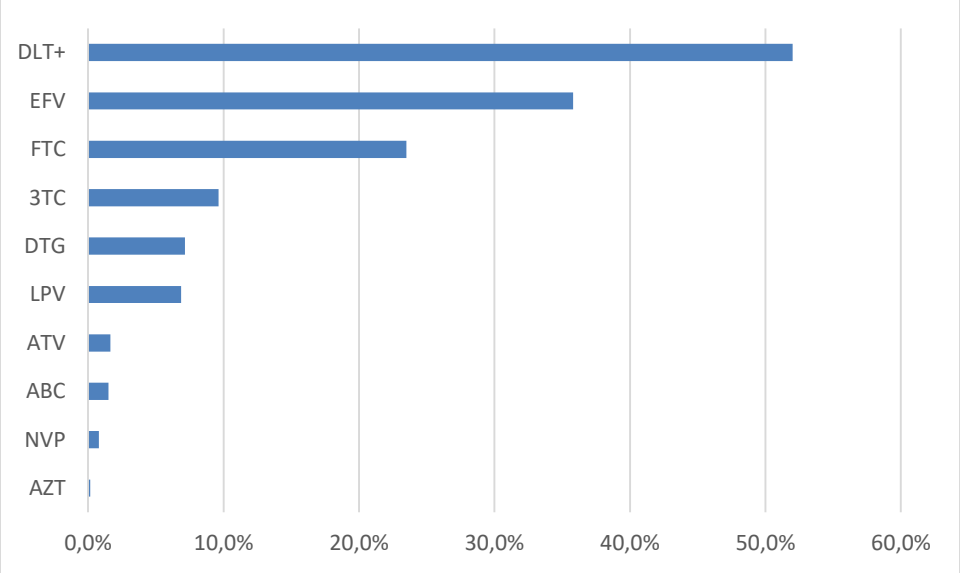

Figure 12.2.1. Proportions of specimens with detectable levels of LPV, ATV, 3TC, FTC, FTC, ABC, AZT, EFV, NVP and DTG in the cross-sectional surveillance study to assess levels of HIV drug resistance (HIVDR) in adults with viraemia, August-September 2021, South Africa.
DLT+: drug level testing positive; LPV: lopinavir. ATV: atazanavir. 3TC: lamivudine. FTC; emtricitabine. ABC; abacavir. AZT; zidovudine. EFV: efavirenz. NVP: nevirapine. DTG; dolutegravir.

12.3. Laboratory testing – HIV drug resistance testing

Of the 621 samples selected for further testing, HIVDR genotyping was successful for 538 (86.8%). HIVDR was detected in 67.6% (95% CI 62.3%–72.4%) of specimens, with resistance to Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI) in 66.4% (61.4%–71.0%), resistance to Nucleoside Reverse Transcriptase Inhibitors (NRTI) in 41.4% (35.3%–47.8%), resistance to Protease Inhibitors (PI) in 4.0% (95% CI 2.3%–6.9% and resistance to Integrase Strand Transfer inhibitors (INSTI) in 0.2% (95% CI 0.2%–1.5%) (Table 7.2). When analyzed according to drug level detection (any ART detected vs not detected), resistance levels were higher in specimens that had detectable ART levels (78.0% (95% CI 73.2%–82.1%) vs 56.2% (95% CI 48.1%–64.1%), p<0.0001).
The prevalence of specific HIVDR mutations is depicted in Figure 7.2. The most frequently detected mutations were at positions K103, M184, V106, and P225.

Table 12.3.1 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, August – September 2021, South Africa

	n/N	%	95% CI		
All specimens					
Resistance any class	367/539	67.6%	62.3%	-	72.5%
Resistance to PI	21/539	4.1%	2.4%	-	6.9%
Resistance to NRTI	224/539	41.4%	35.5%	-	47.8%
Resistance to NNRTI	360/539	66.4%	61.5%	-	71.0%
Resistance to INSTI	1/539	0.2%	0.0%	-	1.5%
ART detected					
Resistance any class	214/275	78.0%	73.2%	-	82.1%
Resistance to PI	14/275	5.0%	2.9%	-	8.6%
Resistance to NRTI	170/275	61.8%	54.4%	-	68.7%
Resistance to NNRTI	209/275	76.1%	71.9%	-	80.0%
Resistance to INSTI	1/275	0.4%	0.0%	-	2.9%
ART not detected					
Resistance any class	153/264	56.2%	48.1%	-	64.1%
Resistance to PI	7/264	3.0%	1.1%	-	8.2%
Resistance to NRTI	54/264	19.1%	13.33	-	26.6%
Resistance to NNRTI	151/264	55.7%	47.8%	-	63.4%
Resistance to INSTI	0/264	0.0%	0.0%	-	0.0%
NNRTI-based regimens					
Resistance any class	141/165	85.1%	76.7%	-	90.9%
Resistance to PI	6/165	3.5%	1.6%	-	7.6%
Resistance to NRTI	114/165	68.4%	56.6%	-	78.3%
Resistance to NNRTI	141/165	85.2%	76.7%	-	90.9%
Resistance to INSTI	0/165	0.0%	0.0%	-	0.0%
PI-based regimens					
Resistance any class	30/39	78.6%	60.8%	-	89.7%
Resistance to PI	7/39	17.2%	7.0%	-	36.6%
Resistance to NRTI	28/39	75.1%	57.8%	-	86.9%
Resistance to NNRTI	27/39	71.0%	52.1%	-	84.6%
Resistance to INSTI	0/39	0.0%	0.0%	-	0.0%
INSTI-based regimens					
Resistance any class	17/38	42.0%	27.2%	-	58.4%
Resistance to PI	1/38	2.2%	0.3%	-	13.5%
Resistance to NRTI	10/38	23.5%	12.2%	-	40.4%
Resistance to NNRTI	17/38	42.0%	27.2%	-	58.4%
Resistance to INSTI	1/38	2.7%	0.3%	-	21.7%

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

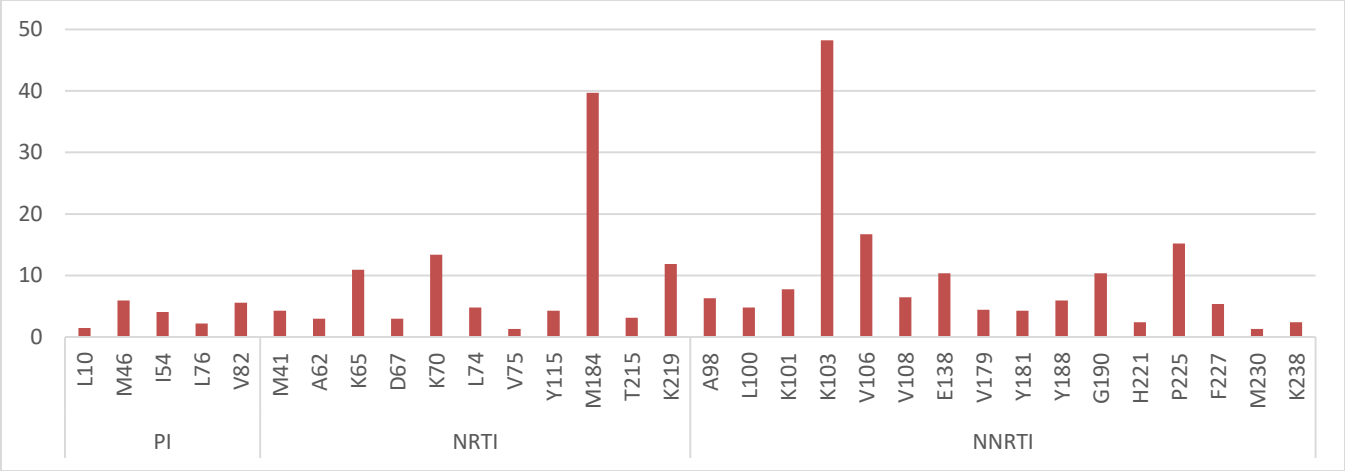

Figure 12.3.1 HIV drug resistance mutations detected in 538 specimens successfully genotyped in the cross-sectional surveillance study to assess levels of HIVDR in adults with viraemia, August – September 2021, South Africa.
PI = Protease Inhibitors; NRTI = nucleoside reverse transcriptase inhibitors; NNRTI = non-nucleoside reverse transcriptase inhibitors. Mutations detected at prevalence <1.0% not depicted in this graph

12.4. Resistance patterns by sex

Of 621 specimens tested, 436 (70.2%) were collected from female patients and 178 (28.7%) were from male patients, whereas 7 were not recorded. Amongst specimens from female patients, 53% were positive for DLT and 50% of specimens from male patients were positive for DLT. HIV drug resistance was detected in 70% of all specimens collected from female patients and 65% of all male patients, with no significant difference noted (p=0.192; Figure 7.3).

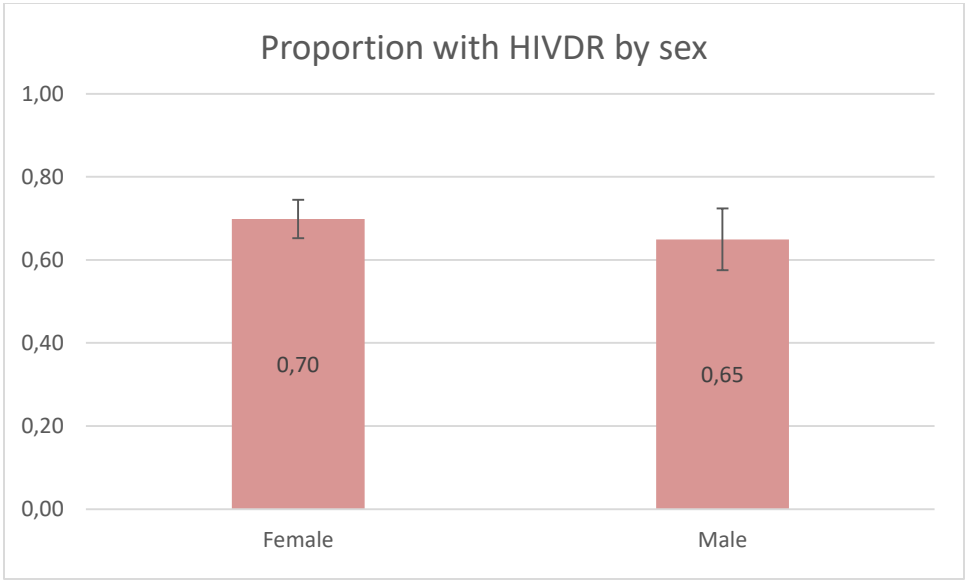

Figure 12.4.1 Proportions of specimens with resistance detected by sex in the cross-sectional surveillance study to assess levels of HIV drug resistance (HIVDR) in adults with viraemia, August-September 2021, South Africa.

12.5. Resistance patterns by age group

Median age at time of enrollment was 37 years (IQR 30–44 years). Whilst a trend was evident towards lower levels of resistance amongst age groups 45 - 64 years, this was not statistically significant (p=0.942, 45-64 years versus 18-44 years, Figure 7.4).

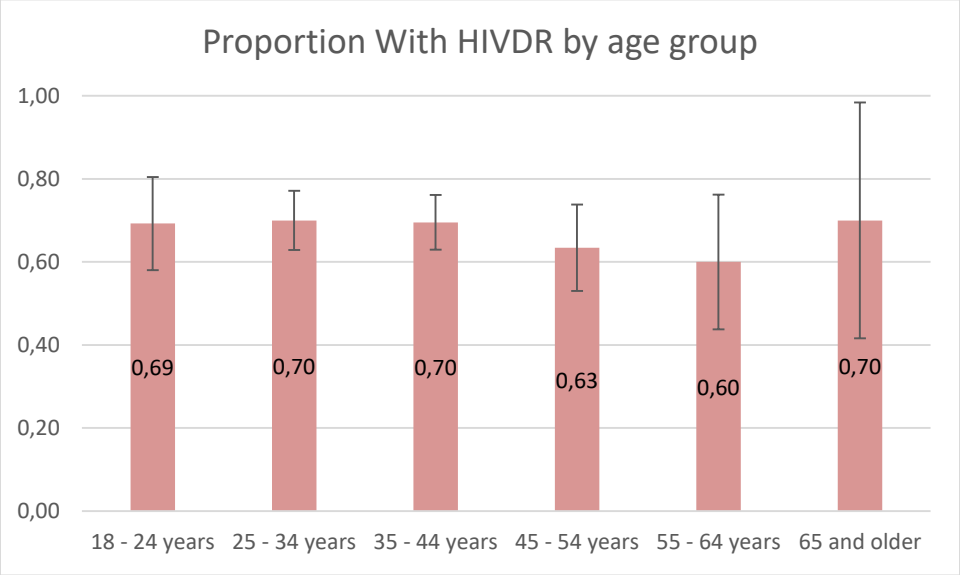

Figure 12.5.1 Proportions of specimens with resistance detected by age group in the cross-sectional surveillance study to assess levels of HIV drug resistance (HIVDR) in adults with viraemia, August-September 2021, South Africa.

13. DISCUSSION

Our current survey showed that 67.6% of HIV positive patients on ART with unsuppressed VL in the public sector harbor resistance to ART, compared to 72.1% in the 2019 survey. The observed drop is not statistically significant. The most common resistance found was to NNRTI, with 66.4% of specimens harboring resistance to NNRTI, 41.4% of specimens harboring resistance to NRTI, 4.1% of specimens exhibiting resistance to PI and 0.2% to INSTI. The low prevalence of INSTI resistance can be explained by multiple factors: the roll-out of TLD in South Africa was delayed and only 57% of those on treatment are on a DTG-based regimen (communication NDoH March 2022) and DTG is known to have a high genetic barrier to resistance. A limitation of this survey, is the lack of treatment regimen details and treatment duration. Although drug levels are used as a proxy for treatment regimen, drug levels were only detected in 52% of the samples; and only 7.2% of patients had detectable DTG levels.

The trend towards lower prevalence of NNRTI and NRTI resistance might be due to the roll-out of DTG-based regimens, which allows adherent patients to suppress viral replication quicker, reducing the risk for development of resistance. It is however still early in the DTG roll-out program to draw any firm conclusions regarding this trend. Although the prevalence of PI resistance doubled from 2.2% in 2019 to 4.1% in the current survey, this difference was also not statistically significant.

HIVDR was lower in patients with undetectable levels of ART, presumably due to lack of drug selection pressure ($p<0.0001$). Notably, 48% of patients on ART and presenting for routine VL testing had undetectable levels of ART, which was no different from the results observed in 2019 (45%).

The use of leftover specimens proved advantageous in that it allowed for proportion to size sampling, and reduced data collection time and cost. However, limited demographic and no clinical data was available through the laboratory information system.

14. CONCLUSION

The observed HIVDR levels in this survey are similar to those observed prior to the roll-out of DTG with frequent NNRTI and NRTI resistance, but low prevalence of PI and INSTI resistance, which is in line with the high genetic barrier of LPV/r and DTG and the recent introduction of DTG at large scale. However, it should be noted that these results should be interpreted cautiously given the low sample size. In addition, based on the sampling strategy, as viral suppression may be higher amongst patient receiving DTG-based regimens, over-sampling of NNRTI-based regimens may have occurred. Regular surveillance efforts are essential to continuously monitor the possible development of DTG resistance in the population.

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16. APPENDIX 1: Curriculum Vitae for investigators