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

2023

PEPFAR

CDC CENTERS FOR DISEASE

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
Ewalde Cutler	National Institute for Communicable Diseases (NICD)	Co- Investigator	ewaldec@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
Sean Currin	National Health Laboratory Service	Co-investigator	sean.currin@nhls.ac.za
Elliot Raizes CITI ID: 47856822; Expiration: 9 Mar 2025 SIQT Expiration: 04-Aug-26	Centers for Diseases Control and Prevention (CDC Atlanta)*	Co-Investigator	gwq0@cdc.gov
Kassahun Ayalew CITI ID: 45007574 Expiration: 8 Dec 2024 SIQT Expiration: 11-Sep-26	CDC South Africa *	Co-investigator - statistician	ylo8@cdc.gov
Jason Bedford CITI ID: 46902990 Expiration: 25-Jan-25 SIQT Expiration: 22-Dec.2026	CDC South Africa	Co-investigator	lgn1@cdc.gov
Kiren Mitruka CITI ID: 50356927 Expiration:13-Oct-2025 SIQT expiration: 31-Aug-26	CDC South Africa	Co-investigator	ylo8@cdc.gov
Rachael Joseph CITI ID:45411047 Expiration: 28-Dec-24 SIQT Expiration: 30-Aug-26	CDC South Africa	Co-investigator	vie5@cdc.gov
** CDC investigators are not considered "engaged" and did not intervene nor interact with participants or have access to identifiable information*			

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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 U.S. Centers for Disease Control and Prevention (CDC).

1.1. NHLS/NICD

Dr Kim Steegen served as the Principal Investigator for this study. She provided leadership, study implementation, specimen processing, data analysis, and reporting of study findings.
Dr Ewalde Cutler served as a co-investigator for this study. She provided coordination of HIV drug resistance testing.
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.
Dr Sean Currin served as a co-investigator for this study. He provided technical assistance in 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 Kassahun Ayalew* served as a statistician during protocol development and was involved in data analysis.
Dr Jason Bedford* was involved in protocol development and provided technical assistance in data analysis and interpretation and reporting of results.
Dr Rachael Joseph* was involved in protocol development and provided technical assistance in data analysis and interpretation and reporting of results.
Dr Kiren Mitruka* was involved in technical assistance in data interpretation and reporting of results.
*CDC investigators are not considered “engaged” and will not intervene nor directly 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.

Acknowledgment

This study has been supported by the President's Emergency Plan for AIDS Relief (PEPFAR) through the Centers for Disease Control and Prevention (CDC) under the terms of CDC-RFA- GH2126

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 using standardized ART regimens. To maximize the effectiveness and sustainability of ART programmes, it is essential to monitor and minimize the further spread of HIV drug resistance (HIVDR). HIVDR can affect the effectiveness of ART regimens, as well as be a source of HIVDR transmission.[1]

In South Africa, it is estimated that there were approximately 7.6 million people living with HIV in 2022.[2] The scale-up of ART has been ongoing since April 2004, and based on the latest figures, 5.7 million people living with HIV in South Africa received ART in 2022.[2] From 2013 to 2019, 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).[3,4] Towards the end of 2019, South Africa released updated national ART guidelines which began being implemented beginning in 2020, wherein first-line regimens for adults and adolescents consist of dolutegravir (DTG)/lamivudine (3TC))/tenofovir (TDF) [TLD]. Dolutegravir replaced efavirenz in 2020 for first-line ART in light of rising regional non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance.[5] In clinical studies, dolutegravir demonstrated excellent tolerability and a formidable resistance barrier,[6] providing cost benefits over efavirenz-based regimens in generic co-formulations in lower and middle income countries (LMICs).[7] A recent meta-analysis of studies assessing efficacy, safety and tolerability of DTG in first-line ART showed a very high suppression rate with none of the patients developing DTG resistance, indicating most failures are due to suboptimal treatment adherence.[8] Likewise, switching patients from a NNRTI-based ART to tenofovir-lamivudine-dolutegravir (TLD) has proven to be a successful strategy, with high levels of viral suppression obtained[9-14]. It is expected that this high population-level suppression rate will reduce the chance for HIV transmission as well as the development of HIVDR. Despite the small number of patients failing DTG-based ART, the prevalence of DTG resistance in patients with treatment failure was higher than expected in recent studies. In Malawi, a programmatic approach was taken to transition clients from a NNRTI based regimen to TLD, irrespective of viral load at switch. Only 14 clients presented with virological failure 12 months after switch and two of those presented with DTG resistance (2/14,14%).[12] In the NADIA trial 3/17 (18%) participants with virological failure by 96 weeks presented with DTG resistance.[11] Another study in Malawi assessed the prevalence of DTG resistance in patients failing treatment. Resistance testing was only requested after review by the HIVDR expert committee and 8/27 (30%) of the clients with approved resistance testing presented with DTG resistance.[15]

The roll-out of TLD in South Africa was initially delayed in 2020 as there were safety concerns regarding the development of neural tube defects in infants born to pregnant women taking DTG-based regimens.[16] 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.[17,18] 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, over 4.7 million people living with HIV in South Africa had been initiated or switched to DTG by March 2023, representing approximately 76% of those on treatment in the public sector.[19]

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).[20] 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. Given that steady increases in HIVDR prevalence have been demonstrated, particularly in Southern and Eastern African countries[1], the WHO Global Action Plan on HIV Drug Resistance includes a series of recommendations aimed at preventing HIVDR from undermining efforts to achieve global targets on management of HIV. 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[21].

5.2. Rationale for programmatic monitoring of HIVDR prevalence

In many LMICs, 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 typically managed by switching to standardized second-line treatment regimens. In these settings, continued and regular surveillance of pre-treatment drug resistance 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 appropriate 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.

Since 2004, WHO has recommended nationally representative surveys be implemented in LMICs to assess levels of pre-treatment drug resistance and ADR. However, uptake of these surveys in countries with high HIV burden has been slow and complex, with limited funding available. 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. Additionally, countries can use convenience cohorts and/or laboratory-based sampling of treatment failures to facilitate cost-effective surveillance outcomes and generate more-timely data.

In South Africa, HIV VL testing has been recommended as a treatment monitoring tool since 2004. At the time of the survey, VL testing was 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 a strong network of 17 HIV VL laboratories that contribute programmatically to VL testing, with coverage rates of >85% across all nine provinces (NHLS data, unpublished).

6. STUDY OBJECTIVES

The objective of the study was to estimate the prevalence of HIVDR among adult patients in 2023 who were receiving ART in public health facilities and presented for routine monitoring with a VL ≥1,000 copies/ml. Samples were obtained using remnant plasma specimens in South Africa.

7. METHODS

7.1. Sampling Strategy

This cross-sectional study used a two-stage sampling approach. For the first stage, a systematic random sample of remnant VL test samples coming from public health facilities were selected at each of the 17 national VL laboratories over a six-day period. The National Health Laboratory Service (NHLS) laboratory information system (LIS) (TrakCare) database was then used to identify each sample and retained 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 by testing volumes and viral non-suppression rates per laboratory and included for drug resistance testing. Due to the lack of ART history on LIS, drug level testing was used as a proxy for treatment exposure.

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
- Blood specimens were sent for routine VL testing
- HIV VL results were already available and authorized (released) in the NHLS LIS
- Leftover sample was available in sufficient amount (>500 ul)
- HIV VL result was ≥1,000 copies/ml

7.2.2. Exclusion criteria:

- Minimal data fields were not available in the laboratory information system, including age, facility, and clinic.
- Remnant plasma specimens from males or females who were <18 years
- HIV VL was <1,000 copies/ml
- Leftover sample was insufficient (<500 ul)

7.3. Sample size calculations

Sample size calculations were performed, based on the assumption that 88.7% of patients with available VL tests had a VL <1,000 copies/mL (unpublished NHLS data from March 2022-February 2023). To select 821 specimens with VLs of ≥1,000 copies/mL, a minimum required sample total of 7,288 had to be collected and stored during Stage 1.

The minimum effective sample size was 385 specimens, after adjusting for a 10% specimen rejection rate, 5% genotyping failure rate, and 4.1% specimen exclusion rate due to age and a design effect of 1.75 (Table 7.3.1).

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, May-June 2022, South Africa

	Statistical Precision		Sample size adjustments					
Proportion Estimated	Error size	95% CI*	Effective Sample Size	Design Effect 1.75	Genotyping failure (5%)	Unusable sample (10%)	Underage sample (4.1%)	VL** suppression (88.7%)
0.5	0.05	1.96	385	674	709	788	821	7288

*CI = Confidence interval

**VL = Viral load

7.4. Specimen collection and randomization

Specimens were selected at each of the 17 NHLS VL laboratories from 13 May 2023 to 10 June 2023 by selecting every 5th specimen once the VL result was authorized on the LIS. Remnant plasma was decanted into a separate tube and allocated a study identification number (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.[22,23] The decanted specimen was labelled with the Study ID only. Only the principal investigator and data manager had access to the linkage component of the database. Specimens were shipped to the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) in Johannesburg for storage at -80^{o}C.

7.5. HIV drug level testing (DLT)

All specimens were tested for the following antiretroviral drugs used in the public sector: EFV, TDF, LPV, atazanavir (ATV), darunavir (DRV), ritonavir (RTV) and DTG, using high performance liquid chromatography tandem mass spectrometry (LC/MS/MS) in a multiplex testing approach. The extraction was performed using acetonitrile or methanol protein precipitation. An Acquity HSS T3 column was used, phase A: water + 0.1% formic acid, phase B: acetonitrile + 0.1% formic acid. A combination of commercial calibrators, controls, and internal standards (Chromsystem MassTox® TDM Anti-HIV set). Where standards were not commercially available, traceable standards were spiked into drug free serum to form calibrators and controls. Mass analysis was performed on a Waters Xevo TQ-S triple quadrupole mass spectrometer in ESI positive mode using quantifier and qualifier transitions. The method is validated and was performed in an accredited laboratory.

Results were reported down to the limit of quantification (LOQ). This analysis was performed at the NHLS Chemical Pathology Laboratory at CMJAH, and these results were used as a proxy for current treatment regimen.

7.6. HIVDR genotyping

Remnant specimens from adult patients 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 (SOP NIC0998, BioMérieux, Marcy l'Étoile, France). PCR amplification of the protease, reverse transcriptase and integrase (PR/RT and IN) regions

of the HIV-1 pol gene was performed using the HIV-1 Genotyping Kit with integrase (SOP NIC1320, Cat No A55120, Thermo Scientific, Waltham, MA, USA). PCR amplicons were purified using AMPure XP beads (Beckman Coulter, Indianapolis, IN, USA). PR/RT and IN amplicons from the same patient, were combined and quantified using the Qubit dsDNA HS (high sensitivity) kit on the Qubit Flex Fluorometer (SOP NIC1288, Thermo Scientific, Waltham, MA, USA). Quantified amplicons were diluted and pooled in equimolar concentrations and libraries were prepared using the Illumina Nextera DNA Flex Library Preparation Kit (Illumina, San Diego, CA, USA), as per manufacturer's instructions (SOP NIC1205). Sequencing was done on the Nextseq 550 (Illumina, San Diego, CA, USA, SOP NIC1205). The genotyping was performed in an accredited laboratory, where the assay was also validated. FastQ sequences were submitted to PASeq (paseq.org) for NGS HIV drug resistance analysis. Consensus sequences were generated at 20%, which has been shown to have the best agreement between standard Sanger Sequencing and Next Generation Sequencing.[24] Consensus sequences were subsequently submitted to the Stanford University HIV Drug Resistance Database (hivdb.stanford.edu) for resistance interpretation. Resistance was defined as at least low-level resistance, 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. The data are weighted by test volumes as well as prevalence of VL≥1000 copies/mL, and the study design was taken into account in the analysis. Comparisons between categorical variables were assessed with the Pearson Chi Square test. Significance was set at p-value of <0.05. All analyses were conducted using STATA version 17 (STATA Corp., College Station, TX, USA).

8. DISSEMINATION OF RESULTS

Upon receiving CDC approval, this survey report will be used to disseminate findings to key stakeholders on the prevalence of HIVDR among patients receiving ART in South Africa. Individual genotyping results were returned to the corresponding provincial HAST (HIV/AIDS, STI's and Tuberculosis) programme managers who will then disseminate the results to the District Medical Officers. Conference abstracts and manuscripts will be developed for dissemination as deemed appropriate by the investigators.

9. ETHICAL CONSIDERATIONS

Ethical approval was obtained from the Human Research and Ethics Committee at the University of the Witwatersrand (M181067). The study was also reviewed in accordance with the US CDC human research protection procedures and was determined to be research not involving human subjects (45 CFR 46.102€). The requirement for individual informed consent was waived as only remnant VL specimens were used from patients undergoing routine VL testing, and all samples were delinked.
The protocol was conducted in accordance with 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 Principal Investigator had access to the linked data, which was required to return the genotyping results to the corresponding HAST programme managers. Electronic data files, computers and other storage devices that contain data were password protected. All NHLS and NICD staff complied with institutional confidentiality policies and agreements, as stated in NHLS Standard Operating Procedure GPQ0061.

10. CONFLICT OF INTEREST

The investigators have no conflicts of interest to declare.

11. OUTCOMES

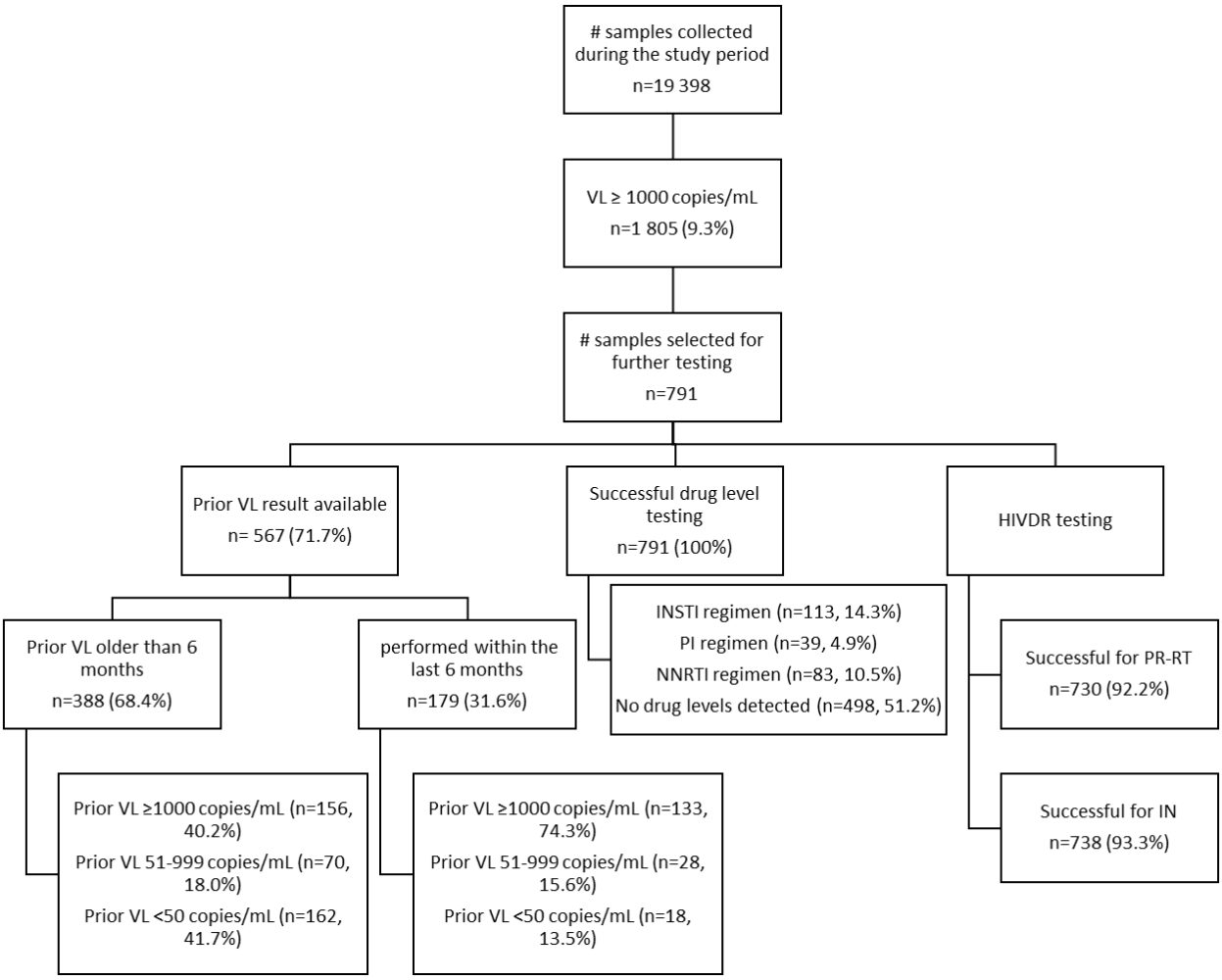
11.1. Specimen collection

Remnant VL plasma specimens were aliquoted on site and shipped on dry ice to the NHLS CMJAH Genotyping laboratory over the collection period from May 13, 2023 to June 10, 2023. In this 4-week collection period, a total of 557,478 VL tests were performed nationwide at the NHLS, of which 53,438 (9.6%) had ≥1,000 copies/ml. A total of 19,398 remnant specimens were collected, exceeding the projected target of 7,288. The collected specimens included 1,805 specimens with a VL ≥1,000 copies/mL, yielding a viral non-suppression rate of 9.3% (Table 12.1.1 and Figure 12.1.1.). After exclusion of specimens from patients <18 years, second stage sampling was performed, ensuring sample selection was proportional to the number of VL tests done and the VS rate at each of the laboratories. A total of 791 samples were selected for further testing. The weighted mean VL of the included specimens was 207,163 copies/ml (95% Confidence Interval (CI): 162,601–251,726 copies/ml). The LIS was used to identify prior VL results for each of the 791 samples included in the survey. For 567 (71.7%) samples, a prior VL result was found; however, only 179 (31.6%) of these prior VL samples had been taken within 6 months before the samples used in the survey were collected. Among samples with a prior VL result within 6 months (n=179), 133 (74.3%) had confirmed virological failure (2 consecutive VL ≥1,000 copies/mL). For another 28 samples (15.6%), the prior VL ranged from 50-999 copies/mL. Among the 388 samples with prior VL results older than 6 months, 156 (40.2%) had a prior VL ≥1,000 copies/mL, 70 (18.0%) had a prior VL between 51 and 999 copies/mL, and the remaining samples (n=162, 41.7%) had a prior VL<50 copies/mL. This data is summarized in Figure 12.1.1.

Table 11.1.1. Number of remnant viral load specimens expected to be and actually collected to assess levels of HIV drug resistance (HIVDR) in adults with viraemia, May to June 2023, South Africa

LAB	Total VL Performed	Total Unsuppressed	Proportion Unsuppressed	Sampling Proportion	Total to be Sampled	Unsuppressed to be Sampled	Samples Collected	Unsuppressed Samples Collected	Proportion Unsuppressed Collected
AD	46,964	3,630	7.7%	8.9%	648	60	1,889	115	6.1%
CM	75,561	6,075	8.0%	13.1%	956	88	2,702	219	8.1%
DG	31,533	4,012	12.7%	6.3%	458	57	990	128	12.9%
FR	24,071	2,975	12.4%	6.6%	484	43	849	104	12.2%
ED	35,041	2,439	7.0%	3.9%	286	42	1,441	111	7.7%
GS	17,752	1,905	10.7%	2.9%	208	25	631	74	11.7%
IA	22,787	1,595	7.0%	3.1%	228	21	566	50	8.8%
MD	16,253	1,177	7.2%	3.3%	238	22	560	36	6.4%
MK	41,562	4,685	11.3%	7.5%	547	72	1,252	134	10.7%
MT	23,611	1,946	8.2%	4.3%	311	32	869	70	8.1%
NG	41,158	3,408	8.3%	9.2%	671	67	1,430	118	8.3%
PE	12,577	2,788	22.2%	7.9%	577	51	498	103	20.7%
NE	57,467	4,792	8.3%	2.1%	151	39	1,118	90	8.1%
TA	26,172	2,041	7.8%	5.1%	369	40	916	67	7.3%
TS	29,202	3,331	11.4%	5.6%	405	60	1,253	145	11.6%
TY	17,478	1,967	11.3%	3.0%	217	29	772	73	9.5%
UN	38,289	4,672	12.2%	7.3%	534	74	1,662	168	10.1%
Total	557,478	53,438	9.6%	100.0%	7,288	822	19,398	1,805	9.3%

VL: Viral Load. copies/ml: copies/milliliter. 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, TA Tambo Memorial Hospital, TS Tshepong Hospital, TY Tygerberg Hospital, UN Universitas Hospital


# samples collected during the study period
n=19 398
VL ≥ 1000 copies/mL
n=1 805 (9.3%)
# samples selected for further testing
n=791
Prior VL result available
n= 567 (71.7%)
Successful drug level testing
n=791 (100%)
HIVDR testing
Prior VL older than 6 months
n=388 (68.4%)
performed within the last 6 months
n=179 (31.6%)
INSTI regimen (n=113, 14.3%)
PI regimen (n=39, 4.9%)
NNRTI regimen (n=83, 10.5%)
No drug levels detected (n=498, 51.2%)
Successful for PR-RT
n=730 (92.2%)
Successful for IN
n=738 (93.3%)
Prior VL ≥1000 copies/mL (n=156, 40.2%)
Prior VL 51-999 copies/mL (n=70, 18.0%)
Prior VL <50 copies/mL (n=162, 41.7%)
Prior VL ≥1000 copies/mL (n=133, 74.3%)
Prior VL 51-999 copies/mL (n=28, 15.6%)
Prior VL <50 copies/mL (n=18, 13.5%)

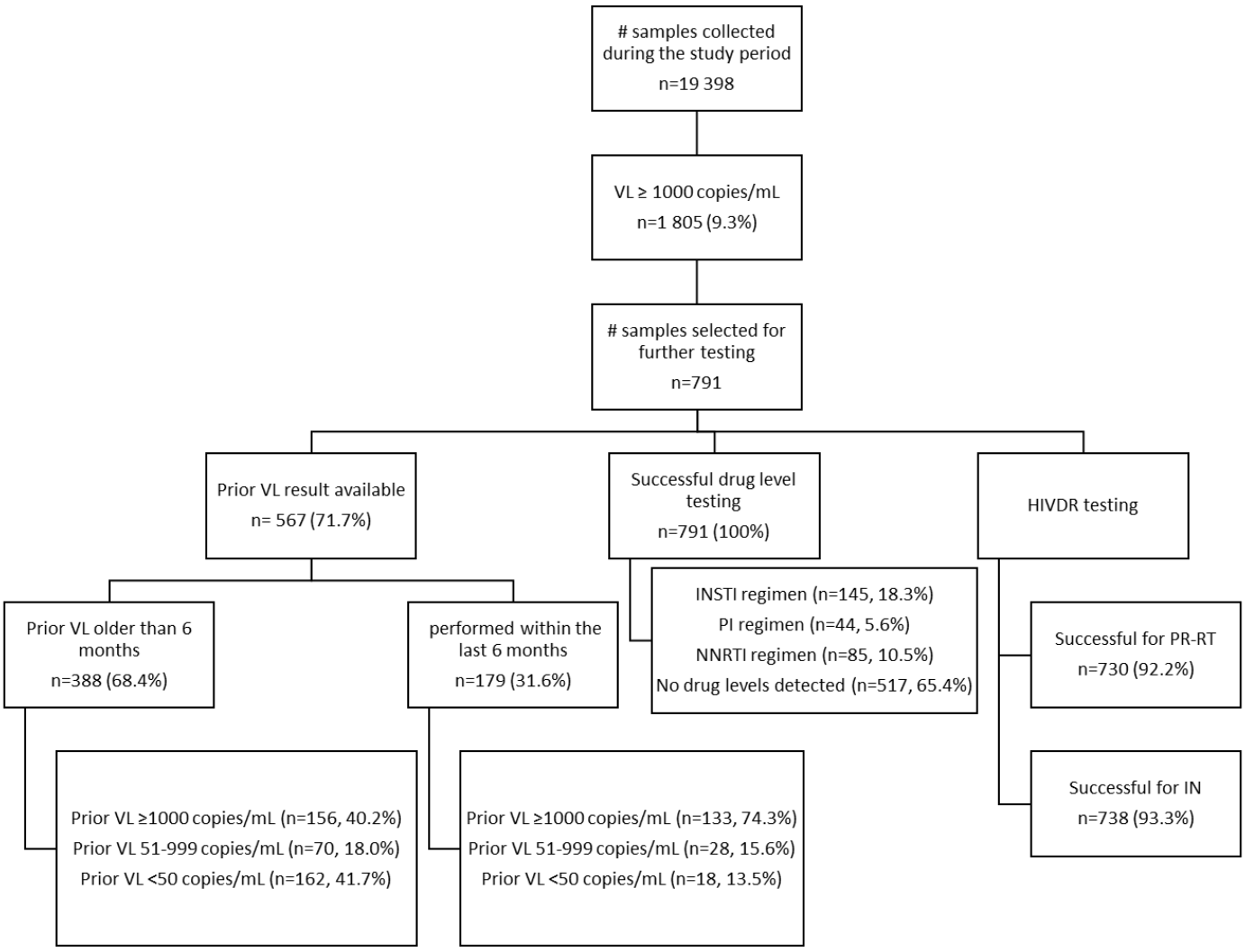


Figure 11.1.1. Flowchart describing specimen collection, HIVDR testing success rate, treatment distribution and prior VL results

VL: viral load; HIVDR: HIV drug resistance; INSTI: integrase strand transfer inhibitors; PI: protease inhibitors; NNRTI: non-nucleoside reverse transcriptase inhibitors; PR-RT: protease and reverse transcriptase genes; IN: integrase gene.

11.2. Laboratory testing – drug level testing

Drug level testing (DLT) was successful for all 791 specimens. ART drugs were detected in 274 (34.6%) specimens. Patients were classified as taking a) an INSTI regimen if DTG levels were detected; 2) a PI regimen if LPV, ATZ, DRV or ritonavir levels was detected; 3) a NNRTI regimen if EFV levels was detected. (Figure 12.1.1.).

The weighted proportion estimate for detection of any ARV was 36.7% (95% CI: 29.7%–44.4%). The most frequently detected drugs were DTG (18.4%, 95% CI: 13.7%–24.2%) and EFV (11.6%, 95% CI: 8.9%–15.0%). There is a notable increase of detectable DTG levels and a yearly decrease of detectable EFV levels from 2019 to 2023 [25-27]. On the other hand, the detection of PIs remained stable around 5% (Figure 12.2.1).

Of the 274 specimens with detectable drug levels, 145 specimens (53.3%, 95% CI: 47.4%–59.2%) had detectable DTG levels.

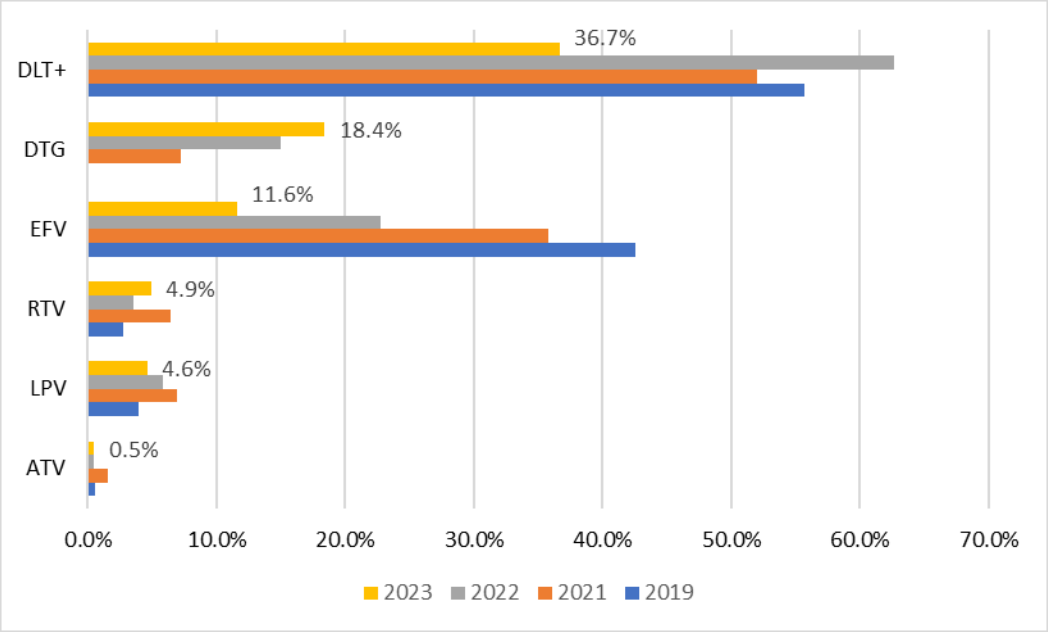


Figure 11.2.1. Weighted proportions of specimens with detectable drug levels, 2019-2023, South Africa.

DLT+: drug level testing positive; LPV: lopinavir. ATV: atazanavir. RTV: ritonavir, EFV: efavirenz. DTG; dolutegravir.

11.3. Laboratory testing – HIVDR testing

Of the 791 samples selected for further testing, HIVDR genotyping was successful for PR-RT in 730 (92.2%) and IN in 738 (93.3%) specimens (Figure 12.1.1.).

Table 12.3.1 depicts the weighted proportion of samples with resistance by drug level exposure. Resistance to at least one drug-class was detected in 53.7% (95% CI: 48.5%–58.9%) of specimens. Resistance to NNRTI was detected in 50.7% (95% CI: 45.6%–55.9%), resistance to Nucleoside Reverse Transcriptase Inhibitors (NRTI) was 25.2% (95% CI: 20.5%–30.6%), resistance to Protease Inhibitors (PI) was detected in 2.2% (95% CI: 1.2%–4.1%) and resistance to Integrase Strand Transfer inhibitors (INSTI) was detected in 2.3% (95% CI: 1.3%–3.8%) (Table 12.3.1). When analyzed according to drug level detection, the proportion of specimens with resistance were significantly higher in specimens that had detectable ART levels (67.8% (95% CI: 60.5%–74.3%)) compared to those without detectable ART levels (47.0% (95% CI: 41.0%–53.1%, p<0.0001)). Among specimens with detectable INSTI levels and the availability of an IN sequence (n=109), 10.5% (95% CI: 6.3%–17.2%) presented with INSTI resistance. In contrast, only four specimens (0.8%, 95% CI: 0.3%–2.2%) had detectable INSTI resistance when no drug levels were present (n=509) (Table 12.3.1).

Among specimens with detectable PI levels and the availability of a PR sequence (n=41), 32.6% (95% CI: 18.6%–50.6%) presented with PI resistance. In contrast, only four specimens (0.7%, 95% CI: 0.2%–2.0%) presented with PI resistance among those where no drug levels were detected (n=509).

Among specimens with detectable NNRTI levels and the availability of a RT sequence (n=80), 84.0% (95% CI: 73.8%–90.7%), presented with NNRTI resistance. The prevalence of NNRTI resistance was 45.0% (95% CI: 39.3%–50.8%) in samples without any detectable drug levels.

The crude prevalence of specific HIVDR mutations is depicted in Figure 12.3.1. The most frequently detected mutations were at positions K103, M184, V106, and E138 in the reverse transcriptase gene. Most common major PI mutations were found at position M46, I54 and V82. Major INSTI mutations were detected at positions R263 (n=7), G118 (n=5), E138 (n=3) and T66 (n=2); however, none of these individual mutations were prevalent in more than 1% of the specimens.

In addition, the prevalence of specific mutations by predicted regimen (based on drug level testing result) are depicted in Figure 12.3.2. Patients were classified as either taking 1) an INSTI regimen if DTG levels were detected; 2) a PI regimen if LPV, ATZ, DRV or ritonavir levels were detected; or 3) a NNRTI regimen if EFV levels were detected. Patients without any ARV levels were classified as drug level testing negative (DLT-).

In the group of patients with a confirmed VF within 6 months prior to sample collection (n=133), 59 had a detectable drug level (44.4%, 95% CI: 36.2% - 52.9%) and were classified as INSTI exposed (n=18, 13.5%, 95% CI: 8.7% - 20.5%), 14 as PI-exposed (10.5%, 95% CI: 6.3% - 17.0%), and 23 (17.3%, 95% CI: 11.8% - 24.7%) as NNRTI exposed.
Successful INSTI resistance testing was obtained in 14/18 patients with confirmed virological failure and detectable DTG levels, of whom 3/14 (21.4%, 95% CI: 6.8% - 48.3%) presented with INSTI resistance.

Table 11.3.1 Proportions of specimens by drug levels in the cross-sectional surveillance study to assess levels of HIV drug resistance (HIVDR) in adults with viraemia, May-June 2023, South Africa

	n/N	%	95% CI		
All specimens					
Resistance to any class	388/720	53.7%	48.5%	-	58.9%
Resistance to PI	18/732	2.2%	1.2%	-	4.1%
Resistance to NRTI	186/732	25.2%	20.5%	-	30.6%
Resistance to NNRTI	374/732	50.7%	45.6%	-	55.9%
Resistance to INSTI	16/736	2.3%	1.3%	-	3.8%
Any drug level detected					
Resistance to any class	161/237	67.8%	60.5%	-	74.3%
Resistance to PI	14/243	5.3%	2.9%	-	9.6%
Resistance to NRTI	120/243	50.0%	40.1%	-	60.0%
Resistance to NNRTI	152/243	63.3%	55.4%	-	70.5%
Resistance to INSTI	12/249	5.4%	3.5%	-	8.1%
No drug level detected					
Resistance to any class	226/481	47.0%	46.9%	-	59.0%
Resistance to PI	4/487	0.7%	0.2%	-	2.0%
Resistance to NRTI	66/487	13.3%	10.4%	-	16.9%
Resistance to NNRTI	221/487	44.7%	38.6	-	51.0%
Resistance to INSTI	4/489	0.8%	0.3%	-	2.2%
NNRTI levels detected					
Resistance to any class	66/78	85.3%	75.4%	-	91.6%
Resistance to PI	0/80			-	
Resistance to NRTI	55/80	70.0%	55.1%	-	80.9%
Resistance to NNRTI	66/80	84.0%	73.8%	-	90.7%
Resistance to INSTI	1/80	1.3%	0.1%	-	11.5%
PI levels detected					
Resistance to any class	32/40	79.7%	63.8%	-	89.7%
Resistance to PI	14/41	32.6%	18.6%	-	50.6%
Resistance to NRTI	29/41	70.0%	52.1%	-	83.7%
Resistance to NNRTI	29/41	69.9%	52.1%	-	83.2%
Resistance to INSTI	1/40	2.7%	0.3%	-	2.0%
INSTI levels detected					
Resistance to any class	53/101	51.2%	42.1%	-	57.9%
Resistance to PI	0/104			-	
Resistance to NRTI	27/104	27.1%	18.0%	-	38.7%
Resistance to NNRTI	49/104	48.0%	39.6%	-	56.4%
Resistance to INSTI	10/109	10.5%	6.3%	-	17.2%

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

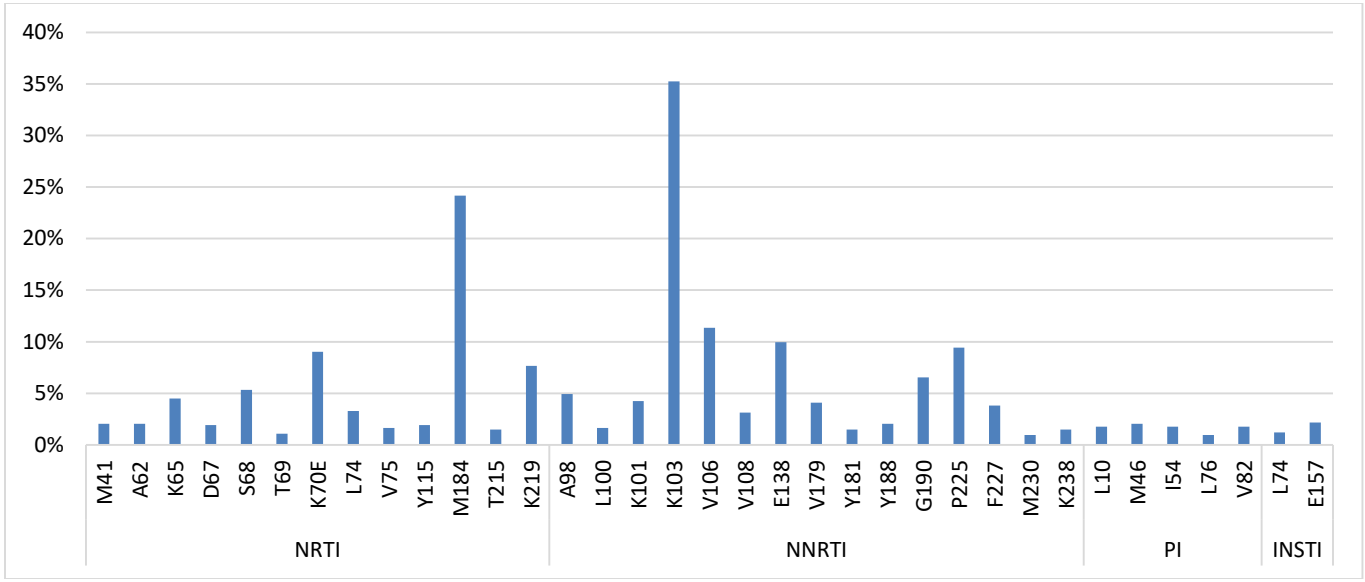


Figure 11.3.1. HIV drug resistant mutations detected in 738 specimens successfully genotyped, May-June 2023, South Africa. This figure depicts mutations that were present in at least 1% of the specimens.
PI: Protease Inhibitors. NNRTI: Non-nucleoside reverse transcriptase inhibitors. NRTI: nucleoside reverse transcriptase inhibitors, INSTI: Integrase strand transfer inhibitors

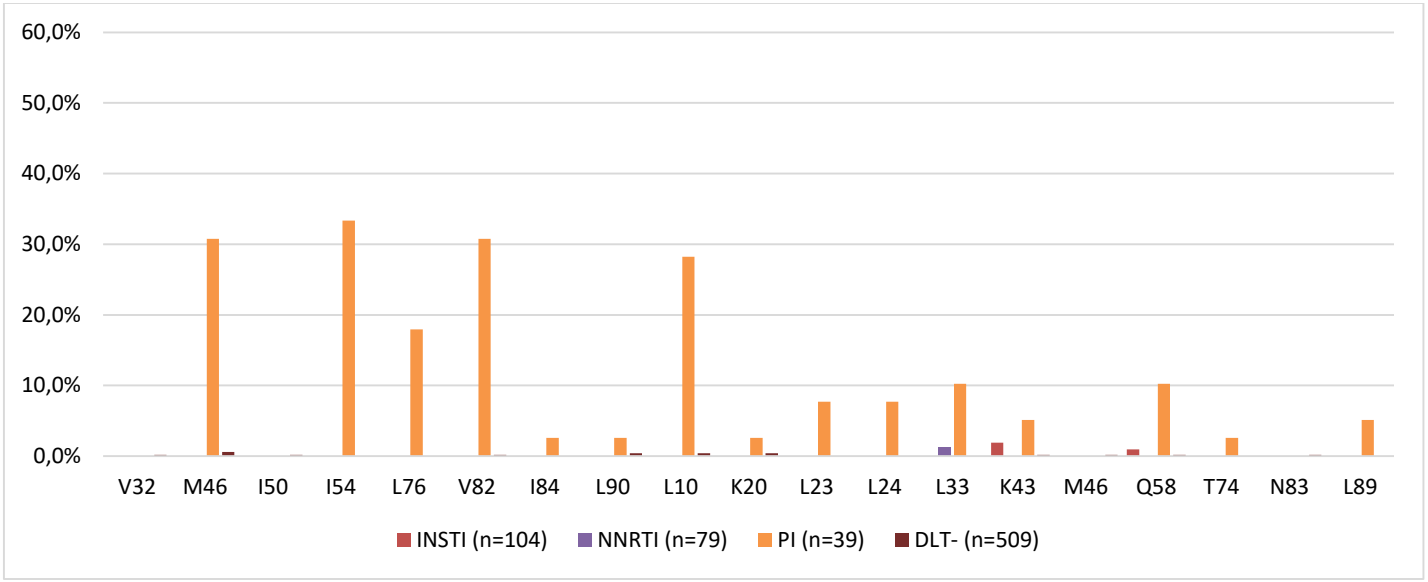


Figure 11.3.2. HIV drug-resistant Protease inhibitor mutations detected in specimens successfully genotyped, by predicted regimen type in the surveillance study, May-June 2023, South Africa.
PI: Protease Inhibitors. NNRTI: Non-nucleoside reverse transcriptase inhibitors, INSTI: Integrase strand transfer inhibitors, DLT-: negative drug level test

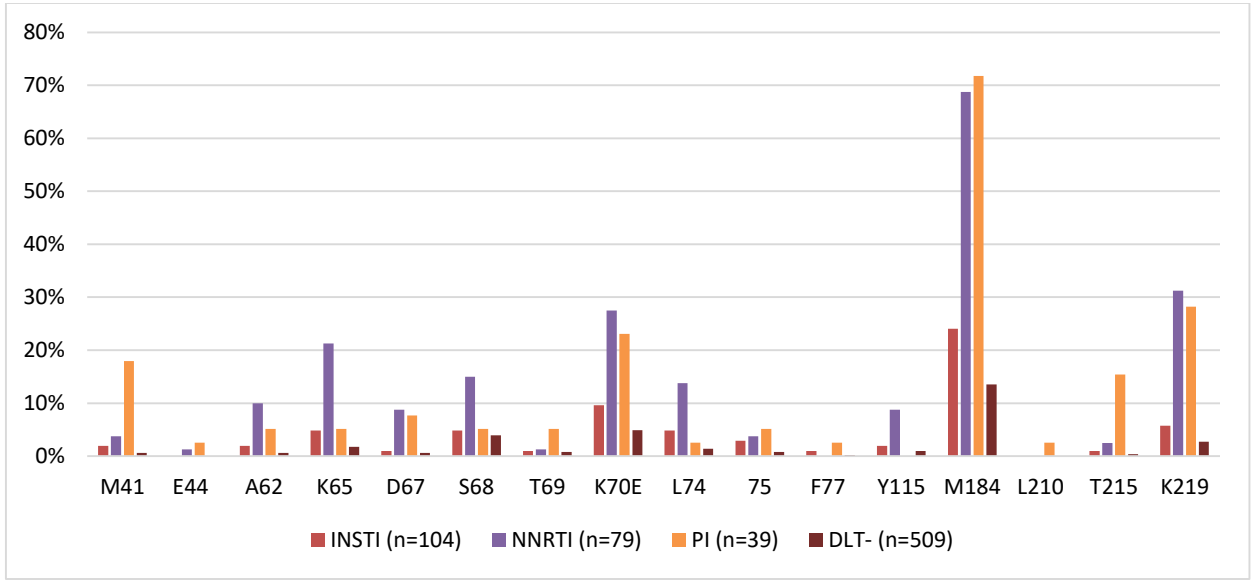


Figure 11.3.3. HIV drug-resistant nucleoside reverse transcriptase inhibitor mutations detected in specimens successfully genotyped, by predicted regimen type in the surveillance study, May-June 2023, South Africa.
PI: Protease Inhibitors. NNRTI: Non-nucleoside reverse transcriptase inhibitors, INSTI: Integrase strand transfer inhibitors, DLT-: negative drug level test

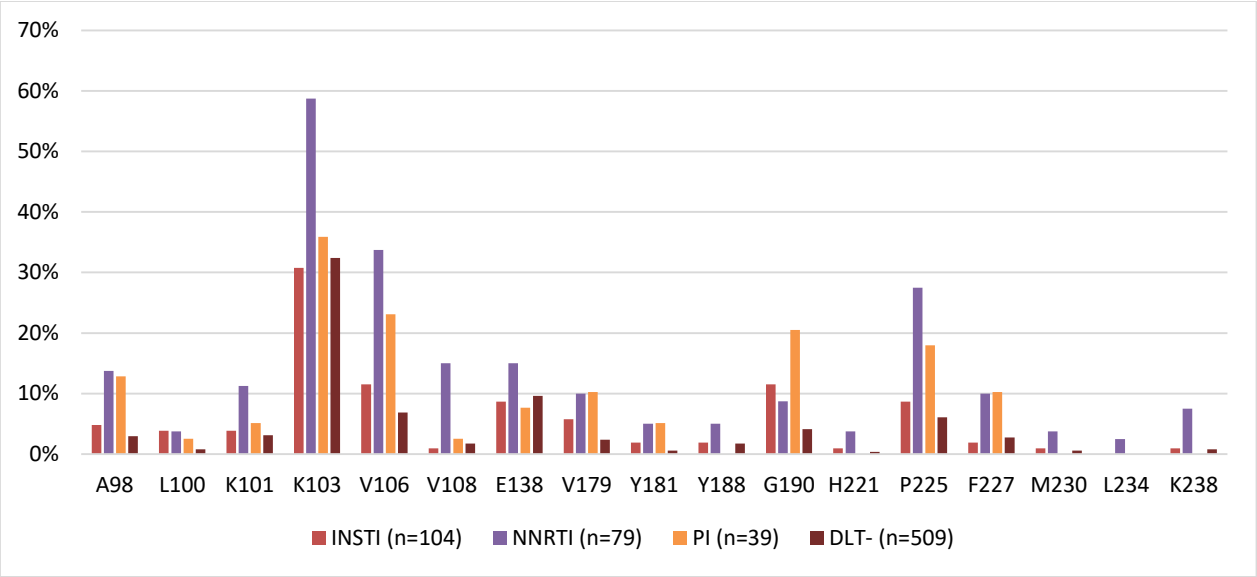


Figure 11.3.4. HIV drug-resistant non-nucleoside reverse transcriptase inhibitor mutations detected in specimens successfully genotyped, by predicted regimen type in the surveillance study, May-June 2023, South Africa.
PI: Protease Inhibitors. NNRTI: Non-nucleoside reverse transcriptase inhibitors, INSTI: Integrase strand transfer inhibitors, DLT-: negative drug level test

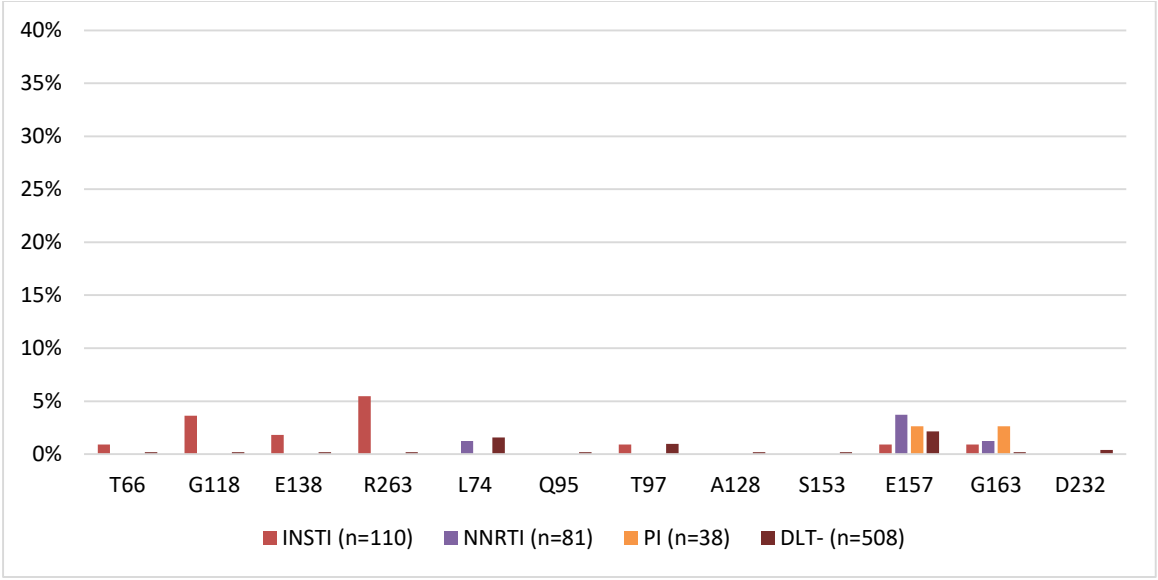


Figure 11.3.5. HIV drug-resistant integrase strand transfer inhibitor mutations detected in specimens successfully genotyped, by predicted regimen type in the surveillance study, May-June 2023, South Africa.
PI: Protease Inhibitors. NNRTI: Non-nucleoside reverse transcriptase inhibitors, INSTI: Integrase strand transfer inhibitors, DLT-: negative drug level test

11.4. Drug levels and resistance patterns by sex

Of 791 specimens tested, 518 (65.5%) were collected from female patients and 262 (33.1%) were from male patients and for 11 (1.4%) patients, gender was not recorded. Amongst specimens from female patients, 32.8% had detectable drug levels and 37.0% of specimens from male patients had detectable drug levels (p=0.372). HIV drug resistance was detected in 54.6% of all successfully processed specimens from female patients and 52.3% of all male patients, with no significant difference noted (p=0.787). Among the 145 specimens with detectable DTG levels, 60.0% were from female patients.

11.5. Drug levels and resistance patterns by age group

Median age at the time of enrollment was 37 years (IQR: 30–45 years). As can be seen in Figure 12.5.1 there was significant (p<0.05) difference in in the proportion of any detectable drug level and DTG drug level by age groups. Lower proportions of any detectable drug level (p<0.001) and detectable DTG (p=0.001) were noted in those less than 45 years of age.

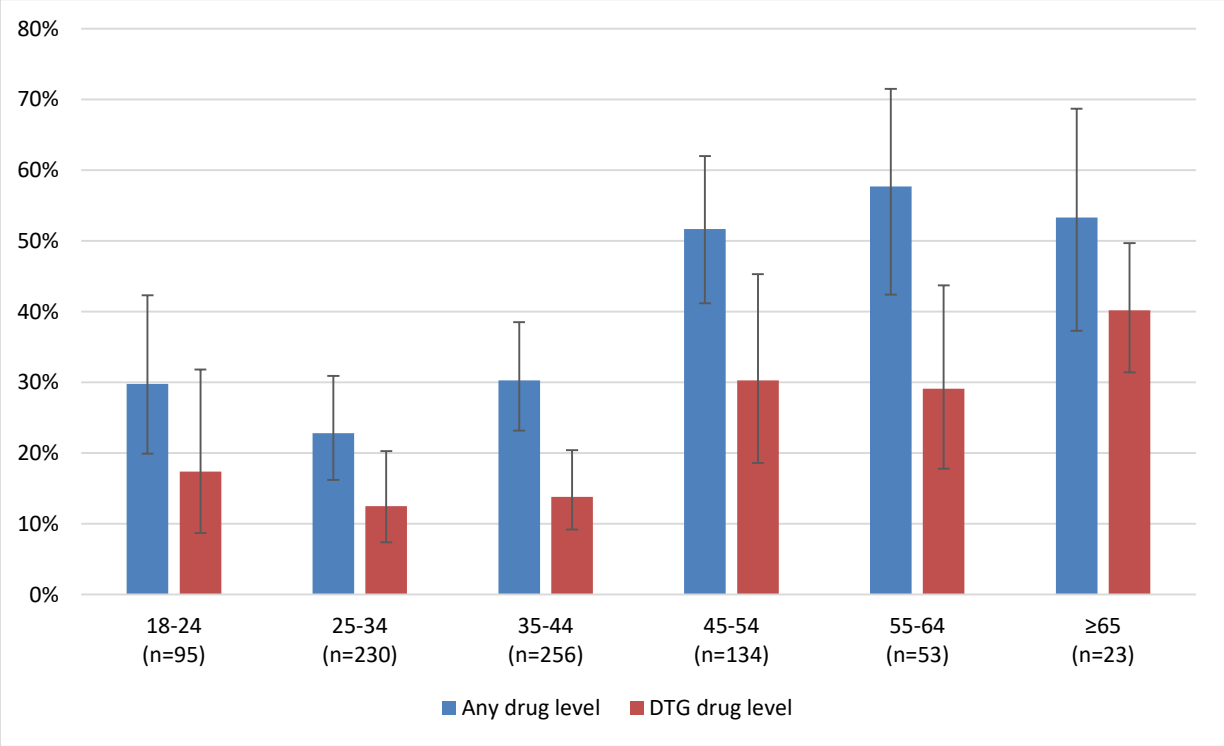


Figure 11.5.1 Weighted proportions of specimens with detectable drug levels by age group, May-June 2023, South Africa. Error bars indicate 95% Confidence intervals
DTG: dolutegravir

The prevalence of INSTI resistance did not differ by age groups (p=0.116), whereas a statistical difference was by age group for the presence of any resistance (p=0.038, Figure 12.5.2).

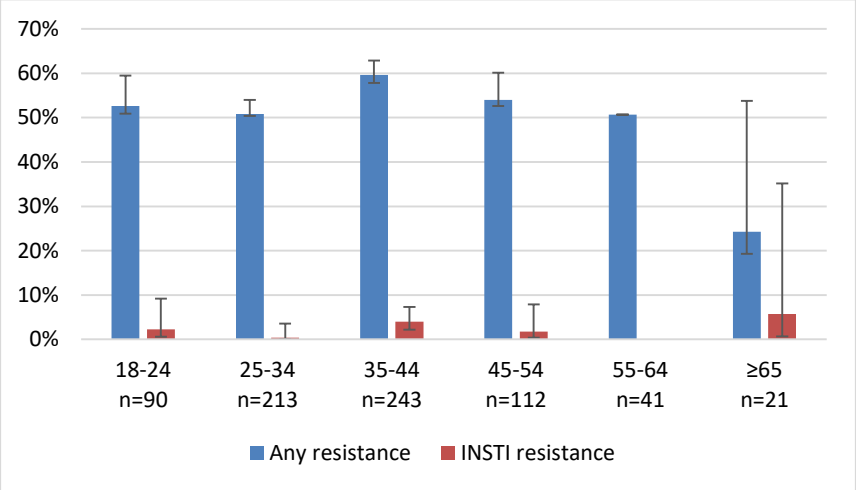


Figure 11.5.2 Weighted proportions of specimens with integrase and any resistance detected by age group, May-June 2023, South Africa. Resistance was defined as low-level resistance or higher as per the Stanford University HIV Drug Resistance Database. Error bars indicate 95% Confidence intervals
INSTI: Integrase strand transfer inhibitors

11.6. Drug levels and resistance patterns by province

The weighted prevalence of any drug level detected by province ranged from 19.9% in the Eastern Cape to 50.0% in Limpopo (p=0.002). The detection of DTG drug levels was most common in Kwazulu-Natal (24.6%) and Limpopo (22.2%) and least common in the Western Cape (8.2%) and the Eastern Cape (8.4%) (p=0.069, Figure 12.6.1). Please note that the study was not powered to adequately assess differences on a provincial level.

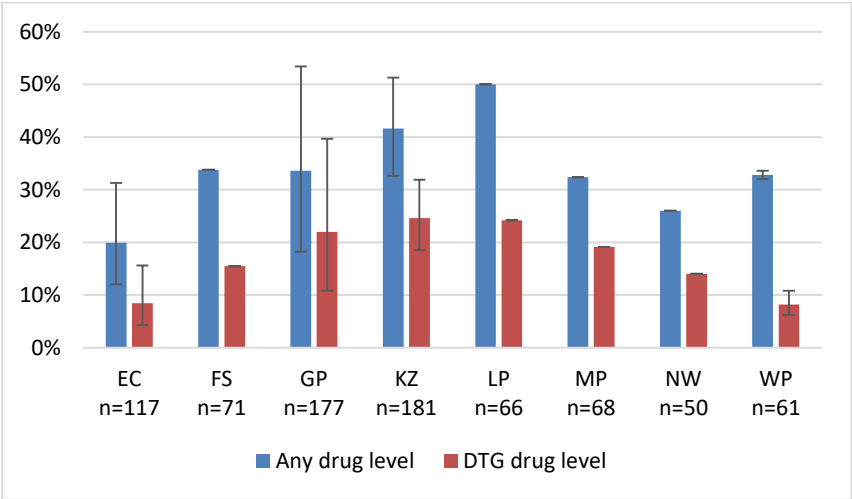


Figure 11.6.1 Weighted proportions of specimens with detectable drug levels by province, May-June 2023, South Africa. Resistance was defined as low-level resistance or higher as per the Stanford University HIV Drug Resistance Database. Error bars indicate 95% CI intervals
EC: Eastern Cape, FS: Free State, GP: Gauteng, KZ: KwaZulu-Natal, LP: Limpopo, MP: Mpumalanga, NW: North West, WP: Western Cape

The prevalence of any resistance detected by province ranged from 42.8% in Gauteng to 68.3% in the Western Cape (p=0.020). The detection of DTG resistance remained very low with no significant differences between provinces (p=0.393, Figure 12.6.2). Please note that the study was not powered to adequately assess differences on a provincial level.

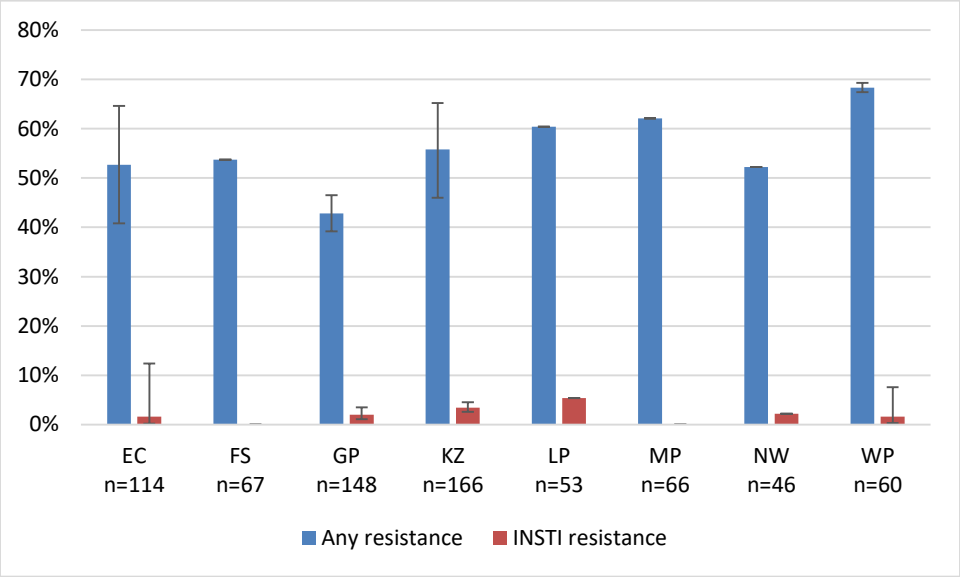


Figure 11.6.2 Weighted proportions of specimens with resistance by province, May-June 2023, South Africa. Resistance was defined as low-level resistance or higher as per the Stanford University HIV Drug Resistance Database. Error bars indicate 95% CI intervals

11.7. Result comparison to previous surveys

The laboratory based HIVDR surveys using the same sampling strategy among clients with VLs >1,000 copies/mL have been conducted in 2019[25], 2021[26], 2022[27] and 2023. Integrase drug level and resistance testing was only performed from 2021 onwards.

In all these prior HIVDR surveys, any drug level was detected in less than 60% of the specimens; with a significant drop to only 36.7% in 2023. The proportion of samples with detectable PI levels remained stable over the years. As per changing treatment guidelines, the proportion of EFV+ specimens decreased over time, while the proportion of DTG+ specimens increased. (Figure 11.7.1).

The prevalence of any drug resistance, regardless of detectable drug levels, declined from 72.1% in 2019 to 53.7% in 2023. The proportion of NNRTI resistance in the survey population followed a similar trend from 70.5% in 2019 to 50.7% in 2023. The prevalence of PI resistance remained stable with a range from 2.2% to 4.1%. INSTI resistance remains low but has steadily increased from 0.2% in 2021 to 2.3% in 2023 (Figure 11.7.2).

Combining drug level results and resistance results show the proportion of specimens with detectable NNRTI levels and NNRTI resistance remained above 84% from 2019 to 2023 (Figure 11.7.3). PI

resistance was found in about a third of samples with detectable PI levels, with the exception of the 2021 survey, where we only detected PI resistance in 17.2% of samples with detectable PI levels (Figure 11.7.3).

The prevalence of INSTI resistance among samples with confirmed INSTI exposure increased from 2.7% in 2021 to 11.1% in 2022, but no further increase was observed in 2023 (10.5%, Figure 11.7.3).

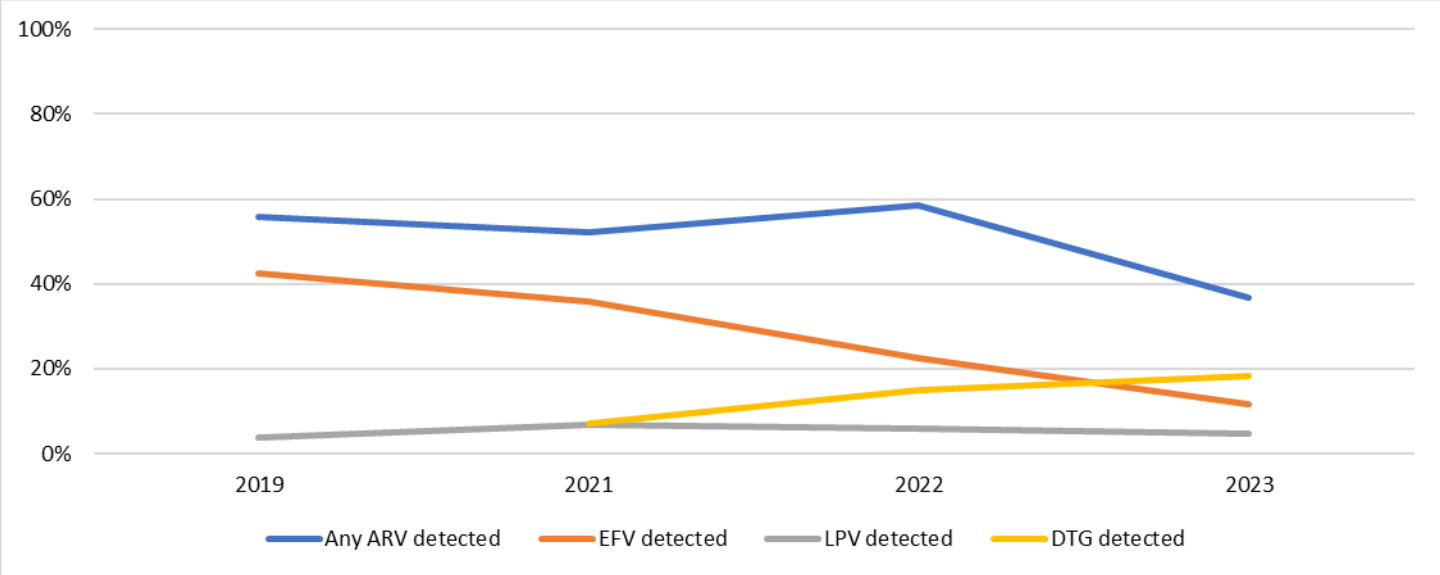


Figure 11.7. 1 Weighted proportions of specimens with detectable drug levels, 2019-2023, South Africa.

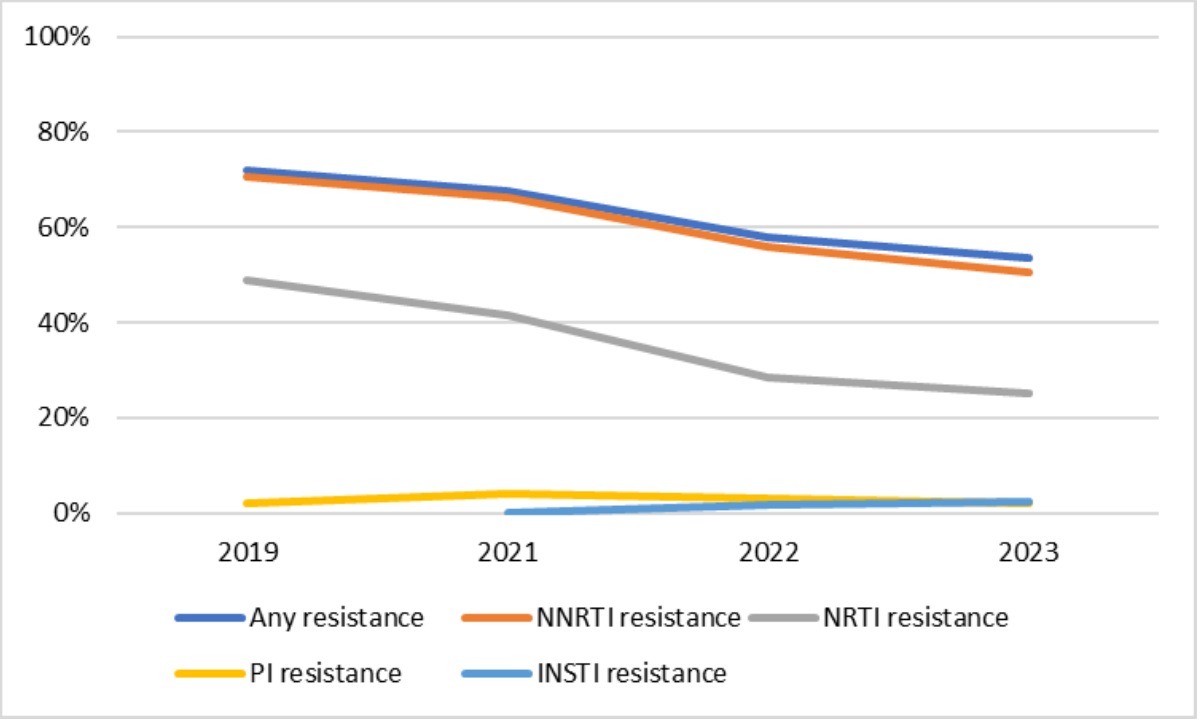


Figure 11.7. 2 Weighted proportions of specimens with drug resistance, 2019-2023, South Africa.

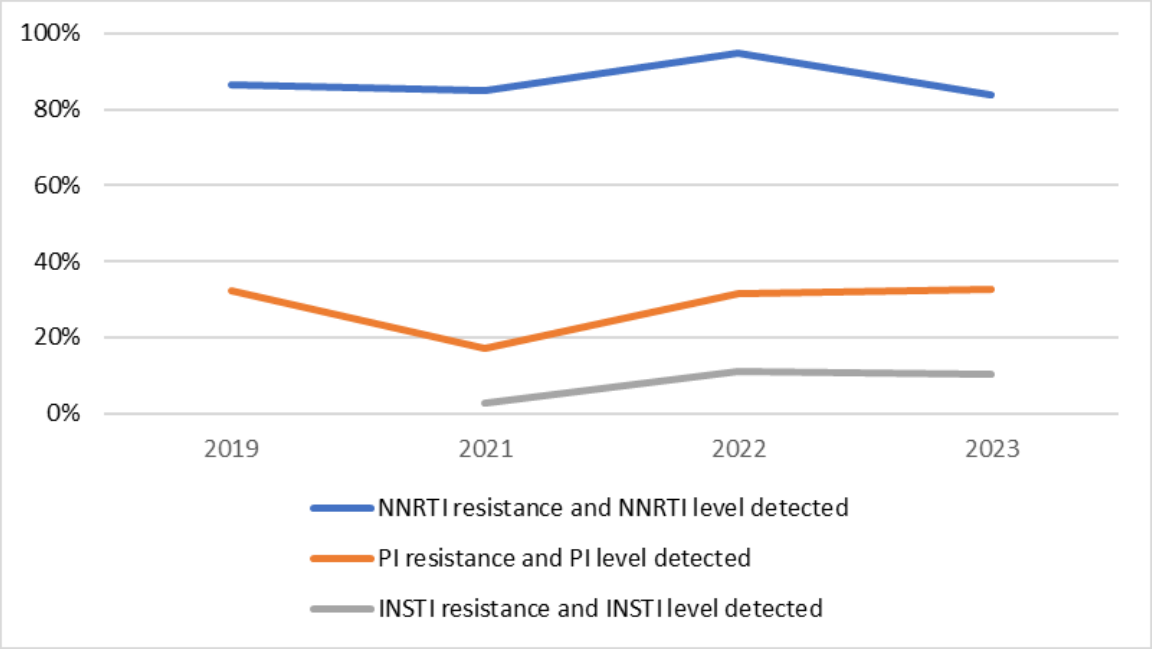


Figure 11.7. 3 Weighted proportions of specimens with drug resistance, 2019-2023, South Africa.

12. DISCUSSION

Our current survey showed that 53.7% of HIV positive patients on ART with unsuppressed VL in the public sector harbor resistance to ART, compared to 72.1%, 67.6% and 59.5% in the 2019[25], 2021[26] and 2022[27] surveys, respectively. NNRTI resistance was still most frequently detected (50.7%), compared to 70.5%, 66.4% and 55.5% in 2019, 2021 and 2022 respectively. Likewise, the prevalence of NRTI resistance has declined over the years: from 49.0% in 2019, to 41.4% in 2021, to 31.6% in 2022 and to 25.2% in the current survey. The overall prevalence of PI and INSTI resistance remains low. The proportion of PI resistance in this recent survey (2.2%) was the same as in 2019 (2.2%), whereas 4.1% and 4.3% of PI resistance was observed in 2021 and 2022, respectively. INSTI resistance has only been measured since the 2021 survey and increased from 0.2% in 2021 to 1.2% in 2022 and 2.3% in 2023.

Despite the roll-out of DTG, NNRTI drug levels and NNRTI resistance were still commonly detected in this population of randomly selected specimens with VLs of ≥1000 copies/mL. The proportion of samples with detectable DTG levels increased from 7.2% in 2021 to 15% in 2022 and 18.4% in 2023. According to the National Department of Health, over 4.7 million people living with HIV in South Africa had been initiated or switched to DTG by March 2023, which is an increase from 57% (March 2022) to 76% (March 2023) of those on treatment in the public sector.

The lack of treatment regimen details and treatment duration is an important limitation of this study. This is especially a concern in the current survey as only 36.7% had at least one drug detected in plasma, which is a significant drop from what was observed in previous surveys (62.6% in 2022, 52.0% in 2021 and 55.7% in 2019). The low prevalence of detectable drug levels, indicate that detectable VLs are often caused by inadequate treatment adherence and not necessarily by drug resistance. It is unclear why we observed a much lower proportion of samples with detectable drug levels in the current survey (36.7%) versus 52.0 to 58.6% in 2019-2022 surveys. In order to ascertain that drug level testing is an adequate proxy for treatment regimens, we will test a random sample of specimens with suppressed VL (VL<50 copies/mL, n=578) and low-level viraemia samples (VL 50-999 copies/mL, n=578) to further validate the use of drug level testing as a proxy for treatment exposure.

The trend towards lower prevalence of NNRTI and NRTI resistance might be explained by the roll-out of DTG-based regimens, which allows adherent patients to suppress VLs faster, and thereby reducing the risk for development of resistance[28]. However, given the lack of information on patient ART regimens on VL testing requisition forms, the proportion of the specimens from clients on DTG-based regimens cannot be discerned.

The prevalence of INSTI resistance remained low (2.3%) in patients with VLs of >1,000 copies/mL. However, the proportion of INSTI resistance was significantly higher in specimens with detectable DTG levels (10.5%). This is similar to the results obtained in 2022 where 11.1% of INSTI resistance was detected in specimens with detectable DTG levels. The proportion of samples with detectable DTG levels and INSTI resistance (10.5%) remains much lower than the proportion of NNRTI resistance in NNRTI exposed clients (84.0%). Also, INSTI resistance remains rare (0.8%) in specimens without detectable DTG levels.

The prevalence of PI resistance in samples with detectable PI levels remained stable in the 2023 survey (32.6%) compared to results obtained from the 2022 survey (31.7%).

The use of remnant specimens continues to be a logistically attractive and cost-effective method for HIV drug resistance surveillance, although the lack of demographic clinical data might limit the interpretation

of the results. On the other hand, the availability of drug level testing provides additional information which may help to identify patients at the highest risk for resistance.

13. CONCLUSION

The observed HIVDR levels in this survey are similar to those observed prior to the roll-out of DTG; however, the overall prevalence of resistance appears to be declining in recent years. This decline likely is driven by less frequent observations of NRTI and NNRTI resistance. However, a substantial proportion of patients with detectable drug levels remain positive for NNRTIs, indicating that the DTG roll-out was not yet fully implemented by May-June 2023, despite treatment guidelines recommending the unconditional switch to TLD for most patients by May 2022.[29]

The prevalence of PI and INSTI resistance remains low, which is in line with the high genetic barrier of LPV/r and DTG and the recent introduction of DTG at large scale.[30,31] Continued monitoring for the development of INSTI resistance in patients with detectable DTG levels is warranted, given that INSTI resistance levels of around 10% was confirmed in the 2022 and 2023 survey.

The sub-analysis of HIVDR resistance relative to the presence or absence of PIs or INSTIs indicates that screening tests for PIs and INSTIs drug levels could be used to triage specimens for HIVDR testing.

Despite the national representativeness of the survey, results should be interpreted cautiously given the limitations of obtaining accurate treatment regimen information. In addition, all sub-analyses should be interpreted with caution as the study was not powered to compare results among different age groups or by province. Also, because viral suppression may be higher amongst patients receiving DTG-based regimens, over-sampling of NNRTI-based regimens may have occurred. Should drug level testing prove to be an adequate measure as a proxy for ART exposure, the increase of patients without any detectable drug levels should be investigated and investments could be made to improve adherence interventions, Regular surveillance efforts are essential to continuously monitor the possible development of DTG resistance in the population.

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