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

PEPFAR

CDC
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NATIONAL HEALTH LABORATORY SERVICE

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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 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
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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 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 7.5 million people living with HIV in 2021.[2] The scale-up of ART has been ongoing since April 2004, and based on the latest figures, 5.6 million people living with HIV in South Africa received ART in 2021.[2] Between 2013 and 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 ARV 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]. 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: 2/14 (14%),[12] 3/17 (18%)[11] and 8/27 (30%).[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 women taking DTG-based regimens during pregnancy.[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, 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 (communication NDoH March 2022).

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).[1] 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, given that steady increases in HIVDR prevalence have been demonstrated, particularly in Southern and Eastern African countries. 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 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 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.

Since 2004, WHO has previously recommended nationally representative surveys be implemented in LMICs 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. 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 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 >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 in public health facilities who present for routine monitoring with a VL ≥1,000 copies/ml during 2022, 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 five-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.

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 87.6% of patients with available VL tests had a VL <1000 copies/mL (NHLS data March 2021-February 2022). To select 833 specimens with VLs of ≥1000 copies/mL, a minimum required sample total of 6 707 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 5.4% 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 (P)	Error size (e)	95% CI (Z0.05/2)	Effective Sample Size	Design Effect 1.75	Genotyping failure (5%)	Unusable sample (10%)	Underage sample (5.4%)	VL suppression (87.6%)
0.5	0.05	1.96	385	674	709	788	833	6707

7.4. Specimen collection and randomization

Specimens were selected at each of the 17 NHLS VL laboratories from May to June 2022 by selecting every 7th specimen once the VL result was authorized 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.[19,20] 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 National Institute for Communicable Diseases 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: 3TC, FTC, EFV, TDF, LPV, atazanavir (ATV), 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 protein precipitation. Then samples were loaded on an Acquity HSS T3 column, 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 Shimadzu 8060 triple quadrupole mass spectrometer in ESI positive mode using quantifier and qualifier transitions. The method is validated and was performed in an accredited laboratory (SOP CHE1920).

Results were reported at the limit of quantification (LOQ). 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 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 and reverse transcriptase (PR/RT) regions of the HIV-1 pol gene was performed using the HIV-1 Genotyping Kit (SOP NIC1190, Cat No A32317, Thermo Scientific, Waltham, MA, USA). The integrase (IN) region was amplified using an in-house nested PCR method

adapted from Van Laethem et al[21] (SOP NIC1090). 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.[22] 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, and the study design was taken into account in the analysis. Significance was set at p-value of <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 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.

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).The study was also reviewed in accordance with the US CDC human research protection procedures and was determined to be research, but CDC investigators did not interact with participants or have access to identifiable data or specimens for research purposes 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. 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

Remnant VL specimens were collected and shipped to the NHLS Genotyping laboratory over the collection period (16th May to 10th June 2022), spanning a 4-week period.
In this 4-week period, a total of 8 419 remnant specimens were collected, exceeding the target of 6 707. After excluding specimens that did not meet the age and VL criteria, 874 specimens remained. This outcome showed that increasing the specimen selection interval from 1:11 (2021 survey) to 1:7 improved the required specimen yield. Second stage sampling was then performed, ensuring sample selection was proportional to the number of VL tests done at each laboratory. The required number of specimens was reached in all but two laboratories (13 and 21 specimens short, at Tshepong Laboratory (TS) and Rob Ferreira Laboratory (NE), respectively). To avoid a decrease in overall samples size while attempting to ensure proportional representation from each of the sites, we decided to perform a second sample collection at the TS and NE sites in the week of 18-22 July 2022. An additional 382 and 559 specimens were selected for site TS and NE, respectively. A total of 9 356 specimens were collected, of which 1 111 (11.9%) samples had a VL of ≥1000 copies/mL. During the complete sample collection period (including the additional sampling week), a total of 497 871 VL tests were performed at the NHLS nationwide, of which 57 184 (11.5%) had ≥1,000 copies/ml (Table 12.1.1, Figure 12.1.1.).
A total of 709 samples were selected for further testing. The weighted mean VL of the included specimens was 193 339 copies/ml (95% Confidence Interval (CI): 122 980–263 698 copies/ml).

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, May to June 2022, 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	39 370	3 854	9.8%	8%	528	53	460	53	11.5%
CM	63 151	5 698	9.0%	11%	725	67	1 117	102	9.1%
DG	33 231	3 872	11.7%	7%	446	51	504	62	12.3%
FR	19 211	2 859	14.9%	5%	357	54	435	61	14.0%
ED	35 065	3 591	10.2%	6%	393	40	549	78	14.2%
GS	17 266	1 959	11.3%	3%	223	25	280	36	12.9%
IA	15 750	1 408	8.9%	2%	160	14	250	14	5.6%
MD	15 929	1 579	9.9%	3%	171	16	231	24	10.4%
MK	37 678	4 527	12.0%	7%	499	62	888	129	14.5%
MT	20 069	2 204	11.0%	4%	274	31	375	52	13.9%
NG	36 734	3 086	8.4%	5%	362	31	648	53	8.2%
PE	10 734	2 767	25.8%	5%	309	81	303	76	25.1%
NE*	49 444	5 177	10.5%	9%	628	66	1 089	84	7.7%
TA	24 088	2 467	10.2%	4%	282	28	347	29	8.4%
TS*	26 055	4 474	17.2%	8%	547	94	814	129	15.8%
TY	17 853	2 286	12.8%	3%	232	30	352	48	13.6%
UN	36 243	5 376	14.8%	8%	569	80	714	81	11.3%
Total	497 871	57 184	11.5%	100%	6 705	823	9 356	1 111	11.9%

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, TA Tambo Memorial Hospital, TS Tshepong Hospital, TY Tygerberg Hospital, UN Universitas Hospital
**At these sites, samples were collected for two weeks*

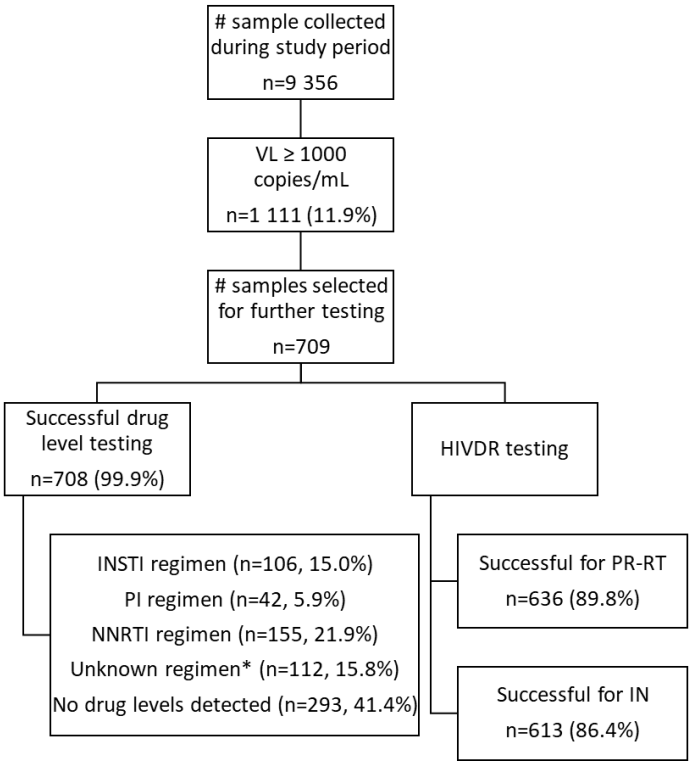

Figure 12.1.1. Flowchart describing specimen collection, HIVDR testing success rate and treatment distribution, in the cross-sectional surveillance study to assess levels of HIV drug resistance (HIVDR) in adults with viraemia.

** Specimens in which only NRTI drug levels were detected, were classified as unknown regimen.*
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.

12.2. Laboratory testing – drug level testing

Drug level testing (DLT) was successful for 708 of the 709 selected specimens. ART drugs were detected in 415 specimens. Patients were classified as taking a) an INSTI regimen if DTG levels were detected; 2) a PI regimen if LPV, ATZ or ritonavir levels were detected; 3) a NNRTI regimen if EFV levels were detected. Patients with only levels of any of the NRTIs, were classified as taking an unknown regimen (Figure 12.1.1.).

The weighted proportion estimate for detection of any ARV was 62.6% (95% CI: 54.4%–70.1%). The most frequently detected drugs (crude analysis) were TDF (23.7%, 95% CI: 20.7%–27.0%), EFV (22.7%, 95% CI: 19.8%–26.0%) and DTG (15.0%, 95% CI: 12.5%–17.8%) (Figure 12.2.1). Detectable DTG levels increased significantly compared to the 2021 survey (7.2% (95% CI 5.3%–9.6%), p<0.0001). Of the 415 specimens with detectable drug levels, 106 specimens (27.5%, 95% CI: 16.0%–43.1%) had detectable DTG levels.

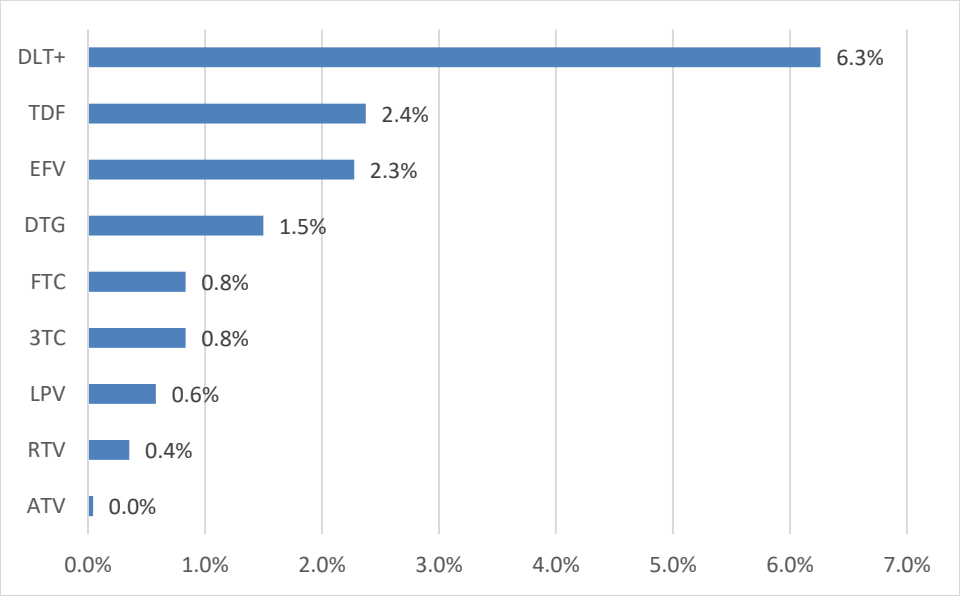

Figure 12.2.1. Weighted proportions of specimens with detectable levels of LPV, ATV, RTV, 3TC, FTC, TDF, EFV and DTG in the cross-sectional surveillance study to assess levels of HIV drug resistance (HIVDR) in adults with viraemia, May-June 2022, South Africa.

DLT+: drug level testing positive; LPV: lopinavir. ATV: atazanavir. RTV: ritonavir, 3TC: lamivudine. FTC; emtricitabine. TDF: tenofovir, EFV: efavirenz. DTG; dolutegravir.

12.3. Laboratory testing – HIVDR testing

Of the 708 samples selected for further testing, HIVDR genotyping was successful for PR-RT in 636 (89.8%) specimens and for IN in 613 (86.4%), Figure 12.1.1.

Table 12.3.1 depicts the proportion of samples with resistance by drug level exposure. Resistance to at least one drug-class was detected in 59.5% (95% CI: 54.2%–64.6%) of specimens. Resistance to NNRTI was detected in 55.5% (95% CI: 50.3%–60.6%), resistance to Nucleoside Reverse Transcriptase Inhibitors (NRTI) was 31.6% (95% CI: 26.9%–36.6%), resistance to Protease Inhibitors (PI) was detected in 4.3% (95% CI: 2.6%–7.2%) and resistance to Integrase Strand Transfer inhibitors (INSTI) was detected in 1.2% (95% CI: 0.5%–2.9%) (Table 12.3.1). When analyzed according to drug level detection, the proportion of specimens with resistance were higher in specimens that had detectable ART levels 69.9% (95% CI: 64.7%–74.1%) versus those without detectable ART levels 44.9% (95% CI: 34.1%–56.2%, p<0.0001). Among specimens with detectable INSTI levels and the availability of an IN sequence (n=64), 11.1% (95% CI: 4.9%–23.2%) presented with INSTI resistance. In contrast, only two specimens (0.3%, 95% CI: 0.0%–2.4%) presented with INSTI resistance when no drug levels were detected (n=273).

Among specimens with detectable PI levels and the availability of a PR sequence (n=38), 31.7% (95% CI: 10.5%–64.9%) presented with PI resistance. In contrast, only three specimens (2.7%, 95% CI: 0.7%–10.4%) presented with PI resistance among those where no drug levels were detected (n=281).

Among specimens with detectable NNRTI levels and the availability of a RT sequence (n=139), 94.7% (95% CI: 87.9%–97.7%), presented with NNRTI resistance. The prevalence of NNRTI resistance was 40.5% (95% CI: 30.9%–50.9%) 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 K70. Major INSTI mutations were detected at positions G118 (n=2), E138 (n=2), G140 (n=1), Q148 (n=1) and R263 (n=4); 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 taking a) an INSTI regimen if DTG levels were detected; 2) a PI regimen if LPV, ATZ or ritonavir levels were detected; 3) a NNRTI regimen if EFV levels were detected. Patients with only levels of any of the NRTIs, were classified as taking an unknown regimen. Patients without any ARV levels were classified as drug level testing negative (DLT-).

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, May-June 2022, South Africa

	n/N	%	95% CI		
All specimens					
Resistance any class	381/651	59.5%	54.2%	-	64.6%
Resistance to PI	20/636	4.3%	2.6%	-	7.2%
Resistance to NRTI	204/636	31.6%	26.9%	-	36.6%
Resistance to NNRTI	359/636	55.5%	50.3%	-	60.6%
Resistance to INSTI	10/613	1.2%	0.5%	-	2.9%
Any drug level detected					
Resistance any class	245/366	69.6%	64.7%	-	74.1%
Resistance to PI	17/355	5.4%	2.8%	-	10.4%
Resistance to NRTI	171/355	46.1%	40.3%	-	51.9%
Resistance to NNRTI	232/355	65.9%	58.3%	-	72.8%
Resistance to INSTI	8/340	1.9%	0.8%	-	4.7%
No drug level detected					
Resistance any class	136/285	44.9%	34.1%	-	56.2%
Resistance to PI	3/281	2.7%	0.7%	-	10.4%
Resistance to NRTI	33/281	10.6%	6.0%	-	18.0%
Resistance to NNRTI	127/281	40.5%	30.9%	-	50.9%
Resistance to INSTI	2/273	0.3%	0.0%	-	2.4%
NNRTI-based regimens					
Resistance any class	127/142	95.0%	88.5%	-	97.9%
Resistance to PI	0/139	0%		-	
Resistance to NRTI	109/139	82.9%	75.0%	-	88.8%
Resistance to NNRTI	126/139	94.7%	87.9%	-	97.7%
Resistance to INSTI	0/130	0.0%		-	
PI-based regimens					
Resistance any class	31/39	79.0%	62.5%	-	89.5%
Resistance to PI	11/38	31.7%	10.5%	-	64.9%
Resistance to NRTI	27/38	74.0%	57.6%	-	85.6%
Resistance to NNRTI	26/38	64.2%	37.8%	-	84.1%
Resistance to INSTI	0/37	0.0%		-	
INSTI-based regimens					
Resistance any class	30/69	56.5%	27.6%	-	60.7%
Resistance to PI	3/63	4.9%	1.2%	-	18.0%
Resistance to NRTI	16/63	31.0%	14.3%	-	54.8%
Resistance to NNRTI	26/63	48.4%	29.7%	-	67.5%
Resistance to INSTI	8/64	11.1%	4.9%	-	23.2%

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

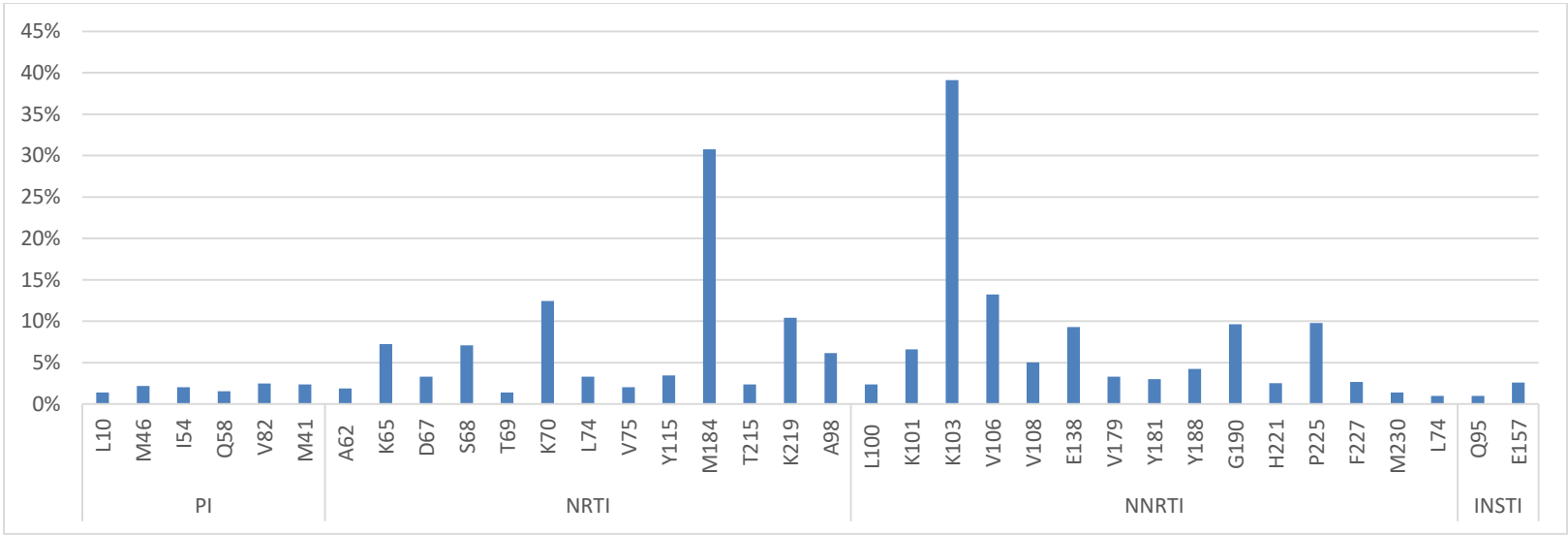

Figure 12.3.1. HIV drug resistance mutations detected in 651 specimens successfully genotyped, in the surveillance study, May-June 2022, South Africa.

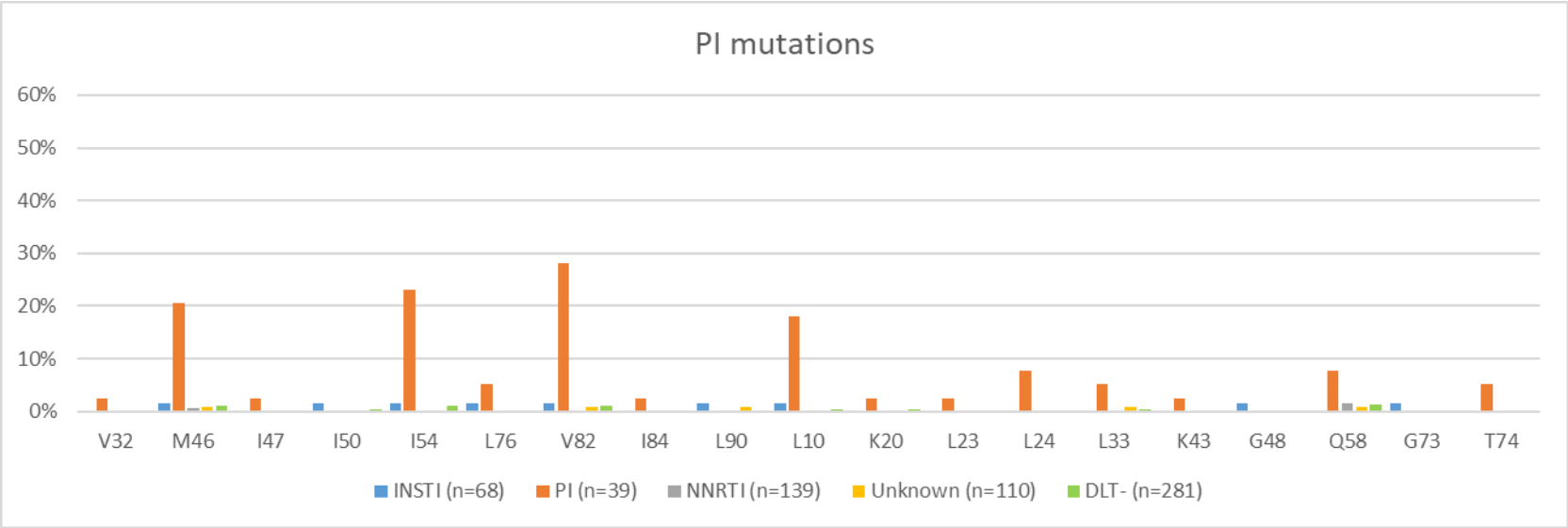

Figure 12.3.2. HIV drug resistance Protease inhibitor (PI) mutations detected in specimens successfully genotyped, by predicted regimen type in the surveillance study, May-June 2022, South Africa.

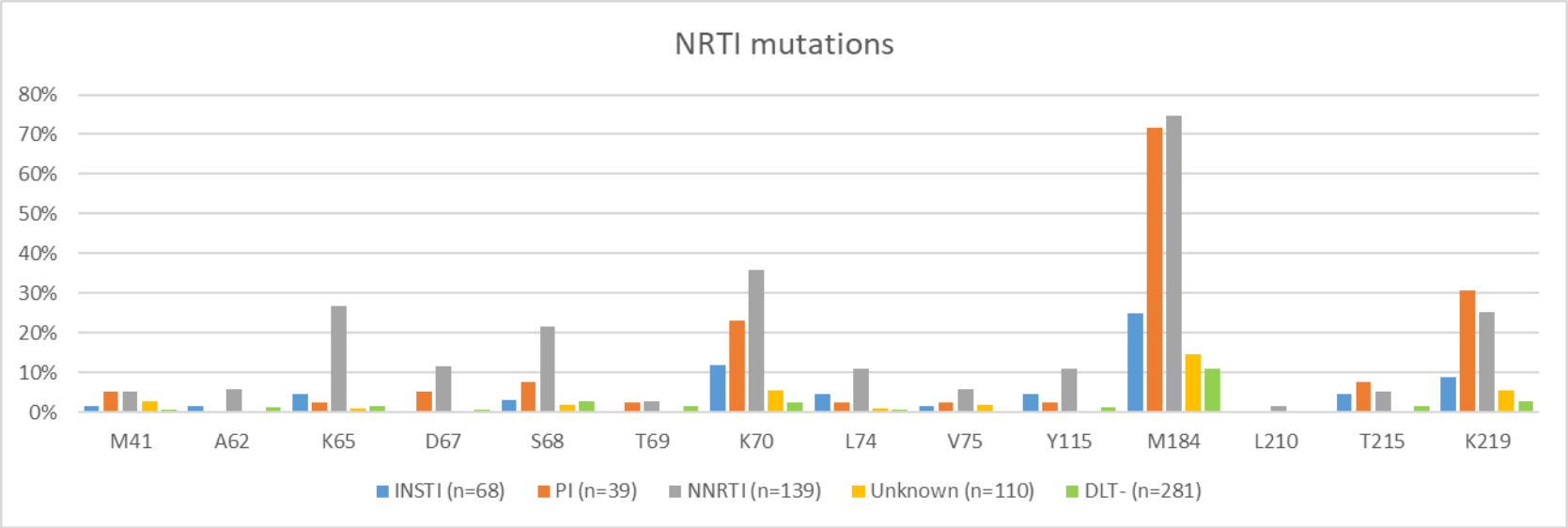

Figure 12.3.3. HIV drug resistance nucleoside reverse transcriptase inhibitor (NRTI) mutations detected in specimens successfully genotyped, by predicted regimen type in the surveillance study, May-June 2022, South Africa.

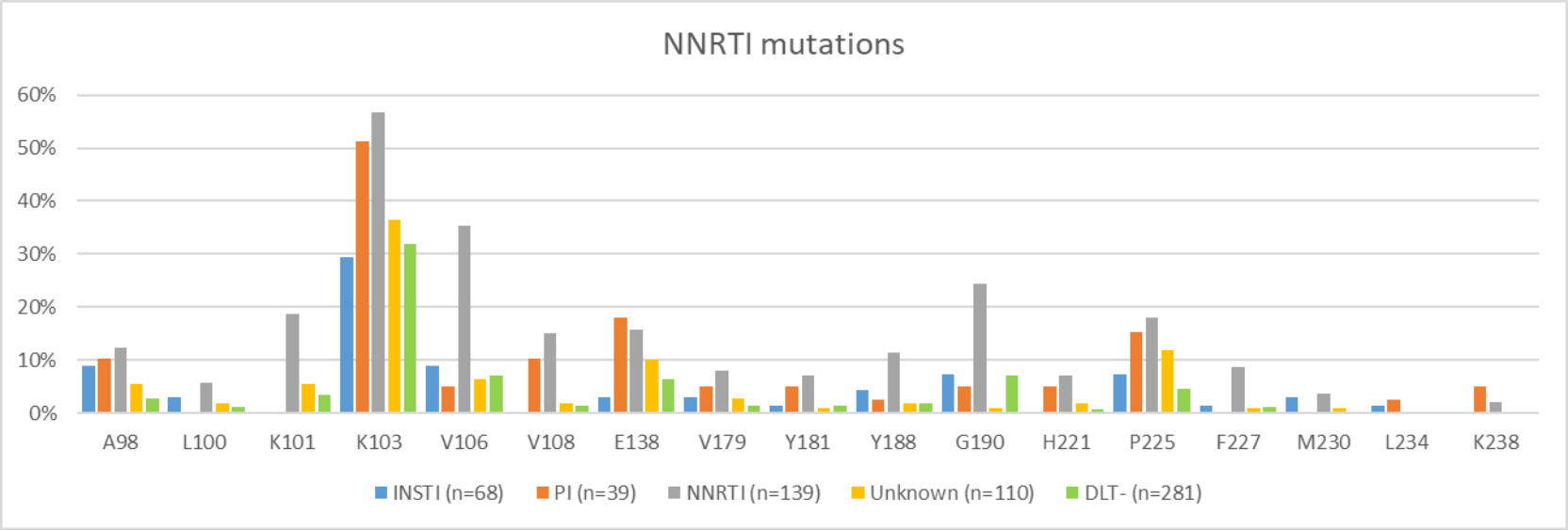

Figure 12.3.4. HIV drug resistance non-nucleoside reverse transcriptase inhibitor (NNRTI) mutations detected in specimens successfully genotyped, by predicted regimen type in the surveillance study, May-June 2022, South Africa.

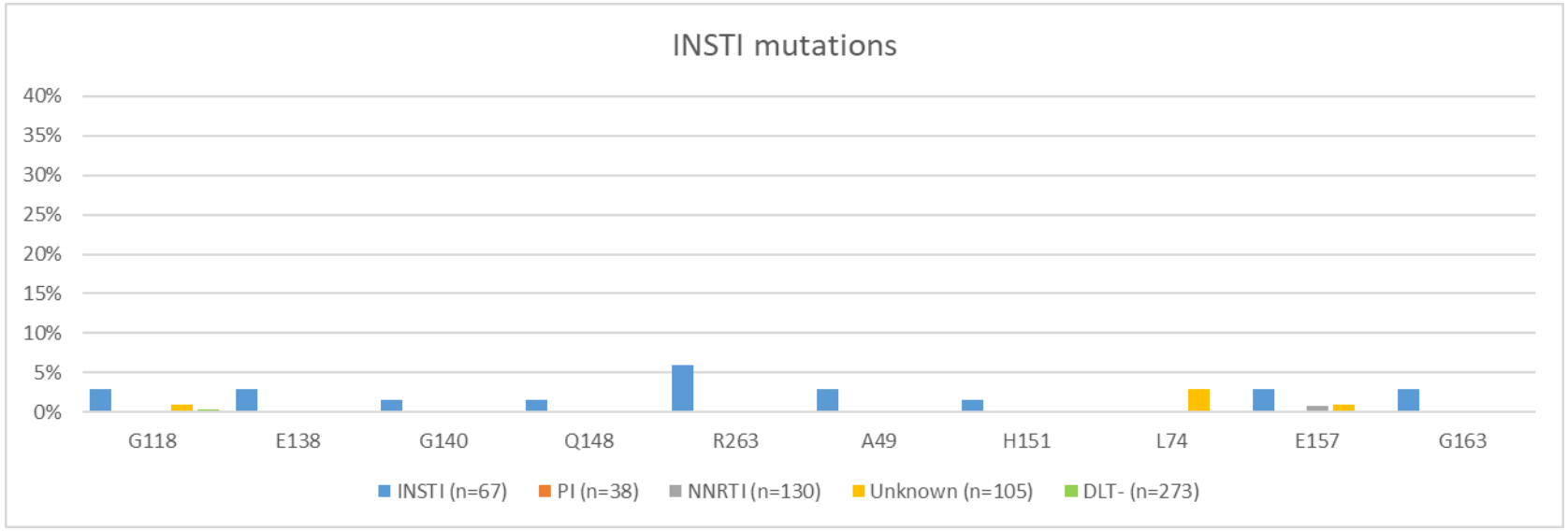

Figure 12.3.5. HIV drug resistance integrase strand transfer inhibitor (INSTI) mutations detected in specimens successfully genotyped, by predicted regimen type in the surveillance study, May-June 2022, South Africa.

12.4. Drug levels and resistance patterns by sex

Of 708 specimens tested, 444 (62.8%) were collected from female patients, 253 (35.7%) were from male patients and for 11 (1.5%) patients, the sex was not recorded. Amongst specimens from female patients, 58.0% had detectable drug levels and 60.5% of specimens from male patients had detectable drug levels (p=0.537). HIV drug resistance was detected in 60.1% of all successfully processed specimens from female patients and 54.0% of all male patients, with no significant difference noted (p=0.574). Among the 106 specimens with detectable DTG levels, 54.7% were from female patients.

12.5. Drug levels and resistance patterns by age group

Median age at time of enrollment was 39 years (IQR: 31–46 years). A trend to lower proportions of detectable DLT and detectable DTG was noted in the younger age groups; however, this did not reach levels of statistical significance across the 6 age groups. A combined analysis of all participants above or below 35 years of age showed that participants aged >=35 years were statistically more likely to test positive for any drug (OR 2.03 (95% CI 1.49 – 2.79), p<0.001) or DTG (OR 2.83 (95% CI 1.69 – 4.78), p<0.001), Figure 12.5.1).

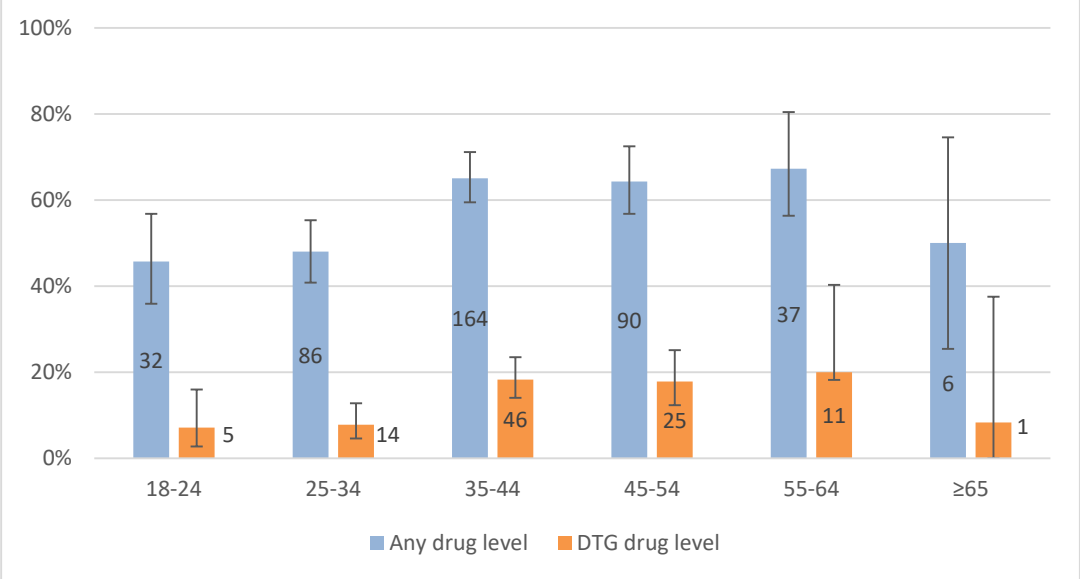

Figure 12.5.1 Unweighted proportions of specimens with detectable drug levels by age group in the cross-sectional surveillance study to assess levels of HIV drug resistance (HIVDR) in adults with viraemia, May-June 2022, South Africa.

Whilst HIVDR prevalence was highest in the 35-44 and 44-54 age groups, no statistical difference could be found among any of the groups (p=0.712, Table 12.5.2).

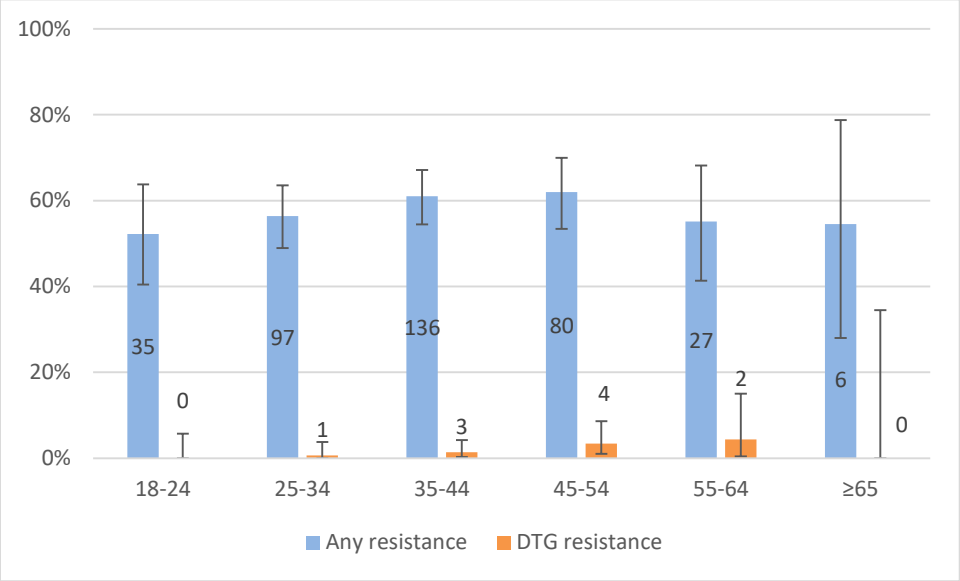

Figure 12.5.2 Proportions of specimens with resistance detected by age group in the cross-sectional surveillance study to assess levels of HIV drug resistance (HIVDR) in adults with viraemia, May-June 2022, South Africa.

12.6. Drug levels and resistance patterns by province

The prevalence of any drug level detected by province ranged from 47.9% in the Free State to 67.9% in Kwazulu-Natal (p=0.048). The detection of DTG levels was most common in Kwazulu-Natal (32.1%) and least common in the Free State (5.5%) and the Western Cape (5.6%, Figure 12.6.1). Please note that the study was not powered to adequately assess differences on a provincial level.

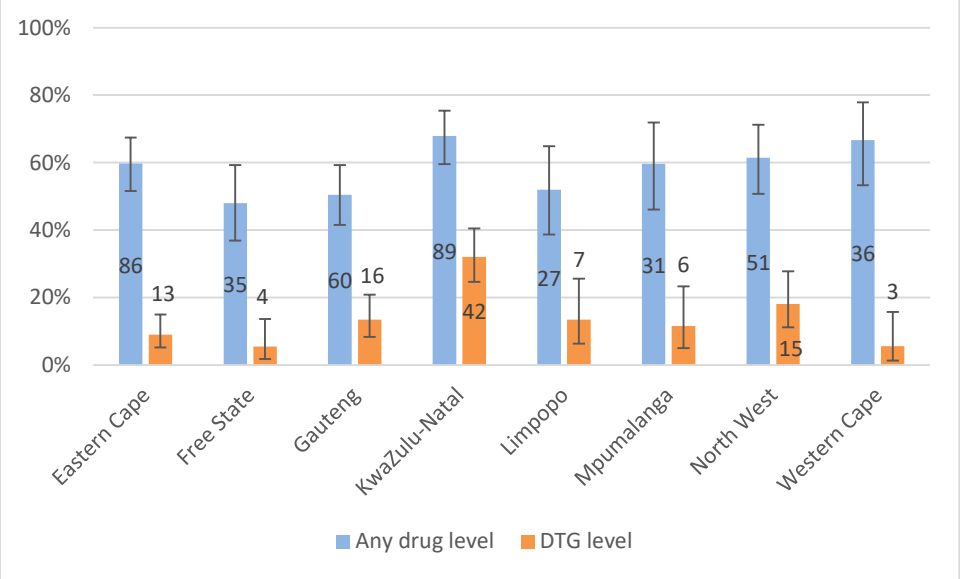

Figure 12.6.1 Unweighted proportions of specimens with detectable drug levels by province in the cross-sectional surveillance study to assess levels of HIV drug resistance (HIVDR) in adults with viraemia, May-June 2022, South Africa.

The prevalence of any resistance detected by province ranged from 51.3% in the North West to 65.7% in the Eastern Cape (p=0.364). The detection of DTG resistance remained very low with no significant differences between provinces (p=0.919, Figure 12.6.2). Please note that the study was not powered to adequately assess differences on a provincial level.

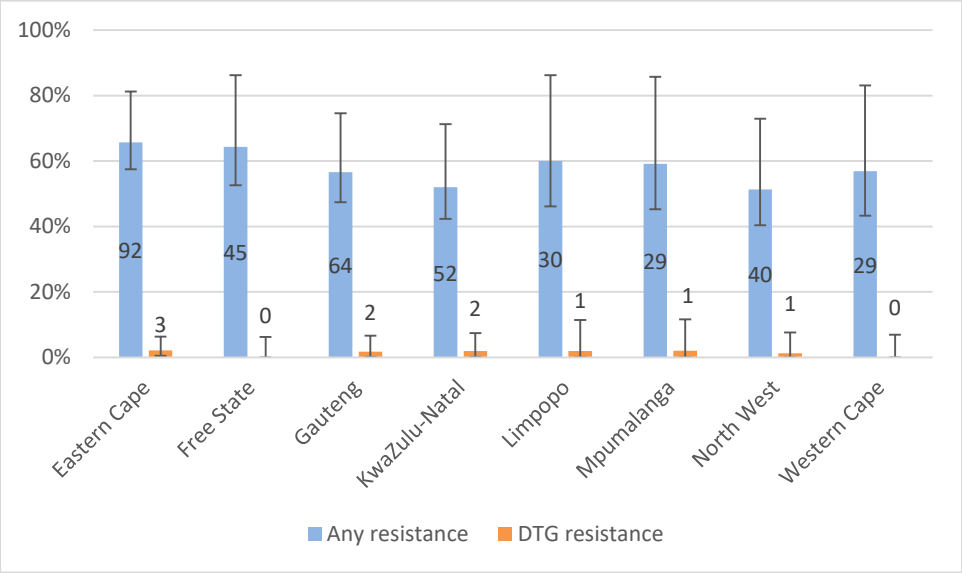

Figure 12.6.2 Unweighted proportions of specimens with resistance by province in the cross-sectional surveillance study to assess levels of HIV drug resistance (HIVDR) in adults with viraemia, May-June 2022, South Africa.

13. DISCUSSION

Our current survey showed that 59.5% of HIV positive patients on ART with unsuppressed VL in the public sector harbor resistance to ART, compared to 72.1% and 67.6% in the 2019[23] and 2021 survey,[24] respectively. NNRTI resistance was still most frequently detected (55.5%), compared to 70.5% and 66.4% in 2019 and 2021, respectively. Likewise, the prevalence of NRTI resistance declined over the years: 49.0% in 2019, 41.4% in 2021 and 31.6% in the current survey. The overall prevalence of PI and INSTI resistance remains low, although PI resistance increased from 2.2% in 2019 to 4.1% in 2021 and 4.3% in 2022. INSTI resistance has only been measured since the 2021 survey and increased from 0.2% in 2021 to 1.2% in the current survey.

Despite the roll-out of DTG, NNRTI drug levels and NNRTI resistance were still commonly detected. The proportion of samples with detectable DTG levels increased from 7.2% in 2021 to 15% in 2022. It was estimated that by March 2022, only 57% of those on treatment were receiving a DTG-based regimen (communication NDoH March 2022). The lack of treatment regimen details and treatment duration is a limitation of this study, especially since drug levels were only detected in 62.6% of the specimens. The previous two surveys similarly showed drug levels were detected in 55.7% of specimens in 2019 and 52.0% in 2021.This consistent finding could indicate either poor adherence in nearly half of the patients with virological failure, or drug-level testing might not be an accurate proxy to assess treatment exposure. We plan to test a random sample of specimens with suppressed VL to further validate the use of drug level testing as a proxy for adequate treatment exposure.

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 VLs faster, reducing the risk for development of resistance. However, it is too early in South Africa's DTG roll-out to draw any firm conclusions regarding this trend.

The prevalence of INSTI resistance remained low (1.2%) in patients with VLs of >1000 copies/mL. However, the proportion of INSTI resistance was significantly higher in specimens with detectable DTG levels (11.1%). In contrast, INSTI resistance was only detected in 0.3% of specimens without detectable DTG levels. In the 2021 survey, INSTI resistance was detected in 2.7% of patients with confirmed DTG exposure, versus no resistance in patients without any detectable ARVs. A similar observation was made for PI resistance, where we found PI resistance in 31.7% of samples with detectable PI levels in the current survey, compared to only 2.7% in specimens without detectable PI levels. In previous surveys, similar outcomes were observed: 32.3% PI resistance in PI DLT+ specimens versus 1.0% in ARV DLT- specimens in 2019. The prevalence of PI resistance was much lower in 2021, with 17.2% PI resistance in PI DLT+ specimens versus 3.0% in ARV DLT- specimens. The drop in PI resistance in 2021 could possibly have been impacted by service disruptions during the SARS-CoV-2 pandemic.

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 current laboratory information system.

14. 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 is driven by less frequent observation 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 2022. At the time, the treatment guidelines recommended to only switch patients from NNRTI-regimens to TLD when they had a suppressed VL.

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.[25,26] Continued monitoring for the development of INSTI resistance in patients with detectable DTG levels is warranted, given that INSTI resistance increased from 2.7% in 2021 to 11.1% in 2022.

The sub-analysis of HIVDR resistance relative to the presence or absence of PIs or INSTIs indicates that screen testing for PIs and INSTIs 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 information. In addition, all sub-analyses should be interpreted with caution as the study was not powered for provincial level analysis. Also, while viral suppression may be higher amongst patients 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.

15. REFERENCES

1. WHO. HIV Drug Resistance Stategy: 2021 Update, 2021.
2. UNAIDS. UNAIDS data 2022, 2022.
3. NDoH. 2019 ART Clinical Guidelines for the Management of HIV in Adults, Pregnancy, Adolescents, Children, Infants and Neonates. 2019.
4. NDoH. 2023 ART Clinical Guidelines for the Management of HIV in Adults, Pregnancy and Breastfeeding, Adolescents, Children, Infants and Neonates. 2023.
5. WHO. HIV drug resistance report 2021, 2021.
6. Walmsley SL, Antela A, Clumeck N, et al. Dolutegravir plus abacavir-lamivudine for the treatment of HIV-1 infection. *N Engl J Med* 2013; **369**(19): 1807-18.
7. Venter WF, Kaiser B, Pillay Y, et al. Cutting the cost of South African antiretroviral therapy using newer, safer drugs. *S Afr Med J* 2016; **107**(1): 28-30.
8. Kanters S, Vitoria M, Zoratti M, et al. Comparative efficacy, tolerability and safety of dolutegravir and efavirenz 400mg among antiretroviral therapies for first-line HIV treatment: A systematic literature review and network meta-analysis. *EClinicalMedicine* 2020; **28**: 100573.
9. Keene CM, Cassidy T, Zhao Y, et al. Recycling Tenofovir in Second-line Antiretroviral Treatment With Dolutegravir: Outcomes and Viral Load Trajectories to 72 weeks. *J Acquir Immune Defic Syndr* 2023; **92**(5): 422-9.
10. Keene CM, Griesel R, Zhao Y, et al. Virologic efficacy of tenofovir, lamivudine and dolutegravir as second-line antiretroviral therapy in adults failing a tenofovir-based first-line regimen. *AIDS* 2021; **35**(9): 1423-32.
11. Paton NI, Musaazi J, Kityo C, et al. Efficacy and safety of dolutegravir or darunavir in combination with lamivudine plus either zidovudine or tenofovir for second-line treatment of HIV infection (NADIA): week 96 results from a prospective, multicentre, open-label, factorial, randomised, non-inferiority trial. *Lancet HIV* 2022; **9**(6): e381-e93.
12. Schramm B, Temfack E, Descamps D, et al. Viral suppression and HIV-1 drug resistance 1 year after pragmatic transitioning to dolutegravir first-line therapy in Malawi: a prospective cohort study. *Lancet HIV* 2022; **9**(8): e544-e53.
13. Paton NI, Musaazi J, Kityo C, et al. Dolutegravir or Darunavir in Combination with Zidovudine or Tenofovir to Treat HIV. *N Engl J Med* 2021; **385**(4): 330-41.
14. Mulenga L FS, Mweemba A, Fwoloshi S, Mweemba A, Siwingwa M, Sivile S, Kampamba D, Engamba DC, Mbewe N, Phiri H, Shibemba A, Simons B, Wester CW, Chirwa L, Hill A. Dolutegravir with recycled NRTIs is non-inferior to PI-based ART: VISEND trial. The 29th Conference on Retroviruses and Opportunistic Infections; 2022; Virtual; 2022.
15. van Oosterhout JJ, Chipungu C, Nkhoma L, et al. Dolutegravir Resistance in Malawi's National HIV Treatment Program. *Open Forum Infect Dis* 2022; **9**(5): ofac148.
16. Zash R, Makhema J, Shapiro RL. Neural-Tube Defects with Dolutegravir Treatment from the Time of Conception. *N Engl J Med* 2018; **379**(10): 979-81.
17. WHO. Update of recommendations on first- and second-line antiretroviral regimens. Geneva; 2019.
18. Zash R, Holmes L, Diseko M, et al. Neural-Tube Defects and Antiretroviral Treatment Regimens in Botswana. *N Engl J Med* 2019; **381**(9): 827-40.
19. Harris PA, Taylor R, Minor BL, et al. The REDCap consortium: Building an international community of software platform partners. *J Biomed Inform* 2019; **95**: 103208.
20. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform* 2009; **42**(2): 377-81.
21. Van Laethem K, Schrooten Y, Covens K, et al. A genotypic assay for the amplification and sequencing of integrase from diverse HIV-1 group M subtypes. *J Virol Methods* 2008; **153**(2): 176-81.

22. Parkin NT, Avila-Rios S, Bibby DF, et al. Multi-Laboratory Comparison of Next-Generation to Sanger-Based Sequencing for HIV-1 Drug Resistance Genotyping. *Viruses* 2020; **12**(7).
23. Hunt G, Steegen K, Hans L, et al. High levels of HIV drug resistance in adult patients with unsuppressed viral load, measured through routine viral load programme monitoring in South Africa, 2019. 23rd International AIDS Conference; 2020; virtual; 2020.
24. Steegen K, Hunt G, MacLeod W, et al. HIV drug resistance surveillance leveraging on routine ART programme monitoring in South Africa. INTEREST 2023; Maputo, Mozambique; 2023.
25. Shuter J, Sarlo JA, Kanmaz TJ, Rode RA, Zingman BS. HIV-infected patients receiving lopinavir/ritonavir-based antiretroviral therapy achieve high rates of virologic suppression despite adherence rates less than 95%. *J Acquir Immune Defic Syndr* 2007; **45**(1): 4-8.
26. Brenner BG, Wainberg MA. Clinical benefit of dolutegravir in HIV-1 management related to the high genetic barrier to drug resistance. *Virus Res* 2017; **239**: 1-9.