2024

ANNUAL SURVEILLANCE OF

HIV DRUG RESISTANCE

IN ADULT AND PAEDIATRIC PATIENTS THROUGH ROUTINE ART PROGRAMME MONITORING

IN SOUTH AFRICA

PEPFAR

CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION

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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).

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

1.3. 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.

2. FUNDING STATEMENT

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3. 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 Organization
3TC	Lamivudine

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6. INTRODUCTION

6.1. Background

Many low- and middle-income countries, including South Africa, have adopted standardized guidelines to ensure effective treatment and management of people living with HIV through recommended antiretroviral therapy (ART) regimens. These programs provide a uniform, evidence-based approach to care; however, to maintain their effectiveness and sustainability, monitoring and minimizing HIV drug resistance (HIVDR) is essential.[1]

In 2024, an estimated 7.7 million people were living with HIV in South Africa, with 81% receiving ART.[2] Dolutegravir (DTG)-based regimens were introduced in 2019, with the recommended first-line regimen for adults and adolescents being tenofovir/lamivudine/DTG (TLD).[3] Initially, TLD rollout prioritized men, adolescent boys, women of childbearing age on reliable contraception, and older women due to safety concerns for infants exposed during pregnancy. A second phase in 2021 expanded access to all women, men, and adolescents regardless of age. By 2023, over 4.7 million people, or 76% of those on treatment, were using DTG-based regimens.[4] This scale-up coincided with improved virological suppression rates (<1,000 copies/mL) from 88.3% in 2019 to 91.3% in 2023.[2]

DTG is available as 50 mg tablets for children ≥20 kg and as pediatric DTG (pDTG) 10 mg dispersible tablets for children 3–20 kg and ≥4 weeks old, approved in June 2022.[5] Before this, pediatric regimens primarily included abacavir + lamivudine + efavirenz (ABC + 3TC + EFZ), tenofovir/lamivudine (TDF/3TC) or emtricitabine/efavirenz (FTC/EFV), or protease inhibitor-based therapy for children under 3 years.[6]

HIV viral load (VL) testing has been recommended since 2004 as a key monitoring tool, performed at 6- and 12-months after ART initiation and annually thereafter[5], and introducing a first post-initiation test performed after three dispensing cycles in the 2023 guidelines.[7] HIVDR testing, however, is not routinely offered at treatment initiation or first-line failure due to cost and systems capacity. Treatment failure is defined as two consecutive VL results ≥1,000 copies/mL, taken a minimum of two to three months apart. Suboptimal VL suppression and HIVDR detection among ART recipients may indicate gaps in program quality, such as inadequate adherence support, drug supply interruptions, and poor retention in care.

6.2. Rates of resistance to DTG may be increasing

DTG is highly effective in managing HIV infection due to its rapid virological suppression, better tolerability, and higher genetic barrier to resistance compared to non-nucleoside reverse transcriptase inhibitors (NNRTIs). However, recent reports indicate that resistance to DTG in resource-limited settings may be more common than initially anticipated,[8] with estimates ranging between 3.9% and 19.6% among viremic individuals on DTG-based regimens reported in selected treated or surveyed populations ,[9] whilst anecdotal evidence from low-middle income countries indicate that emergent DTG resistance appeared at a population rate below 1%, and is associated with treatment adherence challenges.[10] Using modelling approaches, acquired resistance to DTG has been predicted to reach prevalence rates of 41.7% amongst people with virological failure by 2035.[11]

6.3. Rationale for programmatic monitoring of HIVDR prevalence

Since 2004, nationally representative surveys have been implemented in LMICs to assess levels of transmitted, pre-treatment drug resistance and acquired HIVDR. However, uptake of these surveys in countries with high HIV burden has been slow and complex, with limited funding available. More feasible options include using programmatic VL data to estimate HIVDR levels on first-line treatment outcomes or using convenience cohorts and/or laboratory-based sampling of treatment failures to facilitate cost-effective and timely outcomes.

For this survey, remnant samples collected through routine VL monitoring at public health facilities and sent to regional National Health Laboratory Service (NHLS) HIV VL testing laboratories for HIV VL testing were utilized. This laboratory-based sampling approach enables nationally representative surveillance of acquired HIVDR among treatment failures, leveraging South Africa's extensive network of 17 HIV VL laboratories within the NHLS. These laboratories collectively support programmatic VL testing and achieve coverage rates exceeding 85% across all nine provinces (NHLS data, unpublished).

6.4. Preliminary data – findings from adult ADR surveys

A systematic sample of specimens was collected during each of the survey years by identifying all VL specimens with a result that was ≥1,000 copies/ml over a one-week period at each participating laboratory, until sample size was reached. This approach reflected a sample of all specimens submitted for VL testing at each laboratory over the same period. The selected samples were then tested for HIVDR using next generation sequencing and drug level testing (DLT) using liquid chromatography tandem mass spectrometry. VL results and patient age were extracted from the laboratory information system. Treatment history or other relevant clinical data were not available as it was not captured in the laboratory database. The summarized outcomes from the 2019 – 2023 surveys are indicated in Table 6.1.[12]

Table 6.1. Overall proportions of HIVDR among patients with viral non-suppression, estimated in national laboratory-based HIVDR surveillance activities, 2019 – 2023.

	2019	*2021*	*2022*	*2023*
Successful HIVDR	753	538	595	720
Any resistance	72.1%	67.6%	57.9%	53.7%
NNRTI resistance	70.5%	66.4%	56.0%	50.7%
NRTI resistance	49.0%	41.4%	28.5%	25.2%
PI resistance	2.2%	4.1%	3.1%	2.2%
INSTI resistance	ND	0.2%	1.6%	2.3%

PI: Protease Inhibitors; NRTI: nucleoside reverse transcriptase inhibitors; NNRTI: Non-nucleoside reverse transcriptase inhibitors; INSTI: integrase strand transfer inhibitors. ND: not done

6.5. Study Objectives

The objective of the study was to estimate the prevalence of HIVDR among adult and pediatric patients <18 years of age in 2024 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

Sampling was performed by identifying all available remnant samples with HIV VL ≥1,000 copies/ml over a random 6-day consecutive period at each of the 17 VL laboratories using the NHLS Laboratory Information Management System (LIMS). All specimens with a VL ≥1,000 copies/mL were identified, assigned a study number, captured in a REDCAP database and aliquoted on site. The aliquoted specimens were then categorized into the adult group (≥18 years) and pediatric and adolescent group (<18 years). Previous VL history was assessed by submitting the list of retrieved specimens to the Central Data Warehouse.

7.2. Inclusion and exclusion criteria

7.2.1. Inclusion criteria

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

- Leftover sample was from a patient with known age
- Blood specimens had been sent for routine VL testing
- HIV VL results were available and distributed through LIMS
- HIV VL result was ≥1,000 copies/ml

7.2.2. Exclusion criteria

- Minimal data fields not available in LIMS, including age, facility, clinic or hospital record number
- HIV VL was <1,000 copies/ml
- Leftover sample volume was insufficient (<650 ul)

7.3. Sample size calculations

A proportional random sample of specimens was selected from each of the participating VL laboratories to generate a nationally representative sample of all patients who had a VL test performed during the time-period of sampling. The sample size is estimated using an error size of 5% and a two-side 95% confidence interval (CI) of alpha=0.05, and assuming a HIVDR proportion of 50% among adult and pediatric patients with viral loads ≥ 1,000 cp/mL and a genotyping failure rate of 10%.

The minimum effective sample size was calculated as 385 specimens, and after adjusting for a 10% genotyping failure rate and a design effect of 1.75, the final number of samples to be collected was 741 from adult patients and 741 from pediatric patients (Table 7.1).

Table 7.1. Sample size calculations to determine the number of specimens necessary to estimate the proportion of HIVDR in persons with viraemia

Statistical Precision				**Sample size adjustments**	
Proportion Estimated	Error size	95% Confidence Interval	Effective Sample Size	Design Effect 1.75	10% Genotyping failure
0.5	0.05	1.96	385	674	741

7.4. Specimen collection and randomization

Specimens were consecutively selected at each of the 17 participating NHLS HIV VL testing laboratories during the collection period by identifying HIV VL specimens with a VL ≥1,000 copies/ml, once the result had been authorized on the NHLS LIMS. Specimen collection took place over a 6-day period in each of the participating laboratories. Remnant plasma was decanted into a separate 2ml 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. The decanted specimen was labelled with the Study ID only. 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

All specimens were tested for the following antiretroviral drugs used in the public sector: efavirenz (EFV), lopinavir (LPV), atazanavir (ATV), darunavir (DRV), ritonavir (RTV) and DTG, using high performance liquid chromatography tandem mass spectrometry (LCMS/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) were applied. 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. Results were reported as above the limit of quantification. This analysis was performed at the NHLS Chemical Pathology Laboratory at CMJAH, and these results were used as a proxy for current treatment regimen. Patients were classified as taking a) an INSTI regimen if DTG was detected; 2) a PI regimen if LPV, ATZ, DRV or RTV was detected; 3) a NNRTI regimen if EFV was detected.

7.6. HIVDR genotyping

Remnant specimens from all 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 (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 (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 kit on the Qubit Flex Fluorometer (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. Sequencing was done on the Nextseq 550 (Illumina, San Diego, CA, USA. FastQ sequences were submitted to PASeq (paseq.org) for NGS HIV drug resistance analysis. Consensus sequences were generated at 20%. 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 testing volumes and proportions of non-suppressed samples per lab, as well as study design. Significance was set at p-value of <0.05. All analyses were conducted using STATA version 18 (STATA Corp., College Station, TX, USA).

7.8. Returning of results

Individual genotyping results were returned to the corresponding provincial HAST (HIV/AIDS, STI's and Tuberculosis) program managers who disseminated the results to the District Medical Officers.

8. ETHICAL CONSIDERATIONS

Ethical approval was obtained from the Human Research and Ethics Committee at the University of the Witwatersrand (M230984). 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 delinked from personal identifiers and confidentiality maintained in the collection, storage, entry, and analysis of data. The laboratory episode number of the collected specimens was captured in a secure database, which was required to return the genotyping results to the corresponding HAST program 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.

9. SPECIMEN COLLECTION

Remnant VL plasma specimens were aliquoted at each of the participating HIV VL testing laboratories between May – June 2024, whilst specimens from Universitas Hospital were collected in August 2024. Over this same period, a total of 57,238 samples were identified as being from patients aged ≥18 years and having a VL ≥1,000 copies/ml, and 8,027 samples identified as being from patients <18 years and having a VL≥1,000 copies/ml (Table 9.1).For the study, a total of 745 specimens from adult patients and 555 specimens from pediatric patients were collected and stored.

Table 9.1. Number of eligible viral load specimens and ultimately collected to assess levels of HIVDR in adult and pediatric populations

Lab	Total Unsuppressed VL samples (patients aged ≥18 years	Total samples collected and tested (adult cohort)	Weighting (adult cohort)	Total Unsuppressed VL samples (patients aged <18 years)	Total samples collected and tested (pediatric cohort)	Weighting (pediatric cohort)
AD	5,505	65	1.1023	577	32	1.2467
CM	6,548	87	0.9796	643	58	0.7665
DG	2,154	30	0.9345	266	27	0.6812
ED	2,696	35	1.0026	533	65	0.5670
FR	3,214	44	0.9507	451	43	0.7252
GS	2,158	27	1.0403	179	16	0.7735
IA	1,319	17	1.0099	218	10	1.5073
MD	1,552	21	0.9619	263	40	0.4546
MK	5,616	65	1.1246	1071	35	2.1157
MT	2,284	31	0.9590	519	28	1.2816
NE	4,836	67	0.9395	824	64	0.8902
NG	3,245	41	1.0302	604	19	2.1980
PE	2,933	37	1.0318	300	14	1.4816
TA	4,262	62	0.8947	358	6	4.1255
TS	4,397	58	0.9867	611	39	1.0832
TY	1,173	15	1.0178	122	13	0.6489
UN	3,346	43	1.0128	488	46	0.7335
Total	**57,238**	**745**	**0.9988**	**8027**	**555**	**1.2518**

VL: Viral Load. AD Addington Hospital, CM Charlotte Maxeke 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

10. TESTING OUTCOMES IN ADULT PATIENTS

A total of 745 specimens from adult patients were collected across each of the 17 participating laboratories. The mean VL of these collected specimens from adult patients was 19,956 copies ml (IQR: 4,230 – 88,754 copies/ml) whilst mean age from collected specimens was 36 years (IQR: 28 – 44 years).

10.1. Laboratory testing – drug level testing

DLT was successful for all 745 specimens, and the proportion of samples positive for any ARV was 32.4% (95% CI: 29.1 – 35.9%), with 26.1% (23.0 – 29.3%) testing positive for 3TC, 24.8% (21.9 – 28.1%) for DTG and 19.8% (17.1 – 22.8%) for TDF. Notably, EFV was only detected in 1.6% (95% CI: 0.9 – 2.8%) of samples collected in 2024.

10.2. Laboratory testing – HIVDR testing

Of the 745 samples from adult patients selected for further testing, HIVDR genotyping was successful for PR-RT in 684 (91.8%) and IN in 698 (93.7%) specimens. From these, 676 samples

were successfully sequenced in both PR-RT and IN regions, 8 samples were sequenced in the PR-RT region only, 22 in the IN region only, and 39 had no result for both genes.

Table 10.1. Proportions of specimens from adult patients with HIVDR

	n	Proportion with resistance (weighted)	p-value
All samples	676	37.1 [33.5 – 40.8]	
Sex			
Female	466	39.5 [35.2 – 44.1]	0.0494
Male	210	31.6 [25.7 – 38.2]	
Age Group			
19 – 24 years	121	36.0 [27.7 – 45.3]	0.3722
25 - 49 years	506	36.0 [31.7 – 40.5]	
≥50 years	118	43.3 [34.0 – 53.1]	
Province			
Limpopo	56	35.7 [24.3 – 49.0]	0.1246
Gauteng	165	31.1 [24.5 – 38.5]	
Mpumalanga	61	39.4 [28.0 – 52.1]	
North West	55	49.1 [36.2 – 62.1]	
KwaZulu-Natal	164	34.8 [27.9 – 42.4]	
Free State	39	33.3 [20.4 – 49.3]	
Eastern Cape	98	47.4 [37.7 – 57.3]	
Western Cape	38	31.5 [18.9 – 47.8]	
Any Analyte detected			
Yes	200	51.2 [44.2 – 58.1]	0.0000
No	476	31.2 [27.2 – 35.5]	

No difference in prevalence of HIVDR was noted across the different provinces or age groups; however, HIVDR was more prevalent in women (39.5%) relative to men (31.6%, Table 10.1). Notably, there was a significant difference in prevalence of resistance in specimens that tested positive for ARV analytes (any of the six analytes tested, 51.2% vs 31.2%, p=0.0000).

NNRTI resistance was higher among women (37.5%) than men (25.2%), however no differences were noted in other class resistance between specimens collected from males and females (Table 10.2). Any class resistance was more prevalent amongst samples testing positive for ARV analytes; however, the most striking difference was for NRTI class resistance, being detected in 29.8% of samples that tested positive relative to 6.4% of sample testing negative for ARV analytes. NRTI and NNRTI resistance was highly prevalent in samples that tested positive for NNRTI and PI drugs; however, the sample sizes for these categories are small.

Table 10.2. Proportions of specimens with class-specific HIVDR from adult patients (≥18 years) patients

	n	NNRTI Resistance % [95% CI]	NRTI Resistance % [95% CI]	PI Resistance % [95% CI]	n	INSTI Resistance % [95% CI]	DTG Resistance % [95% CI]
All specimens	684	33.7 [30.2 – 37.3]	13.4 [11.0 – 16.1]	1.5 [0.8 – 2.7]	698	2.6 [1.7 – 4.1]	1.8 [1.1 – 3.2]
Sex							
Female	473	37.5 [33.2 – 41.9]	13.2 [10.4 – 16.6]	1.3 [0.6 – 2.9]	480	2.1 [1.2 – 4.0]	1.7 [0.9 – 3.9]
Male	211	25.2 [19.8 – 31.5]	13.7 [9.6 – 19.0]	1.9 [0.7 – 5.0]	218	3.6 [1.8 – 7.1]	2.2 [0.9 – 5.2]
Any Analyte detected							
Yes	205	40.5 [33.9 – 47.3]	29.8 [23.9 – 36.4]	4.0 [2.0 – 7.8]	211	7.7 [4.8 – 12.2]	5.7 [3.2 – 9.7]
No	479	30.8 [26.8 – 34.0]	6.4 [4.5 – 9.0]	0.4 [0.1 – 1.6]	487	0.4 [0.1 – 1.7]	0.2 [0.0 – 1.5]
Assumed Regimen							
NNRTI	7	86.4 [43.2 – 98.1]	86.4 [43.2 – 98.1]	0.0	7	0.0	0.0
NRTI	36	41.7 [26.9 – 58.1]	43.8 [28.7 – 60.1]	0.0	36	0.0	0.0
PI	11	54.2 [26.3 – 79.5]	90.8 [55.9 – 98.7]	65.0 [35.1 – 86.4]	10	0.0	0.0
DTG	151	37.0 [29.7 – 45.0]	19.2 [13.7 – 26.3]	0.7 [0.1 – 4.5]	158	10.3 [6.4 – 16.2]	7.5 [4.3 – 12.9]

PI: Protease Inhibitors; NRTI: nucleoside reverse transcriptase inhibitors; NNRTI: Non-nucleoside reverse transcriptase inhibitors; INSTI: integrase strand transfer inhibitors; DTG: dolutegravir.

Resistance to INSTI and DTG was uncommon, with 18 samples harboring resistance mutations to INSTI and 13 with resistance to DTG. Amongst patients with INSTI resistance, 16 specimens tested positive for DTG, and two specimens tested negative for all ARV analytes (Table 10.3).

Table 10.3. Mutation profile of 18 specimens with resistance to INSTI

Sex	Age	INSTI Resistance pattern	Analytes detected	Resistant to DTG
M	54	T66A+G118R+E138K	DTG	Yes
F	41	L74M+G118R+E157Q	DTG+3TC	Yes
M	49	R263K	DTG+TDF+3TC	Yes
F	56	G118R+E138K	DTG+3TC	Yes
F	39	G118R+E138K	DTG+TDF+3TC	Yes
M	29	R263K	DTG+TDF+3TC	Yes
F	36	E157Q+R263K	None	Yes
F	42	T97A+S147G+N155H+E157Q	DTG+TDF+3TC	Yes
F	44	L74M+G118R+E138K	DTG+TDF	Yes
F	32	G118R	DTG+3TC	Yes
M	37	T66A+G118R+E138K+E157Q	DTG+TDF+3TC	Yes
F	42	T66A+G118R+E138A	DTG+TDF+3TC	Yes
M	55	R263K	DTG+TDF+3TC	Yes
M	30	Q95K+S153A+E157Q+S230R	DTG+TDF+3TC	No
M	36	G163R	DTG+TDF+3TC	No
F	59	G163K	DTG+TDF+3TC	No
M	46	H51Y+Q95K	DTG	No
F	48	H51Y	None	No

INSTI: integrase strand transfer inhibitors; DTG: dolutegravir; 3TC: lamivudine, TDF: tenofovir;

11. TESTING OUTCOMES IN PAEDIATRIC PATIENTS

A total of 555 specimens from pediatric patients were collected from each of the 17 participating laboratories. The mean VL of these collected specimens from adult patients was 13,200 copies ml (IQR: 3,409 – 60,904 copies/ml) whilst mean age from collected specimens was 12 years (IQR: 7 – 16 years).

11.1. Laboratory testing – drug level testing

DLT was successful for all 555 specimens. The proportion of samples positive for any ARV was 43.0% (95% CI: 38.3 – 47.8%), for 3TC it was 25.9% (95% CI: 21.9 – 30.3%), for DTG it was 33.1% (95% CI: 28.6 – 38.0%) and for TDF it was 9.0% (95% CI: 6.6 – 12.0%). NNRTI and PI were infrequently detected, with 2.5% (95% CI: 1.4 – 4.5%) of samples testing positive for EFV, 2.3% (95% CI: 1.3 – 4.0%) testing positive for LPV and 0% for ATZ.

11.2. Laboratory testing – HIVDR testing

Of the 555 samples from pediatric patients selected for further testing, HIVDR genotyping was successful for PR-RT in 495 (89.2%) and IN in 513 (92.4%) specimens. From these, 491 samples were successfully sequenced in both PR-RT and IN regions, four specimens were sequenced in the PR-RT region only, 22 in the IN region only, and 38 had no result.

Table 11.1. Proportions of specimens from pediatric patients with HIVDR

	n	Proportion with resistance (weighted)	p-value
All samples	491	54.3 [49.6 – 58.7]	
Sex			
Female	273	53.3 [46.4 – 60.1]	0.6549
Male	218	55.7 [48.0 – 63.0]	
Province			
Limpopo	30	60.0 [41.9 – 75.7]	0.4256
Gauteng	84	50.6 [36,6 – 64.5]	
Mpumalanga	51	60.8 [46.9 – 73.1]	
North West	32	65.6 [47.9 – 79.9]	
KwaZulu-Natal	153	48.1 [39.0 – 57.4]	
Free State	44	63.6 [48.6 – 76.4]	
Northern Cape	--		
Eastern Cape	73	55.0 [43.0 – 66.5]	
Western Cape	24	41.4 [23.8 – 61.5]	
Any Analyte detected			
Yes	188	64.6 [56.0 – 72.4]	0.0015
No	303	47.2 [40.9 – 53.6]	

No difference in prevalence of HIVDR was noted across the different provinces or different sexes; however, HIVDR was more prevalent in specimens that tested positive for ARV analytes (64.6% vs 47.2%, p=0.0015, Table 11.1).

Table 11.2. Proportions of specimens with class-specific HIVDR from pediatric patients

	n	NNRTI Resistance % [95% CI]	NRTI Resistance % [95% CI]	PI Resistance % [95% CI]	n	INSTI Resistance % [95% CI]	DTG Resistance % [95% CI]
All specimens	495	44.4 [39.4 – 49.5]	22.9 [19.0 – 27.3]	0.9 [0.3 – 2.8]	513	2.2 [1.2 – 4.1]	1.9 [1.0 – 3.7]
Sex							
Female	275	45.3 [38.5 – 52.2]	18.0 [13.5 – 23.5]	0.0	288	2.0 [0.9 – 4.5]	1.5 [0.6 – 3.6]
Male	220	43.3 [36.0 – 50.8]	29.4 [23.0 – 36.5]	2.2 [0.7 – 6.4]	225	2.6 [1.0 – 6.3]	2.6 [1.0 – 6.3]
Age Group							
0-4 years	59	52.2 [37.8 – 66.2]	33.0 [21.0 – 47.6]	0.0	62	0.0	0.0
5-9 years	110	50.1 [39.8 – 60.4]	45.9 [35.7 – 56.4]	3.0 [0.8 – 11.0]	113	2.1 [0.7 – 6.5]	2.1 [0.7 – 6.5]
10-17 years	326	40.8 [34.8 – 47.1]	12.7 [9.5 – 16.9]	0.4 [0.0 – 2.8]	338	2.8 [1.3 – 5.6]	2.3 [1.1 – 5.0]
Analyte detected							
Yes	190	48.6 [40.3 – 56.9]	36.7 [29.3 – 44.7]	2.3 [0.8 – 6.8]	200	3.8 [1.8 – 7.8]	3.8 [1,8 – 7.8]
No	305	41.5 [35.4 – 47.9]	13.3 [9.6 – 18.1]	0.0	313	1.0 [0.3 – 3.5]	0.6 [0.1 – 2.4]
Regimen							
NNRTI	7	0.0	45.4 [15.2 – 79.5]	0.0	7	0.0	0.0
NRTI	30	45.5 [27.8 – 64.3]	61.9 [42.5 – 78.1]	0.0	32	0.0	0.0
PI	12	29.3 [10.6 – 59.0]	61.1 [32.4 – 83.8]	16.3 [3.9 – 48.4]	12	0.0	0.0
DTG	141	48.5 [38.7 – 58.4]	29.2 [21.4 – 38.4]	1.7 [0.3 – 8.3]	149	5.0 [2.4 – 10.2]	5.0 [2.4 – 10.2]

PI: Protease Inhibitors; NRTI: nucleoside reverse transcriptase inhibitors; NNRTI: Non-nucleoside reverse transcriptase inhibitors; INSTI: integrase strand transfer inhibitors; DTG: dolutegravir

Overall, minimal difference in class resistance was noted amongst males and females, however, NRTI resistance was more prevalent in male children (29.4% vs 18.0%, Table 11.2). More distinct differences were noted between children of different age groups: NNRTI and NRTI resistance was lower in adolescent children as compared to children less than 10 years of age, whereas PI resistance and INSTI resistance was only detected in children 5 years and older. NRTI resistance was higher in children testing positive for NRTI (61.9%) and PI (61.1%) analytes. PI resistance was highest in children testing positive for PI analytes (16.3%). Amongst patients with INSTI resistance, nine specimens tested positive for DTG, and three specimens tested negative for all ARV analytes (Table 11.3).

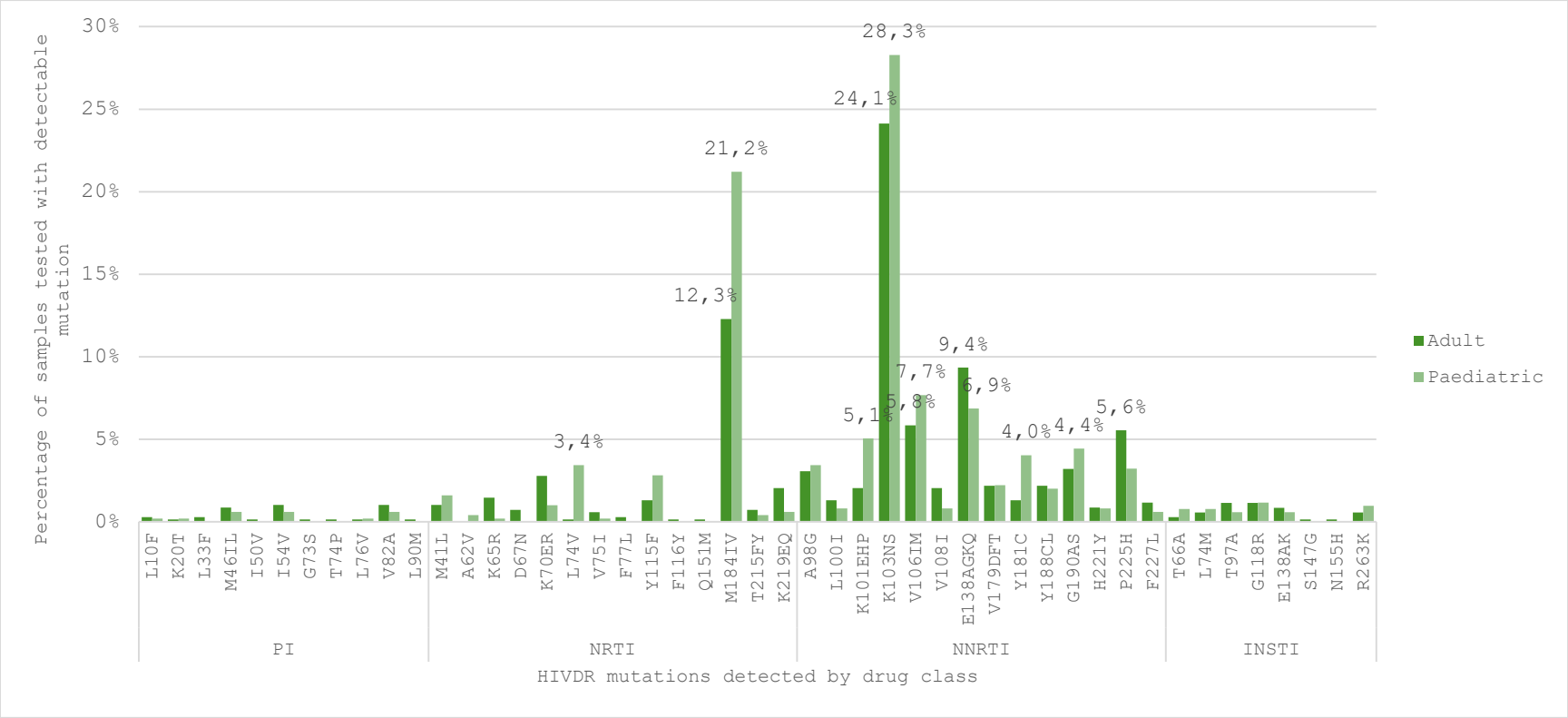
Table 11.3. Mutation profile of 12 specimens from pediatric patients with resistance to INSTI

Sex	Age	INSTI Resistance Pattern	Analytes detected	Resistance to DTG
M	8	G118R	DTG	Yes
F	12	T66I+G118R	DTG	Yes
F	16	T66A+G118R+E138K	DTG+3TC	Yes
F	17	Q95K+R263K	DTG+TDF+3TC	Yes
M	12	R263K	DTG+3TC	Yes
M	4	T66A+G118R+E138K	DTG+3TC	Yes
M	15	T66I+L74M+G118R+E138K	DTG+3TC	Yes
M	16	R263K	DTG	Yes
F	15	R263K	DTG+TDF+3TC	Yes
F	9	R263K	None	Yes
M	17	G118R	None	Yes
F	17	G163K	None	No

12. MUTATION PROFILE AMONGST SOUTH AFRICAN PATIENTS WITH VIRAEMIA

All mutations detected are indicated in Figure 12.1. The K103NS (24.1%, 28.3%) and M184IV (12.3%, 21.2%) mutations were most frequently detected in adult and pediatric patients, respectively, followed by the V106IM and P225H mutations. Notably, the NNRTI E138AGKQ mutations were present in 9.4% and 6.9% of patients with resistance.

Figure 12.1. Profile of mutations detected in 706 specimens from adult patients and 517 specimens from pediatric patients that were successfully genotyped in both or either protease/reverse transcriptase and integrase regions.



PI: Protease Inhibitors; NRTI: nucleoside reverse transcriptase inhibitors; NNRTI: Non-nucleoside reverse transcriptase inhibitors; INSTI: integrase strand transfer inhibitors.

13. DISCUSSION

Our current survey showed that 37.1% of adult patients with unsuppressed VL in the public sector harbor resistance to ART, with marginally higher rates of resistance detected amongst samples from female patients. This prevalence estimate is consistent with a notable decline in HIVDR over five years of surveillance, with 72.1%, 67.6%, 57.9% and 53.7% prevalence estimates noted in 2019, 2021, 2022 and 2023, respectively.[7] Rates of resistance were driven by resistance to NNRTI, with 33.7% of specimens from adult patients harboring mutations to efavirenz and nevirapine. Despite the wide implementation of DTG-based regimens in South Africa, the persistence of NNRTI mutations after NNRTI drug pressure is removed is explained by their minimal fitness cost and not readily replaced by wild-type variants.

NRTI resistance was relatively uncommon, detected in 13.4% of specimens and primarily involving resistance to lamivudine (3TC). However, its prevalence differed significantly between samples with detectable antiretroviral (ARV) analytes (29.8%) and those without (6.4%). This disparity highlights the fitness cost associated with NRTI mutations and their tendency to rapidly revert to wild type in the absence of drug pressure. NRTI resistance was highly prevalent in specimens positive for NNRTI (86.4%) and PI analytes (90.8%), although very few specimens fit this definition and outcomes should be interpreted cautiously. PI resistance remained low at 1.5% but was much higher in patients with detectable levels of PI analytes, with seven of eleven specimens testing positive for PI harboring major resistance mutations PI.

The prevalence of INSTI resistance remained low at 2.6% in adult patients with VL of ≥1,000 copies/mL but has continued to increase over the past four years of surveillance, with rates of resistance to INSTI increasing from 0.2% in 2021, 1.6% in 2022 and 2.3% in 2023. However, the proportion of INSTI resistance was higher in specimens with detectable DTG levels, with 10.3% of specimens positive for DTG harboring mutations to INSTI. Notably, NNRTI resistance was present in 37.0% and PI resistance was present in 0.7% of specimens positive for DTG, suggesting these patients were using DTG-based regimens as second- or third-line regimen options.

The survey included specimens from pediatric patients for the first time in 2024 and showed that resistance was more prevalent in this population, with 54.3% of specimens harboring resistance mutations. Resistance patterns differed by age group, consistent with treatment regimens recommended for each group: NNRTI resistance was more prevalent on children 9 years or younger, and PI resistance was more prevalent in children aged 5-9 years. PI resistance was detected in two of twelve specimens testing positive for PI analytes, and INSTI resistance was detected in children aged 5 years and older, and only in children testing positive for DTG.

Study limitations

The lack of treatment regimen details and treatment duration is an important limitation of this study. Despite efforts to align laboratory and clinical databases, the country has not implemented combined digital platforms such as electronic medical records, hampering access to timely information for patient care and for effective surveillance strategies. In this year of surveillance, two thirds of specimens collected tested negative for ARV analytes, indicating that virological non-

suppression is frequently driven by inadequate treatment adherence and not necessarily by drug resistance.

Despite the national representativeness of the survey, results should be interpreted with caution given the absence of 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. In addition, given that viral suppression may be higher amongst patients receiving DTG-based regimens, over-sampling of NNRTI-based regimens may have occurred. The use of DLT testing provides a novel perspective to HIVDR interpretation, in that the absence of analytes is likely to be indicative of suboptimal adherence and can provide a range of scenarios of partial non-adherence to complete non-adherence. DLT testing can be used as a proxy for adherence, with 51.2% of adults and 64.6% of children with detectable drug levels harboring resistance. However, it should be noted that 31.2% of adults and 47.2% of children testing negative for analytes harbored resistance.

The use of remnant specimens continues to be a cost-effective method for HIVDR surveillance, although the lack of demographic clinical data will 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.

14. CONCLUSION

The observed HIVDR levels in this survey show that the overall prevalence of resistance is declining over the years of surveillance, with the decline likely being driven by less frequent observations of NRTI and NNRTI resistance. The prevalence of PI and INSTI resistance remains low, which is in line with the high genetic barrier of ritonavir-boosted PI and DTG. However, continued monitoring for the development of INSTI resistance, and especially in patients with detectable DTG levels, is warranted.

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