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**GEOSPATIAL AND TEMPORAL ANALYSIS OF HIV-1 VIRAL LOADS AND
DRUG RESISTANCE IN KWAZULU-NATAL, SOUTH AFRICA**

By

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*Thesis submitted in fulfilment of the requirements for the Doctor of Philosophy Degree, in
the School of Laboratory Medicine and Medical Sciences, College of Health Sciences,
University of KwaZulu-Natal*

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Co-supervisor: Professor Raveen Parboosing

Co-supervisor: Dr Kerusha Govender

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Preface

The research described in this thesis was conducted at the Department of Virology (DOV), Inkosi Albert Luthuli Central Hospital, National Health Laboratory Service (NHLS), in the School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal (UKZN), in Durban, South Africa. This work was done between January 2021 and July 2024 under the primary supervision of Dr Benjamin Chimukangara, with additional co-supervisory support from Prof Raveen Parboosing and Dr Kerusha Govender.

This thesis consists of original work, some of which has been published in peer-reviewed journals. I noted that a significant amount of big data was produced during routine HIV diagnostic testing at the DOV, which, after acquiring the necessary ethical and gatekeeper permissions, could be re-purposed for HIV programmatic research. Having a keen interest in HIV and some experience in database management and analysis, I was privileged to have been awarded a 3-year PhD position by Dr Benjamin Chimukangara who was funded by the European and Developing Countries Clinical Trials Partnership (EDCTP) grant number: TMA2019CDF-2728 (HIVDR-MIT), to conduct HIV research in drug resistance, big data and geospatial analysis. My EDCTP-funded PhD project was completed under the supervision of Dr Benjamin Chimukangara, and the findings thereof are described in this thesis.

Declaration by Supervisor

As the candidate's supervisor, I agree to the submission of this thesis.



Signed

Dr Benjamin Chimukangara

Supervisor

28 November 2024

Date

Declaration: Plagiarism

I, Lilishia Gounder, declare that:

- i. The research reported in this dissertation, except where otherwise indicated, is my original work.
- ii. This dissertation has not been submitted for any degree or examination at any other university.
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Declaration: Publications

I, Lilishia Gounder, declare as follows:

- i. That the work described in this thesis has not been submitted to UKZN or any other tertiary institution for the purposes of obtaining an academic qualification, whether by myself or any other party.
- ii. That my contribution to the project was as follows:

1. **Gounder L**, Tomita A, Lessells R, Moodley S, Francois KL, Khan A, Pillay M, Manyana SC, Govender S, Govender K, Moodley P, Parboosing R, Msomi N, Tanser F, Naidoo K, Chimukangara B. Geospatial and temporal mapping of detectable HIV-1 viral loads amid dolutegravir rollout in KwaZulu-Natal, South Africa.

Presented in Chapter 2.

Published in PLOS Glob Public Health. 2024; 4(5): e0003224.

My responsibilities were: Conceptualization, data curation, formal analysis, investigation, methodology, project administration, resources, software, visualization, writing – original draft.

2. **Gounder L**, Khan A, Manasa J, Lessells R, Tomita A, Pillay M, Manyana SC, Govender S, Francois K, Moodley P, Msomi N, Govender K, Parboosing R, Moyo S, Naidoo K, Chimukangara B. Patterns of HIV-1 drug resistance observed through geospatial analysis of routine diagnostic testing in KwaZulu-Natal, South Africa.

Presented in Chapter 3.

Published in Viruses. 2024; 16(10): 1634.

My responsibilities were: Conceptualization, methodology, data curation, software acquisition, method validation, formal analysis, investigation, resource acquisition, writing – original draft, visualization, project administration.

3. **Gounder L**, Archary M, Khan A, Govender K, Parboosing R, Naidoo K, Chimukangara B. Characterization of HIV-1 drug resistance in ART-experienced children and adolescents through geospatial analysis of routine genotypic tests in KwaZulu-Natal, South Africa.

Presented in Chapter 4.

Prepared for submission to Journal of the Pediatric Infectious Diseases Society

My responsibilities were: Conceptualization, methodology, method validation, project administration, data curation, formal analysis, investigation, visualization, resource acquisition, writing – original draft.

iii. Other related publications during the period of study:

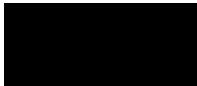
1. Manyana S, **Gounder L**, Pillay M, Manasa J, Naidoo K, Chimukangara B. HIV-1 drug resistance genotyping in resource limited settings: current and future perspectives in sequencing technologies. *Viruses*. 2021; 13(6): 1125.
2. Chimukangara B, Lessells RJ, Sartorius B, **Gounder L**, Manyana S, Pillay M, Singh L, Giandhari J, Govender K, Samuel R, Msomi N, Naidoo K, de Oliveira T, Moodley P, Parboosing R. HIV-1 drug resistance in adults and adolescents on protease inhibitor-based antiretroviral therapy in KwaZulu-Natal Province, South Africa. *Journal of Global Antimicrobial Resistance*. 2022; 29: 468-75.
3. Manyana S, Pillay M, **Gounder L**, Khan A, Moodley P, Naidoo K, Chimukangara B. Affordable drug resistance genotyping of HIV-1 reverse transcriptase, protease and integrase genes, for resource limited settings. *AIDS Research and Therapy*. 2023; 20(1): 1-2
4. Khan A, Pillay M, Chimukangara B, **Gounder L**, Manyana S, Francois KL, Chipango K. Identification of HIV-1 subtype CRF18_cpx in a patient with multidrug resistance in KwaZulu-Natal, South Africa: An epidemiological worry? *Journal of Clinical Virology Plus*. 2023; 3(2): 100143

iv. Related conference presentations during the period of study:

1. **Gounder L**, Chimukangara B. Monitoring trends of HIV-1 drug resistance in KwaZulu-Natal province amid dolutegravir rollout in South Africa. 2022. 24th Association of Medical Councils of Africa (AMCOA) International Conference, North West Province, South Africa. Abstract 12836.
2. **Gounder L**, Chimukangara B. Monitoring trends of HIV-1 drug resistance in KwaZulu-Natal amid dolutegravir rollout in South Africa. 2023. 11th SA AIDS Conference 2023, Durban, South Africa. Oral Abstract 11A-547.
3. **Gounder L**, Pillay M, Manyana S, Khan A, Francois K, Moodley P, Msomi N, Govender K, Parboosing R, Naidoo K, Chimukangara B. Characterization of HIV drug resistance in ART-experienced patients post-dolutegravir rollout in KwaZulu-Natal, South Africa. 2023. 30th International Workshop on HIV Drug Resistance and Treatment Strategies, Cape Town, South Africa. Abstract 63.
4. Chimukangara B, **Gounder L**, Pillay M, Manyana S, Khan A, Francois KL, Govender K, Moodley P, Msomi N, Naidoo K. OA-739 High-levels of HIV drug resistance persist in ART-experienced patients post-dolutegravir rollout in KwaZulu-Natal, South Africa. *BMJ Global Health* 2023;8:A22. 2023. 11th EDCTP Forum, Paris, France. Oral Abstract 739.
5. **Gounder L**, Pillay M, Manyana S, Govender S, Khan A, Francois K, Moodley P, Msomi N, Govender K, Parboosing R, Naidoo K & Chimukangara B. HIV drug resistance in ART-

experienced children post-dolutegravir rollout in KwaZulu-Natal, South Africa. 2023. 13th World Congress of the World Society for Pediatric Infectious Diseases, Durban, South Africa. Oral Abstract 692.

6. **Gounder L**, Pillay M, Manyana S, Govender S, Khan A, Francois K, Moodley P, Msomi N, Govender K, Parboosing R, Naidoo K, Chimukangara B. Assessing HIV-1 drug resistance in patients with virological failure amid the rollout of dolutegravir in hyperendemic KwaZulu-Natal, South Africa. 2024. 6th Edition of World Congress on Infectious Diseases, Paris, France. Abstract P12.



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Dedication

I dedicate this thesis to my parents, family and friends who have been a wonderful source of encouragement throughout my studies; and to my loving husband, Ryan Gounder, who has patiently and compassionately motivated me to be the best version of myself, together with my children, Caleb Timothy, Christopher Luke and Elizabeth Caris, who constantly challenge me to “dive” into the fullness of life. May the grace of the Lord Jesus Christ be with you all.

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I am especially grateful to my family: my father, Mr. George Moodley, my sister, Dr Eshia Moodley-Govender (and family), my brother, Mr Joshua Moodley (and family), my sister-in-law, Mrs Vanessa Nair (and family), and my husband, Dr Ryan Gounder, for all your love, encouragement, patience, prayers and support through every season of my life. I would also like to acknowledge my late mother, Mrs Jaya Moodley and mother-in-law, Mrs. Savy Govender, whose unconditional love and kindness made an indelible impression on me, shaping me into the person that I am today.

Lastly and most importantly, I would like to thank and praise God for this beautiful life that has been loaned to me, and additionally, for the knowledge, understanding and wisdom to carry out every good work entrusted to me. To God be all the glory and honour.

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List of Abbreviations

AIDS	Acquired immunodeficiency syndrome
ALD	Combination of abacavir, lamivudine and dolutegravir
ART	Antiretroviral therapy
ARV	Antiretroviral
ATV/r	Ritonavir-boosted atazanavir
CAPRISA	Centre for the AIDS Programme of Research in South Africa
CD4	Cluster of differentiation 4
CI	Confidence intervals
COVID-19	Coronavirus disease 2019
CRF	Circulating recombinant form
DNA	Deoxyribonucleic acid
DoH	Department of Health
DoV	Department of Virology
DRV/r	Ritonavir-boosted darunavir
DTG	Dolutegravir
EFV	Efavirenz
FDC	Fixed-dose combination
GIS	Geographic information system
GRT	Genotypic resistance test
HIV	Human immunodeficiency virus
HIVDR	HIV-1 drug resistance
HIPSS	HIV Incidence Provincial Surveillance System
ICTV	International Committee on Taxonomy of Viruses
INSTI	Integrase strand transfer inhibitor
kg	Kilogram
KZN	KwaZulu-Natal province
LIS	Laboratory information system
LMICs	Low and middle-income countries
LPV/r	Ritonavir-boosted lopinavir
mg	Milligram
mL	Millilitre
NDoH	National Department of Health
NHLS	National Health Laboratory Service
NNRTI	Non-nucleoside reverse transcriptase inhibitor

NRTI	Nucleoside/nucleotide reverse transcriptase inhibitor
NVP	Nevirapine
PCR	Polymerase chain reaction
PI/r	Protease inhibitor with boosted ritonavir
PLWH	People living with HIV
RNA	Ribonucleic acid
SIV	Simian immunodeficiency virus
TB	Tuberculosis
TDF	Tenofovir disoproxil fumarate
TLD	Combination of tenofovir, lamivudine and dolutegravir
TEE	Combination of tenofovir, emtricitabine and efavirenz
UKZN	University of KwaZulu-Natal
URF	Unique recombinant form
VL	Viral load
WHO	World Health Organization
XTC	Lamivudine or Emtricitabine

Abstract

Human immunodeficiency virus type 1 (HIV-1) remains a global public health problem, with certain hyperendemic geographic regions contributing significantly to the pandemic, such as the province of KwaZulu-Natal (KZN) in South Africa. Introduction of dolutegravir (DTG) into South African first-line antiretroviral therapy (ART) regimens in December 2019, was a huge stride towards achieving the 95-95-95 UNAIDS targets. However, progressive improvements in standard-of-care must be accompanied by equally dynamic treatment monitoring tools that proactively survey HIV-1 viral load (VL) outcomes and emergent HIV-1 drug resistance (HIVDR). Linking VL and HIVDR data to Geographic Information System (GIS) coordinates, could provide visual insights into areas requiring directed interventions, as HIV-1 treatment programmes mature.

In this study, a surveillance database platform was developed to monitor routinely processed VL and HIVDR data from KZN public health facilities and linked to GIS coordinates for geospatial analysis. Over 7 million VLs were spatiotemporally mapped prior to- and during the transition to DTG-based first-line ART in KZN. Despite an overall decrease in VLs observed between 2018 and 2022 (median $\log_{10}$ copies/ millilitre (mL); 4.09 vs. 3.56), approximately one in every eight people living with HIV (PLWH) had detectable VLs ≥ 400 copies/mL, with 80% of those having VLs ≥ 1000 copies/mL. This underscored the importance of closely monitoring VLs in PLWH, including those with low-level viraemia (i.e., VLs 400–999 copies/mL), a potential marker for treatment failure and emergence of INSTI mutations on DTG-based ART.

This study further investigated patterns of HIVDR from across KZN public health facilities and showed that ~35% (9/26) of adults on DTG with INSTI genotypes had DTG-associated resistance mutations. This represents one of the highest reported prevalences of DTG resistance to date, warranting heightened surveillance of DTG-associated mutations, to prevent accumulation of HIVDR mutations, especially given that four out of five genotypes already exhibited dual- or triple-drug class resistance. Moreover, analyses of GRTs from the paediatric population revealed that HIVDR was detected in ~90% of children aged <10 years ($p < 0.05$) and that approximately 3 out of every 4 children aged 5-9 years had HIVDR to >1 ARV drug class.

Through geospatial analysis of ~4,000 HIVDR genotypes, this study also identified geographic differences in the burden of HIVDR in KZN province, providing proof of concept that big data geospatial mapping can be leveraged for data-driven public health decision making and health systems strengthening. Overall, the findings presented in this thesis send a broader message on the need to direct efforts to known communities with high levels of viraemia on ART and/or HIVDR, enhancing VL re-suppression efforts, improving HIV-1 prevention activities, and ultimately implementing strategies to curb the emergence of HIVDR. This is particularly crucial in resource-limited HIV-hyperendemic settings, where epidemic intelligence can guide the strategic allocation of limited resources for maximum impact in controlling the HIV-1 epidemic.

Chapter 1: Background and Literature Review

1.1. Background

Human immunodeficiency virus (HIV) is a retrovirus that was discovered by Francoise Sinoussi, Luc Montagnier, and Robert Gallo as the causative agent of acquired immunodeficiency syndrome (AIDS) in 1983 [1]. This critical discovery initiated the AIDS research expedition during which numerous diagnostic tests were developed and more than 30 antiretroviral (ARV) drugs from several different drug classes were developed [2], saving the lives of millions of people living with HIV (PLWH) globally. HIV type 1 (HIV-1) is the most common viral type, being responsible for over 95% of all HIV infections globally [3]. Figure 1 summarizes HIV classification according to the International Committee on Taxonomy of Viruses (ICTV) [4].

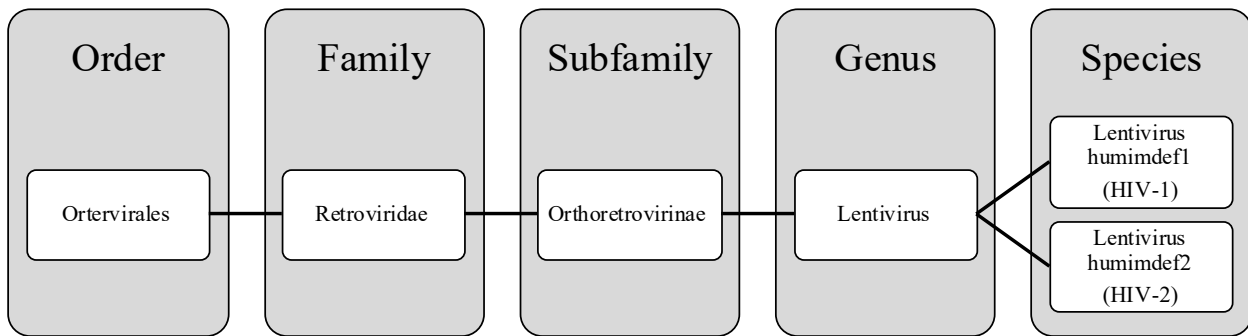


Figure 1. ICTV classification of HIV.

Data from ICTV Virus Taxonomy: 2023 Release.

The HIV virion measures around 100nm in diameter and its genomic RNA is housed in a conical capsid of p24 protein, enveloped by a lipid membrane with glycoproteins gp41 and gp120 on the surface, crucial for eliciting host immune responses and attaching to CD4⁺ T cells [5].

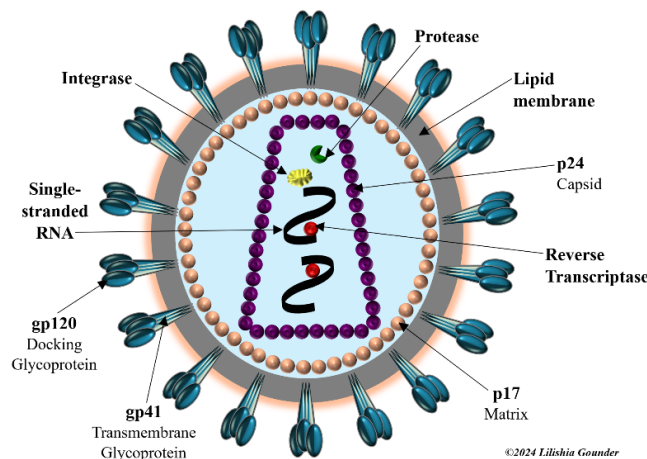


Figure 2. The structure of the HIV-1 virion.

Although HIV-1 is approximately 60% homologous to HIV-2, the latter is less virulent than HIV-1 [6]. Both HIV-1 and HIV-2 are related to simian immunodeficiency viruses (SIV) which infect non-human primates (chimpanzee and gorilla) and Sooty mangabeys, respectively [7]. HIV is generally transmitted by the exchange of bodily fluids, which can occur through sexual contact, sharing needles among intravenous drug users, and exposure to contaminated blood or blood products, while pregnant mothers infected with HIV can spread the virus to the newborn during gestation, at the time of delivery (perinatal transmission), or while breastfeeding (post-natal transmission) [5]. Because of the higher infectivity of HIV-1 compared with HIV-2, there is a striking difference in the global distribution (Figure 3), with HIV-1 found throughout the world and HIV-2 localized to West Africa, with clusters or sporadic cases in certain parts of India, the Americas and Europe [8–11].

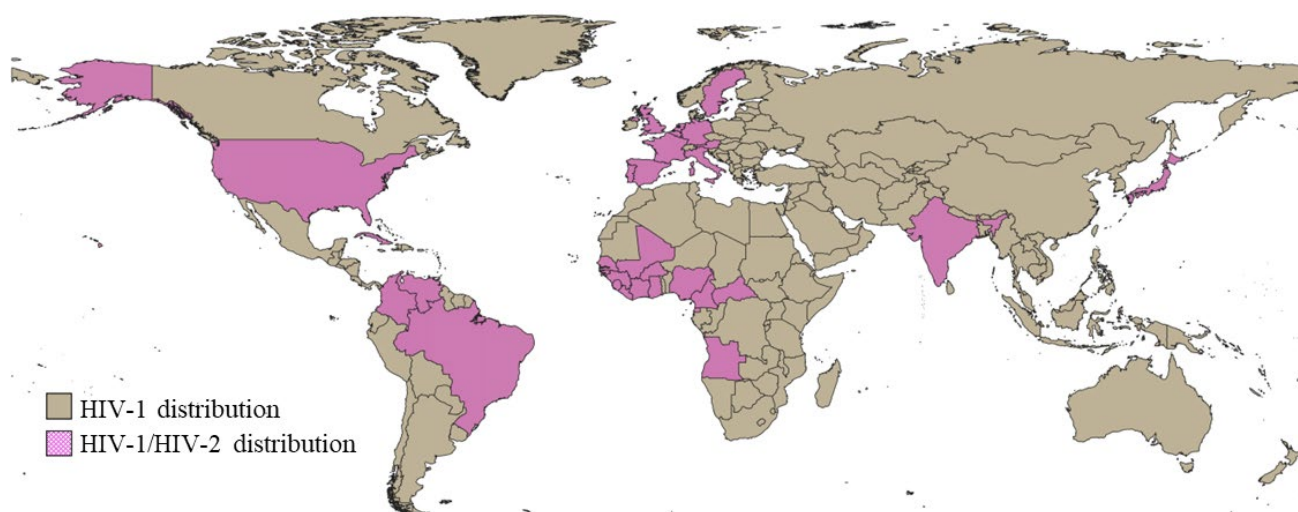


Figure 3. Global distribution of HIV.

Choropleth map of worldwide distribution of HIV, based on data from the Los Alamos HIV Database. Basemap downloaded from: <https://public.opendatasoft.com/explore/dataset/world-administrative-boundaries/export/> and used within the conditions of the Open Government Licence of public sector information.

HIV-1 has four major groups i.e., M, N, O and P, of which M is the most common, accounting for most cases in the global HIV-1 epidemic [12]. HIV-1 group M is further classified into subtypes or clades, circulating recombinant forms (CRFs) and unique recombinant forms (URFs). A CRF can be defined if recombinant virus is identified in three or more epidemiologically unlinked individuals, while a URF is defined if it is found only in one or two individuals [13]. Currently, there are 154 HIV-1 CRFs documented in the Los Alamos HIV database [11], however, only a few CRFs result in widespread transmission and prevalence [13]. Figure 4 is a geographic “snapshot” of HIV-1 subtypes and CRF sampling frequencies from two decades i.e. 1995-2004 and 2015-2024, obtained using the Geography Search Interface Tool within the Los Alamos HIV Database [11,14].

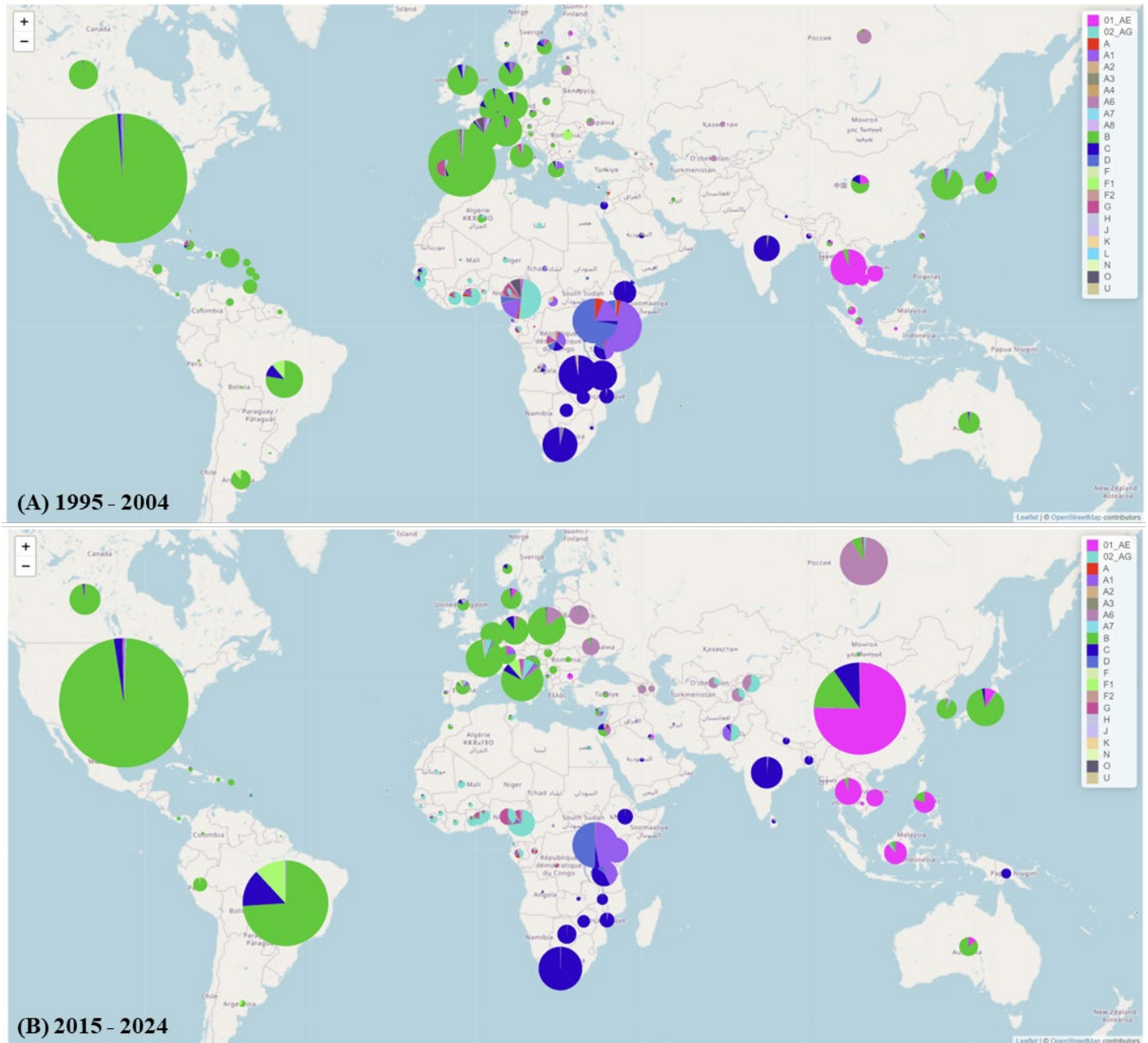


Figure 4. Comparing HIV-1 subtypes and CRFs across different decades.

Time period represented: (A) 1995-2004 and (B) 2015-2024. The size of each circle reflects the approximate number of sequences sampled from each region. Maps were obtained using the Geography Search Interface Tool within the Los Alamos HIV Database. OpenStreetMap is licensed under the Creative Commons Attribution-ShareAlike 2.0 license (CC BY-SA 2.0).

Subtype C is the most prevalent HIV-1 subtype in PLWH worldwide [12], accounting for 98.4% of all subtypes in Southern Africa [15,16]. In South Africa, the total number of PLWH currently is estimated at 8 million and HIV-1 prevalence has increased from 8.9% in 2002 to 12.7% in 2024 [17], partly due to improved life expectancy of PLWH on ART [18]. The HIV-1 prevalence, however, varies across the nine provinces and 52 districts of South Africa, with hyperendemicity in eastern areas such as KZN province, as shown in Figure 5.

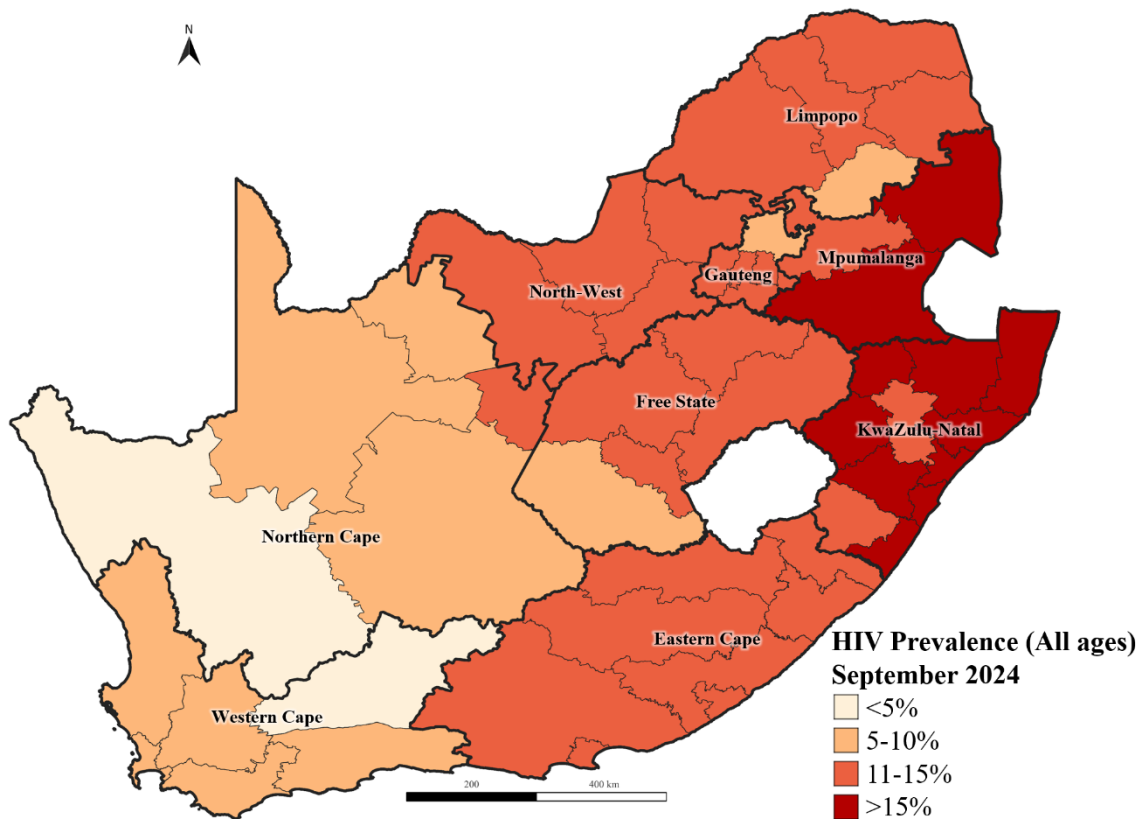


Figure 5. Prevalence of HIV-1 in South Africa.

The mean prevalence per district as at September 2024 was used to generate the choropleth map, with lighter colours representing lower prevalences and darker colours representing higher prevalences. Data was obtained from South Africa District HIV Estimates which incorporates the Thembisa and Naomi models. Data can be downloaded from <https://www.hivdata.org.za/> and the basemap file “boundaries.geojson” contains the geographic boundaries of South Africa.

Overall, the estimated number of AIDS-related deaths has largely declined since 2006 from 286 454 to 68 406 in 2024 [17], evident of the successful South African ART programme which commenced in the public sector in 2004 [16]. Currently, South Africa has the largest ART programme globally [19] with 1 in every 5 ART-treated individuals living in South Africa [20,21]. Life-long HAART has several benefits, including viral suppression which results in immune reconstitution for PLWH and additionally, halts sexual transmission of HIV to others [22]. Currently HIV-1 plasma viral load (VL) testing is used to monitor response to HAART and has been crucial to the success of ART programmes. However, it is only meaningful if VL results are reviewed timeously for appropriate clinical decisions, especially for PLWH who are unable to achieve viral suppression [23]. Factors contributing to virological failure include poor adherence with ongoing viral replication and/or HIV-1 drug resistance (HIVDR) [24].

There has been an increase in HIVDR following the expansion of ART coverage, compounded by restricted access to HIV genotypic resistance testing and delayed transition to more potent ART regimens [25]. With a

notable increase in global NNRTI pre-treatment drug resistance (PDR), the World Health Organisation (WHO) revised first-line ART guidelines, and South Africa adopted those guidelines, rolling-out dolutegravir (DTG) as first-line ART in December 2019 [26,27]. Moreover, treatment guidelines were updated in 2023 to include dispersible DTG tablets for dosing in paediatric patients [28], another success for the South African HIV-1 treatment programme.

Despite these advancements, persistently high levels of VLs and HIVDR in highly treatment experienced PLWH remains a major cause of concern, as these individuals could be largely responsible for new HIV-1 infections. Real-time interactive visualization of the changes in VLs and HIVDR at public health level is essential to ensure timely decision making, to curb HIV-1 transmission and accumulation of HIVDR. Several geospatial studies have demonstrated the heterogeneity of the HIV-1 epidemic in South Africa [29–31]. Beyond characterizing HIV-1 prevalence, geospatial analyses have been used to explore associations between HIV-1 prevalence and sociodemographic factors [32], or co-morbidities such as tuberculosis [33], or non-communicable diseases such as depression [34]. Given that geospatial analyses have also been used to identify differences in HIV-1 care and treatment outcomes across South African facilities [35] it could serve as a valuable tool for treatment monitoring, particularly when combined with community surveillance data [36]. Therefore, this study used at a granular level geospatial data visualization to examine spatiotemporal changes in VLs and HIVDR in KZN overtime, highlighting an effective, inexpensive and timely HIV treatment monitoring tool, that can be utilized to inform public health decision making, using routinely collected HIV-1 programmatic data.

1.2. Literature Review

1.2.1. Antiretroviral therapy in South Africa

The UNAIDS 95-95-95 targets aim for 95% of PLWH to know their HIV status, 95% of those to receive life-long ART, and 95% of those on ART to achieve VL suppression, by 2030 [37], translating to 95–90–86 when expressed as a cascade [38]. In 2023, there were 7.7 million (Confidence interval (CI): 7.0 – 8.3 million) PLWH in South Africa, of which 7.3 million knew their status, 5.9 million were on ART and 5.4 million were virally suppressed, translating to 95% [CI: 88% - >98%], 77% [CI: 71% - 84%] and 71% [CI: 65% - 77%], respectively [21]. This shows that South Africa has made significant progress towards reaching the UNAIDS 95-95-95 targets. However, there remains a notable lag in reaching the second and third 95% targets, which may be reflective of poor linkage to and retention on ART [39]. To date, ART has been the most effective strategy used to control the HIV epidemic, both in preventing viral transmission (i.e., treatment as prevention) and reducing HIV associated morbidity and mortality.

South Africa, like most low-to-middle income countries, uses a public health approach to HIV treatment that involves ART-line regimens [40]. Prior to 2004, ART was only available to patients who were receiving healthcare from the private sector through medical aid schemes, private funding, or research/workplace

programmes [41], and over the years, there has been a notable gap in treatment recommendations between public and private sector programmes in South Africa [42]. Since 2004, the ART programme has transitioned several times, with a shift from twice-daily ARV drug dosing associated with adverse drug reactions, to better tolerated once-a-day fixed-dose combination (FDC) drugs for both adults and children [41]. Previously, the first-line regimen for adults and children older than 3 years of age combined two nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) with a non-nucleoside reverse transcriptase inhibitor (NNRTI), while the regimen for children younger than 3 years of age included two NRTIs with a ritonavir-boosted protease inhibitor (PI) [43]. Adults failing first-line regimens were switched to second-line regimens that included a ritonavir-boosted-PI and two NRTIs (usually AZT if the patient had TDF exposure), with HIV-1 genotypic resistance testing indicated only after failing the second-line regimen [44].

However, as ART coverage improved, the problem of HIVDR emerged and resistance to NNRTIs increased significantly [45]. With the rising threat of HIVDR and potential compromise of first-line ART regimens, South Africa adopted the WHO recommended first- and second-line regimens that included DTG as the primary drug of choice [27]. Currently, the first-line ART regimen in South Africa is a FDC containing tenofovir, lamivudine and dolutegravir (TLD), available immediately after diagnosis to all eligible adults, adolescents and children ≥ 10 years weighing ≥ 30 kilograms (kg) [28]. For children between ≥ 3 kg and < 30 kg or between ≥ 4 weeks and 9 years of age, the preferred first-line ART regimen is a combination of abacavir, lamivudine and dolutegravir (ALD), with paediatric weight-adjusted dosing made easier through the availability of 10mg dispersible DTG tablets [28]. It is hoped that the availability of more potent and palatable regimens will improve adherence and ART coverage among children, where only 63% [CI: 49% - >98%] of those in need were receiving ART in South Africa [21]. Figure 6 contrasts ART coverage among PLWH aged 0-14 years with that of PLWH aged 15-64 years, indicating that the younger age group has lower ART coverage in multiple regions of South Africa.

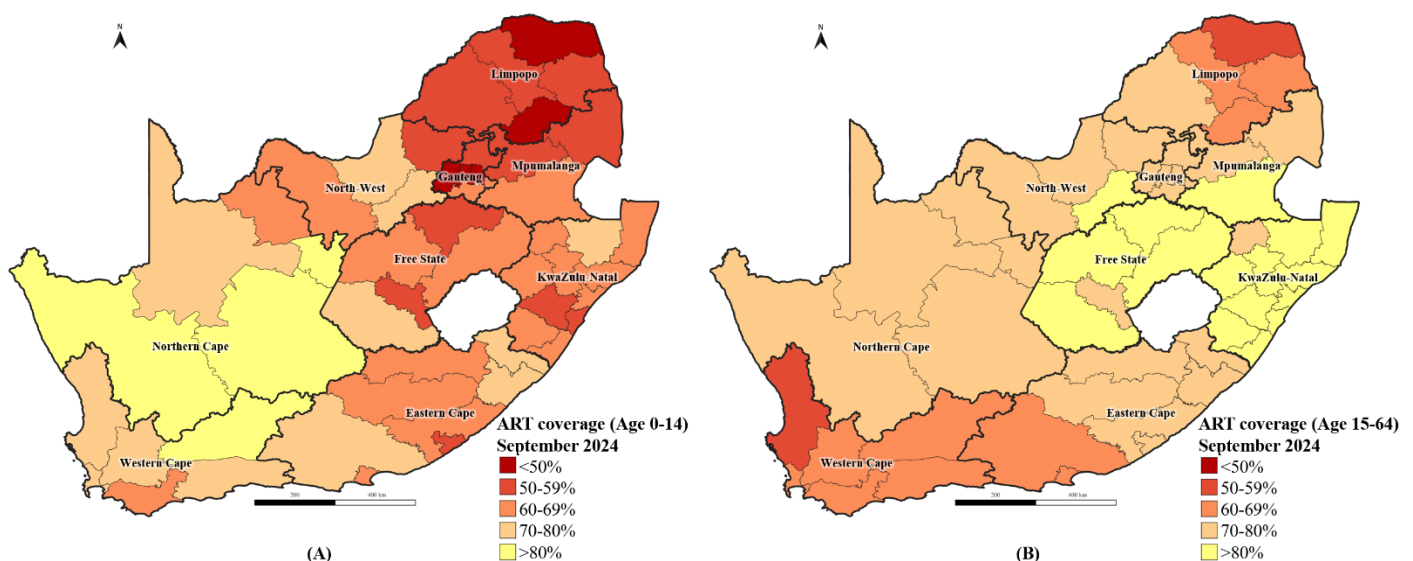


Figure 6. Comparing ART coverage between younger and older people living with HIV in South Africa

The mean ART coverage per district as at September 2024 was used to generate choropleth maps for (A) PLWH aged 0-14 years and (B) PLWH aged 15-64 years. Data was obtained from South Africa District HIV Estimates which incorporates the Thembisa and Naomi models. The lighter colours represent higher ART coverage, whilst darker colours represent lower ART coverage. Data can be downloaded from <https://www.hivdata.org.za/> and the basemap file “boundaries.geojson” contains the geographic boundaries of South Africa.

In the last few years, South Africa like most countries was affected by the coronavirus disease 2019 (COVID-19) pandemic, which caused nationwide lockdowns that slowed the transition to DTG-based ART regimens [46]. However, as we return to pre-COVID-19 pandemic conditions, it is unacceptable to have ART coverage as low as 42% for children in certain regions of South Africa [47]. Ultimately, PLWH who remain untreated despite being eligible for ART, generally become viraemic and could potentially contribute to ongoing HIV-1 transmission, derailing the significant efforts that have been made to end the HIV epidemic.

1.2.2. Monitoring response to antiretroviral therapy

Given that HIV is a chronic disease with no cure, monitoring treatment is essential to ensure sustainable ART. In the early stages of HIV treatment programs, CD4 count testing was used to monitor ART response [48], however, changes in CD4 count alone could not reliably predict ART outcomes [49]. Consequently, and given that ART outcomes are influenced by VL monitoring rather than CD4 counts, routine CD4 monitoring post-ART initiation is no longer recommended in settings where routine VL testing is available [50,51].

In 2013, the WHO recommended the use of VL tests to quantify HIV RNA in blood plasma for monitoring response to ART and diagnosing ART failure, while CD4 counts were limited to regions lacking VL testing capabilities [52]. Currently, VL testing is the standard of care for monitoring patients on ART. Among PLWH receiving ART, virological failure is defined as having two consecutive VLs ≥ 1000 copies/mL measured at least 3 months apart with enhanced adherence support, or a single VL ≥ 1000 copies/mL if on an NNRTI-based regimen [53]. However, PLWH receiving first-line DTG-based ART in South Africa are considered as failing ART only when they have two VLs ≥ 1000 copies/mL after ≥ 24 -months of treatment [28].

Although the majority of people on ART achieve sustained viral suppression, some are unable to fully suppress the virus, resulting in detectable VLs that remain below 1000 copies/mL. These individuals are classified as having low-level viraemia (LLV), typically defined as VLs ranging between 50 - 999 copies/mL [54]. Individuals with LLV require close monitoring, as persistent LLV has been linked to adverse clinical outcomes and/or virological failure [53]. Currently, the South African public health system offers VL monitoring to public health facilities through the National Health Laboratory Service (NHLS), which provides laboratory services to 80% of the population [55]. The standard of care in South Africa is that all patients who have a VL ≥ 50 copies/mL undergo a comprehensive assessment, including evaluation of adherence, co-infections, appropriate

drug dosing, potential drug interactions, and if these are excluded, HIVDR [28]. A key challenge in VL monitoring in South Africa is the delay in switching patients with viraemia to an effective ART regimen [56]. Prolonged use of ineffective ART leads to the development and accumulation of HIVDR mutations, which further compromise the effectiveness of subsequent ART regimens.

1.2.3. HIV-1 drug resistance and genotypic resistance testing

HIV-1 drug resistance (HIVDR) poses a significant challenge to achieving sustained viral suppression on ART and ultimately curbing the transmission of HIV-1. HIVDR occurs when the virus develops mutations, making ARV drugs ineffective in inhibiting viral replication. The problem of HIVDR leads to adverse virological, immunological, and clinical outcomes for PLWH, and poses a substantial threat to the efficacy of HIV-1 treatment programmes [57]. HIVDR may be exacerbated by inadequate HIV service delivery, comprising insufficient adherence support, loss to follow-up, medication stockouts, inappropriate off-label ART usage, or the historic monotherapy from single-drug treatment for the prevention of mother-to-child transmission [58]. In South Africa, a genotypic resistance test (GRT) may be requested in people with confirmed virological failure, and if clinically authorised by a nominated provincial expert, a clinical virologist, a member of the National Third-line committee or a helpline consultant [28]. The designated virology laboratory for public sector HIVDR testing in KwaZulu-Natal is the NHLS Department of Virology (DoV), located at the Inkosi Albert Luthuli Central Hospital. Once a GRT request is authorized, 2mL of blood plasma is sent to the DoV, together with a detailed request form documenting the timeline of previous and current ART regimens. Sanger sequencing is used to detect mutations in the *protease* (PR), *reverse transcriptase* (RT) and *integrase* (IN) genes as outlined in Figure 8, while the Stanford University HIV Drug Resistance Database is used to identify HIVDR mutations and ARV drug susceptibilities [59].

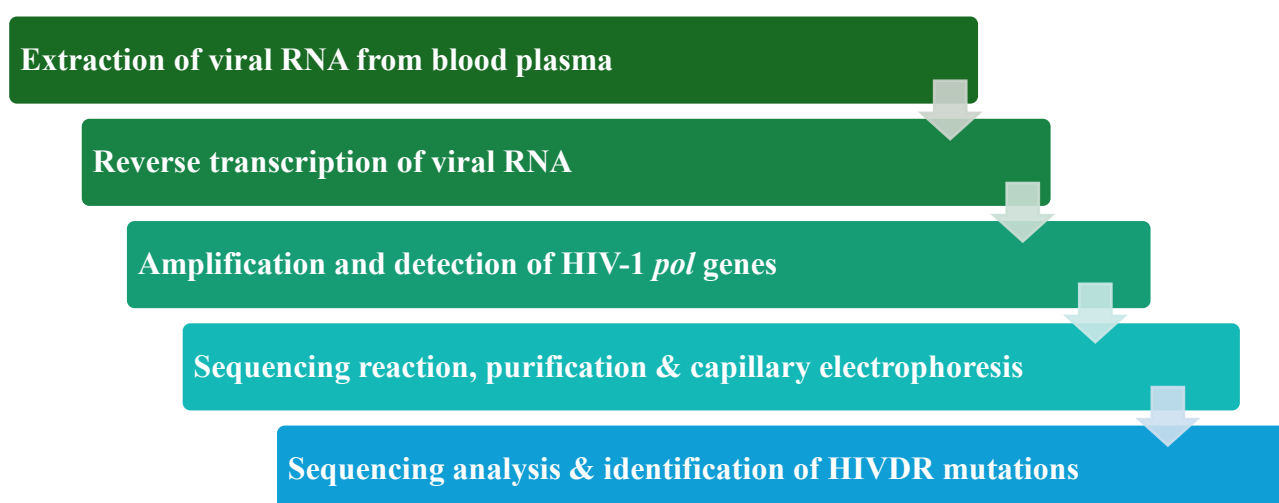


Figure 7. Outline of steps in HIV drug resistance genotypic testing

The flow diagram in Figure 7 outlines key steps involved in HIVDR genotypic testing. The complex, labour-intensive and costly laboratory test is available to a fraction of patients with virological failure.

1.2.4. Geospatial and big data analysis

Data dashboards facilitate the communication of data trends and are important in disease surveillance, enabling the rapid dissemination, comprehension, and interpretation of health-related datasets [60]. The interest in record linkage methodologies among health researchers is growing due to the rise of big data, particularly data generated through routine interactions rather than for research purposes [61]. Moreover, during the COVID-19 pandemic, GIS-linked healthcare data were extensively employed to analyse COVID-19 transmission dynamics, forecast spatiotemporal trends, and focus containment measures in designated "hotspot" areas [62]. The integration of prevalence data with GIS through interactive web-based dashboards allowed the global community to monitor the COVID-19 epidemic in near-real time [62]. Geospatial analysis has provided invaluable insights into several other diseases, including drug resistant tuberculosis (TB) in South Africa [33,63–66]. The use of big data in HIV-1 has largely been constrained by structural and methodological obstacles related to data collection, analysis, and interpretability [67]. Big data science is crucial for improving health outcomes for PLWH, identifying biological, behavioural, and societal variables influencing HIV transmission, comorbidities, and VL control [67].

Several prevalence studies have used geospatial analysis as a tool for evaluating the HIV-1 epidemic in South Africa and highlighting the problem of hyperendemicity in certain regions [29,32,68–72]. A notable hyperendemic region in South Africa is the province of KwaZulu-Natal, often referred to as the epicentre of the South African HIV epidemic, within which there are localized “hotspots” of higher HIV-1 prevalence [73–75]. In addition to identifying "hotspots" of HIV-1 prevalence, geospatial analysis has the ability to characterize viral suppression rates and HIVDR patterns, providing a more comprehensive understanding of the HIV-1 epidemic in near-real time and informing future treatment strategies [76]. Although linkage of large datasets is possible in South Africa, as evidenced by a national HIV cohort built from routine laboratory data [61], the application of big data science to VL and HIVDR genotypic data has not been well described.

1.3. Problem Statement

The problem we identified is the lack of comprehensive geospatial HIV-1 treatment monitoring strategies to assess VL and HIVDR patterns in KZN, which impedes timely responses and the implementation of targeted programmatic interventions.

1.4. Research Questions

1. What are the geospatial distributions of HIV-1 VLs and HIVDR in KZN, and how have the patterns evolved over time?

2. What are the patterns of HIVDR mutations among people with virological failure in KZN, and how do the mutations impact susceptibility to ARV drugs commonly used as standard of care in South Africa?
3. How can we use insights from big data analysis to guide HIV-1 prevention and treatment services?

1.5. Justification

As the ART program evolves and new treatment regimens are implemented, real-time knowledge regarding shifts in VL and HIVDR patterns amongst PLWH is crucial for evaluating the effectiveness of ART programmes. Currently, we primarily rely on cross-sectional studies to evaluate the patterns of HIV-1 viraemia and levels of HIVDR. However, these types of studies require considerable time and financial resources to recruit participants, collect specimens, and perform VL and HIVDR tests. Typically, the results are only generated and published several years after the time of specimen collection with a limited number of participants. This approach does not provide a complete understanding of the present trends in HIV-1 viraemia and drug resistance, especially in the context of evolving ART regimens. Furthermore, the introduction of DTG-based first-line regimens necessitates a closer look at how ART regimens could be modified in the future, particularly for the reutilization of NRTI drugs.

Although the NHLS has developed a surveillance approach which integrates public sector patient-level CD4 and VL test result data from the CDW with data from the health information system [8], these data dashboards lack hotspot visualization and have not incorporated HIVDR data. Therefore, we developed an interactive near-real-time database and linked dashboard that integrates with the relevant NHLS CDW data for monitoring and mapping VLs and HIVDR in KZN, using data generated from routine laboratory tests, to inform the provincial public health response to ART. This database is innovative in that it leverages VL and HIVDR data from routine laboratory tests, without the need for additional testing, and employs geospatial analysis as a monitoring tool to inform policymakers and guide the design of targeted interventions. This approach also generates graphical outputs that are easily interpretable, helping to illustrate the relationship between HIVDR and viraemia.

The use of routine HIVDR genotypic data within the geographic context of PLWH in South Africa represents a novel approach that will enhance the role of big data analysis in HIV-related research. Continuous review of this resource will generate a referenceable database containing high-priority, actionable data drawn from consolidated patient healthcare records, with distinct epidemiological, clinical, and laboratory relevance. Geospatial analyses of the data will inform public health, clinical and laboratory practices, as well as guide future research. The use of an anonymized database of results generated for routine purposes offers several advantages over other types of data and research:

1. Research studies are typically confined to one or a few sites, whereas the database is representative of all public health facilities across the province.

2. The number of specimens tested for routine purposes exceeds that of most research studies.
3. The alternative would require additional testing of patients utilizing fresh or residual samples. Utilizing the database instead of doing additional tests conserves resources, reduces discomfort for patients and increases convenience.
4. Database analysis can be conducted continuously, unlike studies that are limited to a specific timeframe.
5. Database analysis will provide both historical and “real-time” data, allowing for retrospective or prospective analysis. In contrast, research studies are often restricted to one or the other design.
6. While this is a “convenience sample” with inherent bias, it represents the typical range of specimens encountered in a public health facility. In contrast, research studies have specific inclusion and exclusion criteria tailored to answer a particular research question, limiting the ability to repurpose the data.
7. Data from the database may help in sample size calculations and the informed planning of research studies. Without this, such decisions may rely on data from other locations, populations or sources, which may be inaccurate.

Furthermore, there is a lack of data on the patterns of HIVDR mutations in patients receiving INSTI-based regimens in South Africa. Genotypic resistance data from patients experiencing treatment failure on INSTI-based regimens, will help identify patterns of HIVDR mutations among these individuals in KZN. Given the limited treatment options, and with most patients being highly ART-experienced, this approach may highlight the need for newer drug classes in South Africa, including long-acting ARVs.

1.6. Main Objective

To develop a database that integrates GIS data to monitor HIV-1 VLs and HIVDR detected through routine testing of ART-experienced patients with virological failure (i.e., VLs ≥ 1000 copies/mL) in KZN, South Africa, and evaluate geospatial patterns of VLs and HIVDR mutations among adults and children with unsuppressed VLs on ART.

1.6.1. Specific Objectives

1. To design a database with linked geographic data, to monitor and characterize VL and HIVDR mutation patterns among patients with viraemia in KZN.
2. To create a near “real-time” dashboard with geospatial mapping that highlights “hotspots” of high VLs and ARV drug class susceptibility patterns across KZN.
3. To determine the geographic distribution of HIVDR and its association with rates of HIV-1 viraemia.
4. To characterize patterns of HIVDR mutations in patients experiencing virological failure in KZN.
5. To use the database knowledge to advise on the allocation of health resources for HIV-1 prevention and treatment services.

1.7. Research Methodology

1.7.1. Study area

This study used routinely processed VL and HIVDR genotypic data obtained from the NHLS Central Data Warehouse (CDW). The data, which included virology test results linked to demographic information, were curated from patients attending public health care facilities across the 11 districts of KZN province; i.e. Ugu, uMgungundlovu, uThukela, uMzinyathi, Amajuba, Zululand, uMkhanyakude, King Cetshwayo, iLembe, Harry Gwala and eThekweni districts (Figure 8) [77]. Within these geographic areas, there are 610 primary health care institutions and 71 public hospitals that serve the medically uninsured, which accounts for ~9 million or 90% of the population in KZN [29,77]. The NHLS serves as the sentinel provider of diagnostic pathology services for these facilities, as well as for all other public health facilities across South Africa [55].

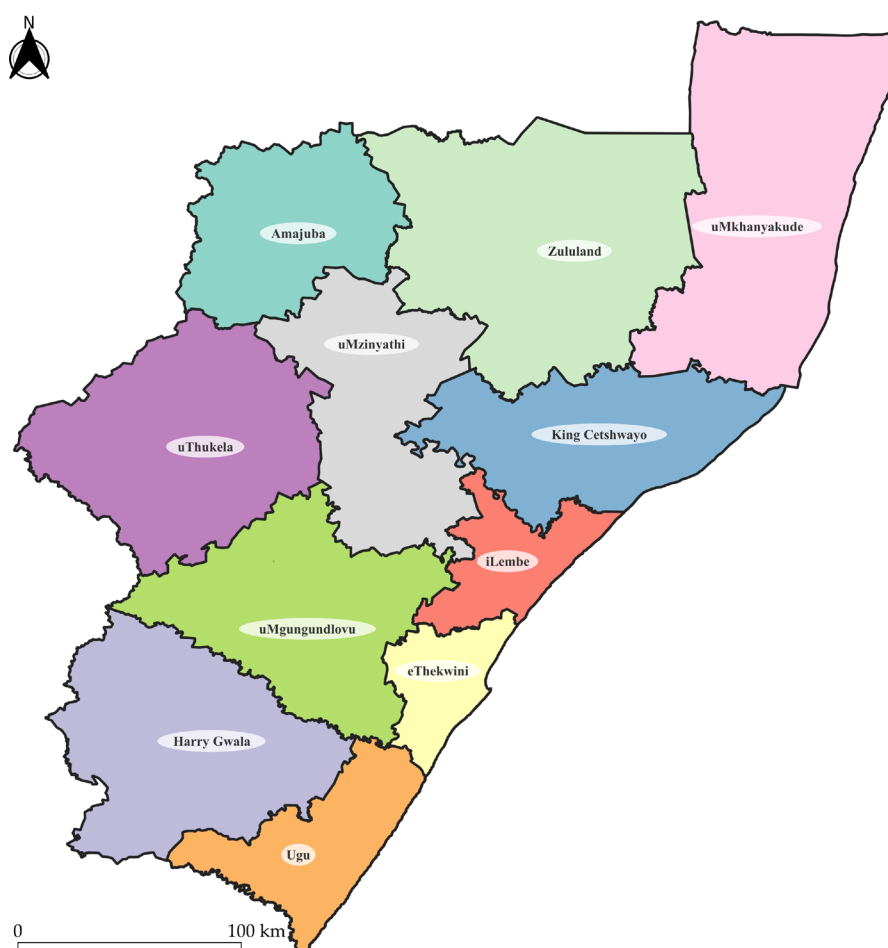


Figure 8. Map of KwaZulu-Natal province, in South Africa

The thick black lines delineate the 11 districts of KwaZulu-Natal province. The basemap of KZN province has been republished under a CC BY license with consent from Carto Builder user Lilishia Gounder, original copyright 2024. Available at: <https://pineapp.app.carto.com/map/4d4c56c1-f82d-4409-b190-ea9ced309005> (accessed on 03 November 2024).

1.7.2. Data analysis methods

A database was developed using routine de-identified VL and HIVDR test results obtained from NHLS CDW. The healthcare facility where the patient's specimen was collected was used to generate GIS coordinates for linking VL and HIVDR results to a geographic region in KZN. The variables included: age, gender, location (i.e. healthcare facility), VLs, HIVDR results (mutations and ARV susceptibilities), dates that tests were performed, treatment regimens and dates that ART was initiated/switched. The database was access-controlled and maintained at NHLS DoV, and data was retrieved as required for peer-review journals, workshops and conferences.

1.7.3. Geospatial analysis methods

Inverse distance weighted (IDW) interpolation maps and choropleth maps were created at a healthcare facility level and subdistrict level, respectively, in QGIS 3.30 using the associated GIS coordinates from individual result records in the VL and HIVDR databases. Each record was represented by the corresponding GIS coordinates of the facility where the patient specimen was taken. From analysis of the records in Stata and QGIS, VL and HIVDR values were calculated and assigned per healthcare facility as follows:

1. Ratio of suppressed VLs (i.e., VLs <400 copies/mL) to detectable VLs (i.e., VLs $\geq$ 400 copies/mL)
2. Median $\log_{10}$ VLs for detectable VLs (i.e., VLs $\geq$ 400 copies/mL)
3. Spatial autocorrelation Moran's I statistic using median $\log_{10}$ VLs
4. Getis-Ord G_i^* statistically significant values from hotspot and coldspot analysis of median $\log_{10}$ VLs
5. ARV drug susceptibility levels for specific drugs contextualized to the South African ART programme

For 1, 2 and 5, a deterministic spatial model (IDW interpolation) was used to estimate what the unknown VL or HIVDR values would be in regions adjacent to the healthcare facilities. The interpolation method proposed that the "ratio of suppressed to unsuppressed VLs" or the "median $\log_{10}$ VLs" or the "ARV drug susceptibility levels" in populations within unsampled regions (i.e., areas adjacent to healthcare facilities) would correspond to the weighted average computed across facilities. Regions adjacent to the healthcare facilities would have values that were equivalent to the actual values recorded at the nearby facility.

For 3, a cluster analysis was done in Stata using the median $\log_{10}$ VL per facility, and Moran's I statistic and p-values were illustrated on Moran scatterplots for each year of the study period. Moran's I statistic measured spatial autocorrelation, with index values ranging from -1 to +1. A positive index value signified positive spatial autocorrelation.

For 4, using the Getis-Ord G_i^* hotspot analysis values derived from both QGIS hotspot analysis and Stata, a facility was identified as being in a hotspot if the p-value was <0.05 and z-score $\geq$ 1.96. Similarly, a facility was identified as being in a coldspot if the p-value was <0.05 and z-score $\leq$ -1.96. The statistical values were

then linked to the respective facility GIS coordinates within the map layer as follows: the red, orange, and yellow circles indicated the statistical significance of primary, secondary, and tertiary hotspots at confidence intervals of 99%, 95%, and 90%, respectively, while the light to dark blue circles represented the statistical significance of tertiary, secondary, and primary coldspots at confidence intervals of 90%, 95%, and 99%, respectively. Lastly, choropleth maps were used to illustrate subdistrict level data, such as proportions of HIVDR specimens obtained from each subdistrict or categorization of detectable viral loads per subdistrict.

1.7.4. Ethical approval

Ethical approval was obtained from the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (reference number: BREC/00003120/2021) on 10 September 2021 and was renewed annually from 2022-2024 (Appendix 1). We obtained additional permission from the NHLS Academic Affairs and Research Management System to use routinely processed retrospective de-identified VL and HIVDR data from CDW for research purposes. This study did not obtain informed consent from individuals due to the use of retrospective anonymized patient data. The research ethics council that approved this study waived the need for participant consent.

1.8. Thesis Outline

This thesis consists of a background, literature review and justification in chapter 1, manuscripts in chapters 2 to 4, and a synthesis consolidating the thesis findings in chapter 5. The manuscript formats and referencing styles utilized in chapters 2 to 4 adhere to specific journal requirements, and the published papers are shown in their current publication format. The supplementary material for chapters 2, 3 and 4 can be found in appendices 2, 3 and 4, respectively.

Chapter 1: Background and Literature Review

This chapter presents an overview of the research work conducted and provides a comprehensive literature review on viraemia, drug resistance and geospatial analysis within the context of the South African HIV-1 epidemic and introduces the research questions/research gaps and the research objectives.

Chapter 2: Manuscript: “Geospatial and temporal mapping of detectable HIV-1 viral loads amid dolutegravir rollout in KwaZulu-Natal, South Africa”

This chapter examines the problem of HIV-1 viraemia across the 44 subdistricts of KZN using geospatial analyses. “Hotspots” of high-level viraemia were explored in greater detail, while also considering the impact and significance of LLV.

Chapter 3: Manuscript: “Patterns of HIV-1 drug resistance observed through geospatial analysis of routine diagnostic testing in KwaZulu-Natal, South Africa”

This chapter assessed HIVDR in adult ART-experienced patients attending public health facilities in KZN. It examines the frequencies of HIVDR mutations, maps ARV drug susceptibilities across the province, and uses genotypic susceptibility scores (GSS) to determine the effectiveness of current drug regimens for adult patients.

Chapter 4: Manuscript: “Characterization of HIV-1 drug resistance in ART-experienced children and adolescents through geospatial analysis of routine genotypic tests in KwaZulu-Natal, South Africa.”

This chapter described the HIVDR mutations detected in ART-experienced children and adolescents attending public health facilities in KZN. It also provides additional analyses to contextualize HIVDR both at patient and facility level.

Chapter 5: Synthesis of the thesis

This concluding chapter offers an overall synthesis of the thesis, consolidating the main findings from the manuscripts in chapters 2 to 4, along with recommendations for policy and future research, based on research findings.

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1.10. Bridging Text

The literature review presented in Chapter 1 cited several studies describing the use of geospatial analysis to characterize HIV-1 prevalence, addressing the first 95% of the UNAIDS 95-95-95 targets, which aims to have 95% of PLWH knowing their HIV status. In addition, it summarized the HIV epidemic in SA focusing on treatment monitoring and on VL and HIVDR. It also gave justification for this work and set out the main study objectives, highlighting the use of big data and geospatial analysis for HIV programmatic treatment monitoring. However, there is a paucity in knowledge regarding the geospatial analysis of HIV-1 viraemia levels and viral suppression, which relate to the third 95% of the UNAIDS 95-95-95 targets, aiming for 95% of people on ART achieving viral suppression. Chapter 2 describes geospatial and temporal analysis of HIV-1 VLs in adults and children in KwaZulu-Natal province, the epicentre of the HIV epidemic in South Africa, amid the transition to DTG-based first-line ART. The chapter is based on a manuscript that was published in PLOS Global Public Health Journal in 2024. The manuscript supplementary materials are provided in Appendix 2.

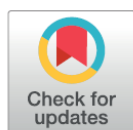
Chapter 2: Geospatial and temporal mapping of detectable HIV-1 viral loads amid dolutegravir rollout in KwaZulu-Natal, South Africa

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RESEARCH ARTICLE

Geospatial and temporal mapping of detectable HIV-1 viral loads amid dolutegravir rollout in KwaZulu-Natal, South Africa

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Abstract

South Africa rolled out dolutegravir (DTG) as first-line antiretroviral therapy (ART) in December 2019 to overcome high rates of pretreatment non-nucleoside reverse transcriptase inhibitor drug resistance. In the context of transition to DTG-based ART, this study spatio-temporally analysed detectable HIV viral loads (VLs) prior to- and following DTG rollout in public-sector healthcare facilities in KwaZulu-Natal (KZN) province, the epicentre of the HIV epidemic in South Africa. We retrospectively curated a HIV VL database using de-identified routine VL data obtained from the National Health Laboratory Service for the period January 2018 to June 2022. We analysed trends in HIV viraemia and mapped median log₁₀ HIV VLs per facility on inverse distance weighted interpolation maps. We used Getis-Ord Gi* hotspot analysis to identify geospatial HIV hotspots. We obtained 7,639,978 HIV VL records from 736 healthcare facilities across KZN, of which 1,031,171 (13.5%) had detectable VLs (i.e., VLs ≥ 400 copies/millilitre (mL)). Of those with detectable VLs, we observed an overall decrease in HIV VLs between 2018 and 2022 (median 4.093 log₁₀ copies/mL; 95% confidence interval (CI) 4.087–4.100 to median 3.563 log₁₀ copies/mL; CI 3.553–3.572), $p < 0.01$ (median test). The downward trend in proportion of HIV VLs ≥ 1000 copies/mL over time was accompanied by an inverse upward trend in the proportion of HIV VLs between 400 and 999 copies/mL. Moreover, specific coastal and northern districts of KZN had persistently higher VLs, with emergent hotspots demonstrating spatial clustering of high median log₁₀

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HIV VLs. The overall decrease in HIV VLs over time shows good progress towards achieving UNAIDS 95-95-95 targets in KZN, South Africa. The DTG-transition has been associated with a reduction in VLs, however, there is a need for pre-emptive monitoring of low-level viraemia. Furthermore, our findings highlight that specific districts will need intensified HIV care despite DTG rollout.

Introduction

The commitment to end the global AIDS epidemic by 2030, albeit shadowed by the COVID-19 pandemic, must be accompanied by accelerated action in upscaling effective HIV services [1]. Although the UNAIDS 95-95-95 strategy aims at ensuring that 95% of all people living with HIV (PLWH) are diagnosed, 95% of all PLWH diagnosed receive antiretroviral therapy (ART), and 95% of people on ART achieve sustained viral suppression, South Africa is still lagging behind in achieving these targets [1–4]. In KwaZulu-Natal (KZN), the province with the greatest burden of HIV in South Africa [5, 6], there are HIV-hyperendemic areas where sustained viral suppression among PLWH is substantially lower (53.9%) than the UNAIDS target [7]. Studies have shown that population prevalence of detectable viraemia could infer the effectiveness of HIV treatment programmes as well as community HIV risk potential [8–10].

Critical to the success of ART programmes is HIV viral load (VL) monitoring, and a sustained VL <50 copies/millilitre (mL) of plasma is considered treatment success [11]. HIV VL testing is recommended every 6–12 months, with annual VL monitoring in patients with viral suppression at follow-up [12]. Over the years, virological failure has been defined as two consecutive VLs ≥ 1000 copies/mL, 3 months apart, in patients on ART for at least 6 months. However, patients receiving first-line dolutegravir (DTG)-based ART are only considered for second-line ART when they have at least two VLs ≥ 1000 copies/mL after 24 months of DTG-based treatment, or have other signs of immunologic or clinical failure [13, 14]. While a plasma VL between 200 and 500 copies/mL significantly predicts future virological failure [15], VLs between 400 and 999 copies/mL are associated with an increased 10-year risk of death [16].

Routine HIV VL testing is only beneficial if accompanied by appropriate and timely clinical action, especially since prolonged treatment regimen failure significantly increases the risk of multiple HIV drug resistant mutations and compromises the efficacy of second-line ART in PLWH [17]. Challenges to VL monitoring of patients on ART include lack of infrastructure and poor service delivery, particularly in rural communities [18]. Moreover, facility-based VLs do not necessarily correlate well with population-based VLs and may not be predictive of HIV incidence [9]. Technologies, such as spatial information management systems with accurate HIV and infrastructure data, could play a significant role in improving the quality of care for PLWH and enhancing accessibility to healthcare services [19, 20].

The advancement of spatial statistics has allowed the application of disease mapping and spatial analyses in epidemiological research [21]. Geographic information systems (GIS) and spatial scan statistics have been applied within the context of the HIV epidemic in South Africa to help healthcare workers better understand levels of VL suppression [22] and HIV-related mortality patterns [21] in space and time. GIS has also been used as a means of visualizing the spread and transmission of diseases in South Africa. However, spatiotemporal tools have not yet been used to describe HIV VL monitoring in KZN with respect to changing treatment regimens [18]. We therefore aimed to describe spatiotemporal changes in HIV VLs in KZN,

during the rollout and subsequent implementation of DTG in ART regimens as per the standard of care in South Africa.

Methods

Study design

This was a retrospective study of HIV VL data obtained from the South African National Health Laboratory Service (NHLS) Central Data Warehouse (CDW). The NHLS is the largest diagnostic pathology service provider in South Africa, delivering cost-efficient health laboratory services to all public-sector healthcare facilities that serve approximately 80% of the population [23]. The NHLS CDW stores data on laboratory testing with linked patient demographic data, which is vital for monitoring national and provincial Department of Health programmes [24].

We obtained de-identified data for all people with HIV who had HIV VLs between 1 January 2018 and 30 June 2022, at 736 public-sector healthcare facilities in KZN Province (Fig 1). This represents a period during the rollout and subsequent implementation of DTG in ART regimens in public-sector healthcare in South Africa. The anonymized data were accessed for research purposes from 21 September 2022 to 22 November 2023. All HIV VLs were tested at NHLS virology laboratories that actively subscribe to external quality assurance programmes (i.e., Quality Control for Molecular Diagnostics, Glasgow, Scotland, UK).

The automated molecular diagnostic analysers used for VL testing were the Abbott m2000 RealTime System (Abbott Molecular Diagnostics, Des Plaines, Illinois, United States), Abbott Alinity m System (Abbott Molecular Diagnostics, Des Plaines, Illinois, United States) and Roche Cobas 6800/8800 System (Roche Molecular Diagnostics, Pleasanton, California, United States). The limit of detection (LOD) varied depending on the analyser and the input volume used. For the Abbott m2000 RealTime System, the LODs were <40 and <150 copies/mL for 0.6 mL and 0.2 mL specimens, respectively. For the Abbott Alinity m System, the LODs were <20 and <50 copies/mL for 0.6 mL and 0.2 mL specimens, respectively. For the Roche Cobas 6800/8800 System, the LODs were <20 and <50 copies/mL for 0.5 mL and 0.2 mL specimens, respectively. In view of the variability in LOD, we defined detectable HIV VLs as any plasma VL ≥ 400 copies/mL and suppressed HIV VLs as any plasma VL <400 copies/mL.

HIV viral load database

We created an in-house HIV VL database at the NHLS Department of Virology, Inkosi Albert Luthuli Central Hospital in Durban, South Africa, using de-identified HIV VL data obtained from the NHLS CDW. VL records originating from facilities outside of KZN were excluded from further analysis. Our analyses included records with suppressed VLs i.e., <400 copies/mL and detectable VLs, i.e., ≥ 400 copies/mL. We further categorized detectable VLs into two groups, i.e., (i) low-level viraemia; defined as any plasma VL 400–999 copies/mL and (ii) high VL; defined as any plasma VL $\geq 1,000$ copies/mL. The database included the following meta-data for each VL record: age, sex, specimen collection date, KZN district, facility name and facility type. We assigned global positioning system (GPS) coordinates to each VL record based on facility location.

Geospatial mapping

We generated maps with linked GPS coordinate locations from individual records in the HIV VL database using QGIS 3.30 software [25]. Each HIV VL record was represented by a linked GPS coordinate of the facility where the specimen was collected. The ratio of suppressed and

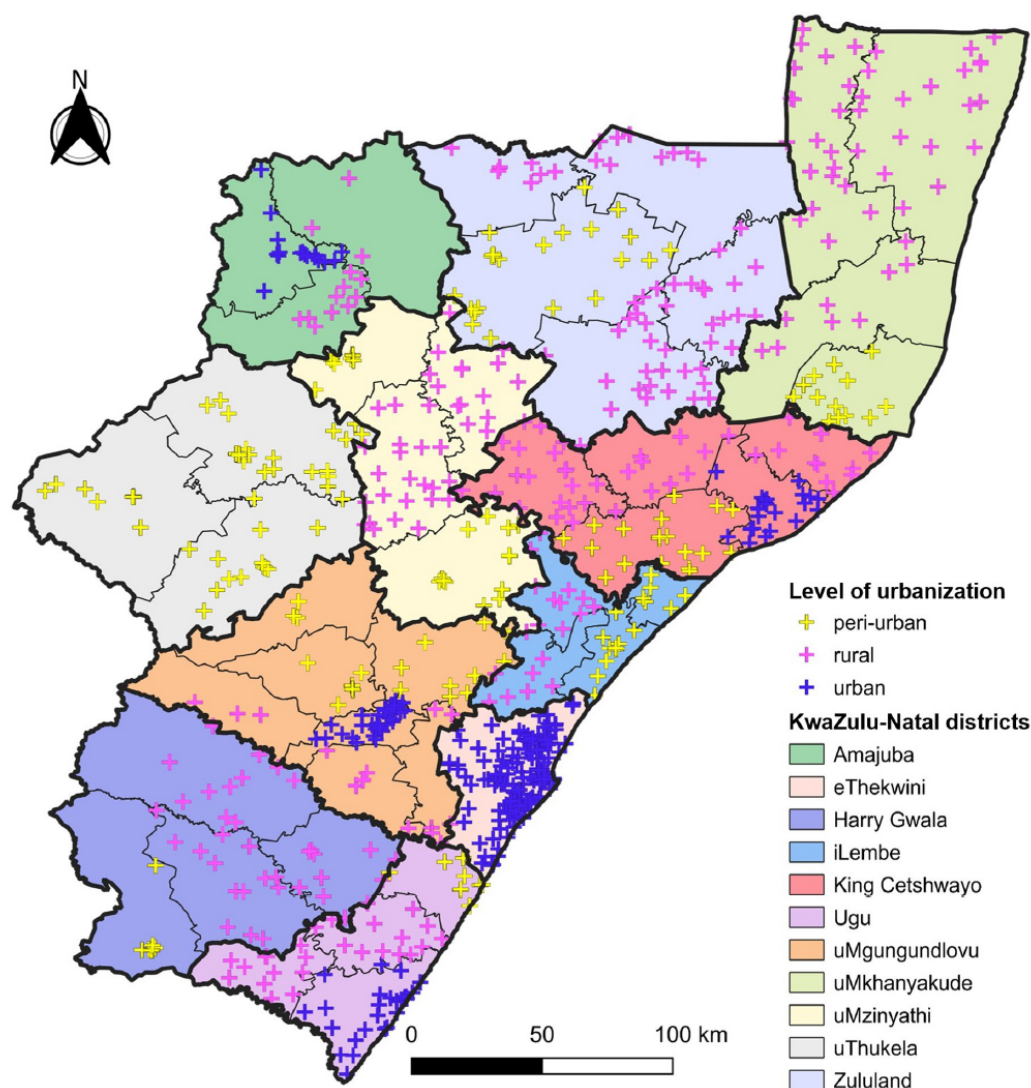


Fig 1. Healthcare facilities by district in KwaZulu-Natal province, South Africa. Cross symbols represent the location of each healthcare facility that had HIV VL data included in this study. The colour of the cross symbols denotes whether the facility location is within a peri-urban, rural or urban subdistrict. Districts are represented by individual colours. Thick and thin black outlines represent the borders of the districts and subdistricts, respectively. Republished from <https://pineapp.carto.com/map/4d4c56c1-f82d-4409-b190-ea9ced309005> under a CC BY license, with permission from Carto Builder user Lilishia Gounder, original copyright 2024.

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detectable VLs was determined for each healthcare facility and used for geospatial mapping. We created inverse distance weighted (IDW) interpolation maps to map the ratio of suppressed VLs (i.e., VLs <400 copies/mL) and detectable VLs (i.e., VLs $\geq$ 400 copies/mL), over the study period.

To better understand spatiotemporal changes in levels of detectable HIV VLs (i.e., VLs $\geq$ 400 copies/mL) per facility, we used a deterministic spatial model (IDW interpolation) to interpolate the median log₁₀ VLs in areas surrounding the healthcare facilities where the VLs were

unknown. The method assumes that the median $\log_{10}$ VLs in populations within unsampled areas (i.e., areas surrounding the healthcare facilities) will correlate to the weighted average calculated between facilities. Areas closest to the healthcare facilities display median $\log_{10}$ VLs comparable to the actual median $\log_{10}$ VL measured at the neighboring facility location.

We used QGIS hotspot analysis 2.0 software [26] to determine spatial clustering (hot- or coldspot identification) of detectable HIV VLs. In summary, we applied local indicators of spatial association (LISA) statistics to perform Getis-Ord G_i^* hotspot analysis [27], linking each statistical result to its respective facility location within the map layer. Using the Getis-Ord G_i^* hotspot analysis values derived from both QGIS and Stata, we considered a facility to be in a hotspot if the p-value was <0.05 and z-score $\geq +1.96$. Similarly, we considered a facility to be in a coldspot if the p-value was <0.05 and z-score ≤ -1.96 .

We calculated spatial autocorrelation Moran's I statistic using median $\log_{10}$ VLs per facility, and graphically represented index values for each year of the study period on Moran scatterplots using Stata 18.0 software. Moran's I statistic measures spatial autocorrelation and index values for Moran's I statistic range from -1 to +1. A positive index value indicates positive spatial autocorrelation. Positive spatial autocorrelation means that the median $\log_{10}$ VLs tend to be similar for facilities geographically nearby each other, i.e., high values located near high values and low values located near low values.

Statistical analysis

HIV VLs were $\log_{10}$ transformed and reported as median VLs with interquartile ranges (IQRs) and or 95% confidence intervals (CIs). The median test was used to compare median VL across demographic groups and year of specimen collection. The Cochran-Armitage test was used to assess the overall trend in proportion VL ≥ 1000 copies/mL over the study period. We used the Jonckheere-Terpstra test to assess the trend in $\log_{10}$ VL over the study period, for records with VLs ≥ 400 copies/mL. All statistical analyses were performed using Stata 18.0 software SE (StataCorp. 2023, College Station, Texas, United States).

Ethical considerations

This study was approved by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (reference number: BREC/00003120/2021). We obtained additional permission from the NHLS Academic Affairs and Research Management System to use routinely processed retrospective de-identified HIV VL records for research purposes. This study did not request informed patient consent because anonymized data was used.

Results

In total we curated 7,639,978 HIV VL records from 736 healthcare facilities in KZN. The majority of VLs (7,273,492; 95.2%) were from outpatient departments or primary healthcare facilities, with a smaller proportion (333,743; 4.4%) coming from inpatient wards and emergency departments, as well as patients institutionalized in correctional facilities and frail care centres (32,743; 0.4%). Approximately half (3,941,234/ 7,639,978; 51.6%) of all VL records were from facilities within urban subdistricts, with smaller proportions from rural (1,943,486; 25.4%) and peri-urban (1,755,258; 23.0%) subdistricts. Fig 2 shows a summary of HIV VL records obtained and analysed.

The overall proportion of VLs from patients who had achieved VL suppression (<400 copies/mL) was 86% in the period 2018 to 2020, increasing to 87% in 2021 and returning to 86% in 2022. Females had higher VL suppression rates (4,579,540/ 5,209,487; 87.9%) than males (1,834,681/ 2,204,485; 83.2%). The average proportion of VLs ≥ 1000 copies/mL decreased

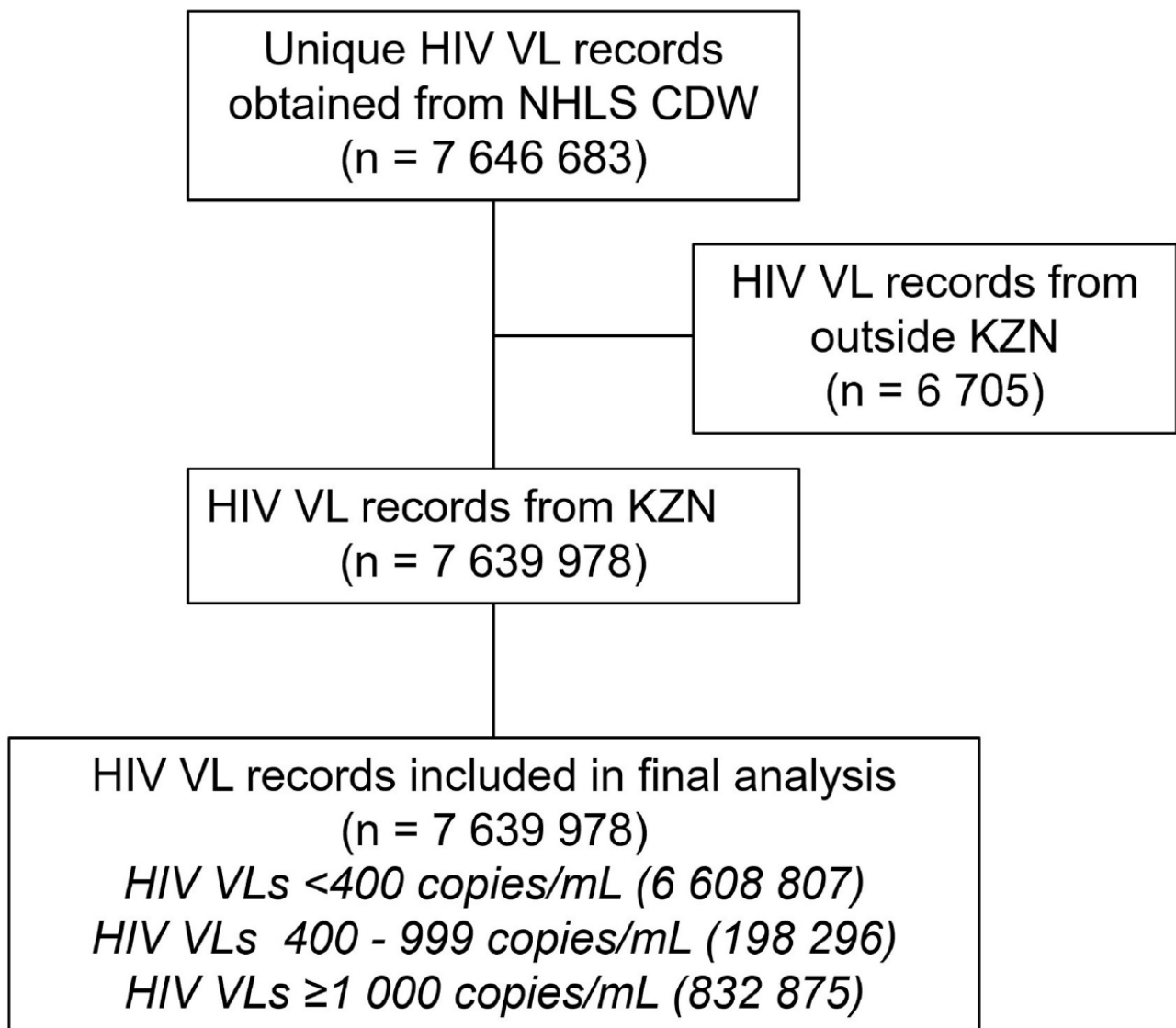


Fig 2. Flow diagram of HIV VL records obtained and included in final analysis. CDW, central data warehouse; HIV VL, HIV viral load; KZN, KwaZulu-Natal; mL, millilitre; NHLS, National Health Laboratory Service.

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significantly from 12% in the period 2018 to 2019, to 11% in 2020, with a further reduction to 10% in 2021 and 2022, $p < 0.05$ Cochrane-Armitage test. The average proportion of VLs from patients who had low-level viraemia (400–999 copies/mL) was 2% in 2018 and 2019, increasing to 3% in 2020 and 2021, and up to 4% in 2022 (Fig 3).

The overall proportions of VLs per subdistrict were heterogeneous, with fluctuating trends noted during the period from 2018 to 2022 (S1 Table). We observed a general trend of increasing VL suppression rates between 2018 and 2022, with high ratios of suppressed to unsuppressed VLs. Despite the overall increase in VL suppression rates over time, there was a

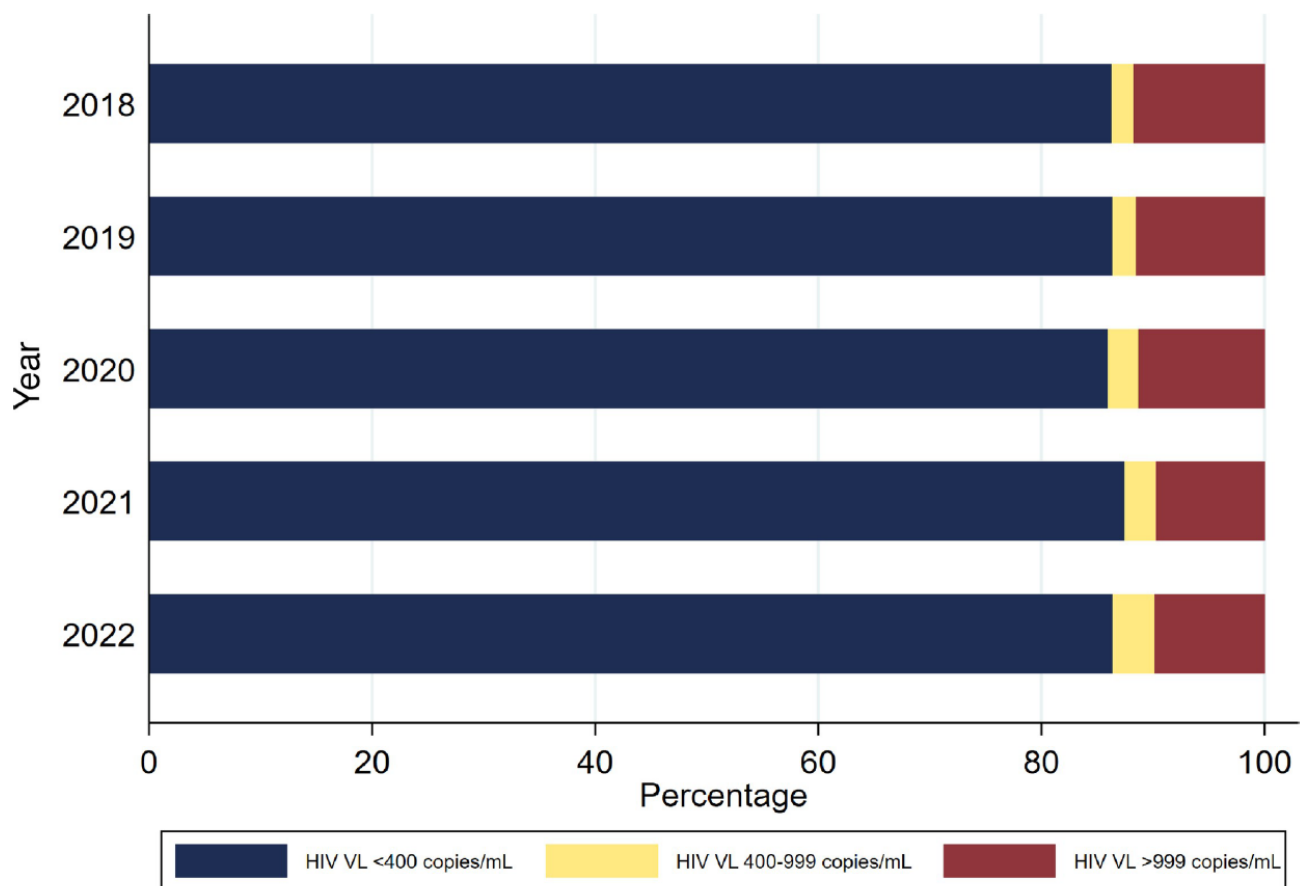


Fig 3. Proportion of all HIV viral loads by year in KwaZulu-Natal province, South Africa. mL, millilitre; VL, viral load.

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notable decrease in the proportion of suppressed VLs in northern KZN by 2022 (S1 Fig).

Table 1 shows characteristics of all patients with HIV VLs, categorized by the level of HIV-1 viraemia (i.e., <400 copies/mL, 400–999 copies/mL and ≥ 1000 copies/mL).

To further understand the spatiotemporal changes in detectable VLs (which could potentially impact ART), from this point onwards we only included VL records ≥ 400 copies/mL in downstream analyses. For the detectable VLs (i.e., ≥ 400 copies/mL), the median $\log_{10}$ VL was lower in outpatients (3.856 $\log_{10}$ copies/mL; IQR 3.121–4.678) than in inpatient and institutionalized patients (4.495 $\log_{10}$ copies/mL; IQR 3.508–5.353), $p < 0.001$ median test. Males had significantly higher median $\log_{10}$ VLs (4.097 $\log_{10}$ copies/mL; IQR 3.220–4.948) than females (3.809 $\log_{10}$ copies/mL; IQR 3.107–4.610), $p < 0.001$ median test. The majority of detectable HIV VLs (904,625/ 1,031,171; 87.7%) were from primary healthcare clinics, community healthcare centres and district hospitals. Notably, nearly half (505,933/ 1,031,171; 49.1%) of all detectable HIV VLs were from patients attending healthcare facilities in urban districts, which includes eThekweni; the metropolitan municipality of KZN.

There was a downward trend in the proportion of HIV VLs ≥ 1000 copies/mL between 2018 and 2022, with an inverse upward trend in the proportion of HIV VLs between 400 and 999 copies/mL (Fig 4). Overall, the median $\log_{10}$ VLs decreased significantly from 4.093 $\log_{10}$

Table 1. Characteristics of database records by level of HIV-1 viraemia.

Variable	All VL (n = 7 639 978)	VL <400 copies/mL (n = 6 608 807)	VL 400–999 copies/mL (n = 198 296)	VL ≥1000 copies/mL (n = 832 875)
Sex				
Female	5 209 487 (68.19%)	4 579 540 (69.29%)	128 476 (64.79%)	501 471 (60.21%)
Male	2 204 485 (28.85%)	1 834 681 (27.76%)	63 996 (32.27%)	305 808 (36.72%)
Unknown	226 006 (2.96%)	194 586 (2.94%)	5 824 (2.94%)	25 596 (3.07%)
Age in years, median (IQR)	37 (29–45)	37 (30–46)	36 (27–44)	33 (24–41)
<5	37 833 (0.50%)	21 262 (0.32%)	2 057 (1.04%)	14 514 (1.74%)
5–14	222 266 (2.91%)	146 383 (2.21%)	12 055 (6.08%)	63 828 (7.66%)
15–24	710 242 (9.30%)	539 469 (8.16%)	25 468 (12.84%)	145 305 (17.45%)
25–49	5 404 631 (70.74%)	4 750 906 (71.89%)	129 445 (65.28%)	524 280 (62.95%)
>50	1 210 106 (15.84%)	1 105 594 (16.73%)	27 686 (13.96%)	76 826 (9.22%)
Unknown	54 900 (0.72%)	45 193 (0.68%)	1 585 (0.80%)	8 122 (0.98%)
Collection year				
2018	1 518 316 (19.87%)	1 309 792 (19.82%)	29 838 (15.05%)	178 686 (21.45%)
2019	1 640 031 (21.47%)	1 416 364 (21.43%)	34 740 (17.52%)	188 927 (22.68%)
2020	1 749 167 (22.89%)	1 503 704 (22.75%)	47 492 (23.95%)	197 971 (23.77%)
2021	1 769 600 (23.16%)	1 547 152 (23.41%)	49 888 (25.16%)	172 560 (20.72%)
2022 ^a	962 864 (12.60%)	831 795 (12.59%)	36 338 (18.33%)	94 731 (11.37%)
Healthcare department				
Outpatients	7 273 492 (95.20%)	6 336 424 (95.88%)	186 822 (94.21%)	750 246 (90.08%)
Inpatients	333 743 (4.37%)	242 672 (3.67%)	10 949 (5.52%)	80 122 (9.62%)
Other ^b	32 743 (0.43%)	29 711 (0.45%)	525 (0.26%)	2 507 (0.30%)
Level of urbanization^c				
Rural subdistricts	1 943 486 (25.44%)	1 674 192 (25.33%)	49 834 (25.13%)	219 460 (26.35%)
Peri-urban subdistricts	1 755 258 (22.97%)	1 499 314 (22.69%)	53 172 (26.81%)	202 772 (24.35%)
Urban subdistricts	3 941 234 (51.59%)	3 435 301 (51.98%)	95 290 (48.05%)	410 643 (49.30%)

IQR, interquartile range; mL, millilitre; VL, viral load

^a Data are for half the year because study period ended on 30 June 2022^b Records from correctional facilities or frail care facilities^c Obtained from annual reports and integrated development plans for individual subdistricts<https://doi.org/10.1371/journal.pgph.0003224.t001>

copies/mL (CI 4.087–4.100) to 3.563 log₁₀ copies/mL (CI 3.553–3.572) between 2018 and 2022, $p < 0.01$ Jonckheere–Terpstra test (S2 and S3 Figs).

We observed heterogeneous spatiotemporal changes in detectable HIV VLs per subdistrict, with most subdistricts reflecting a decrease in median log₁₀ VL between 2018 and 2022 (Fig 5 and S4 Fig). Despite the overall reduction in HIV VLs over time, most districts along the coastal belt and in northern KZN maintained VLs ≥1000 copies/mL throughout the study period (S5 Fig).

Getis-Ord Gi* hot- and coldspot analysis measured the spatial clustering of high and low median log₁₀ VLs per facility, from 2018 to 2022. The relationship between geographical location and median log₁₀ VLs was evaluated using the critical value standard deviation (z-score) and statistical significance (p-value) calculated per facility. Primary (z-score < -2.58 or > +2.58), secondary (z-score ±1.96 to ±2.58) and tertiary (z-score ±1.65 to ±1.96) intensity clusters are shown in Fig 6. Moran's I statistic was calculated for median log₁₀ VLs per facility, and the index value from 2018 to 2022 was >0.15, $p < 0.001$, indicating positive spatial autocorrelation (S6 Fig).

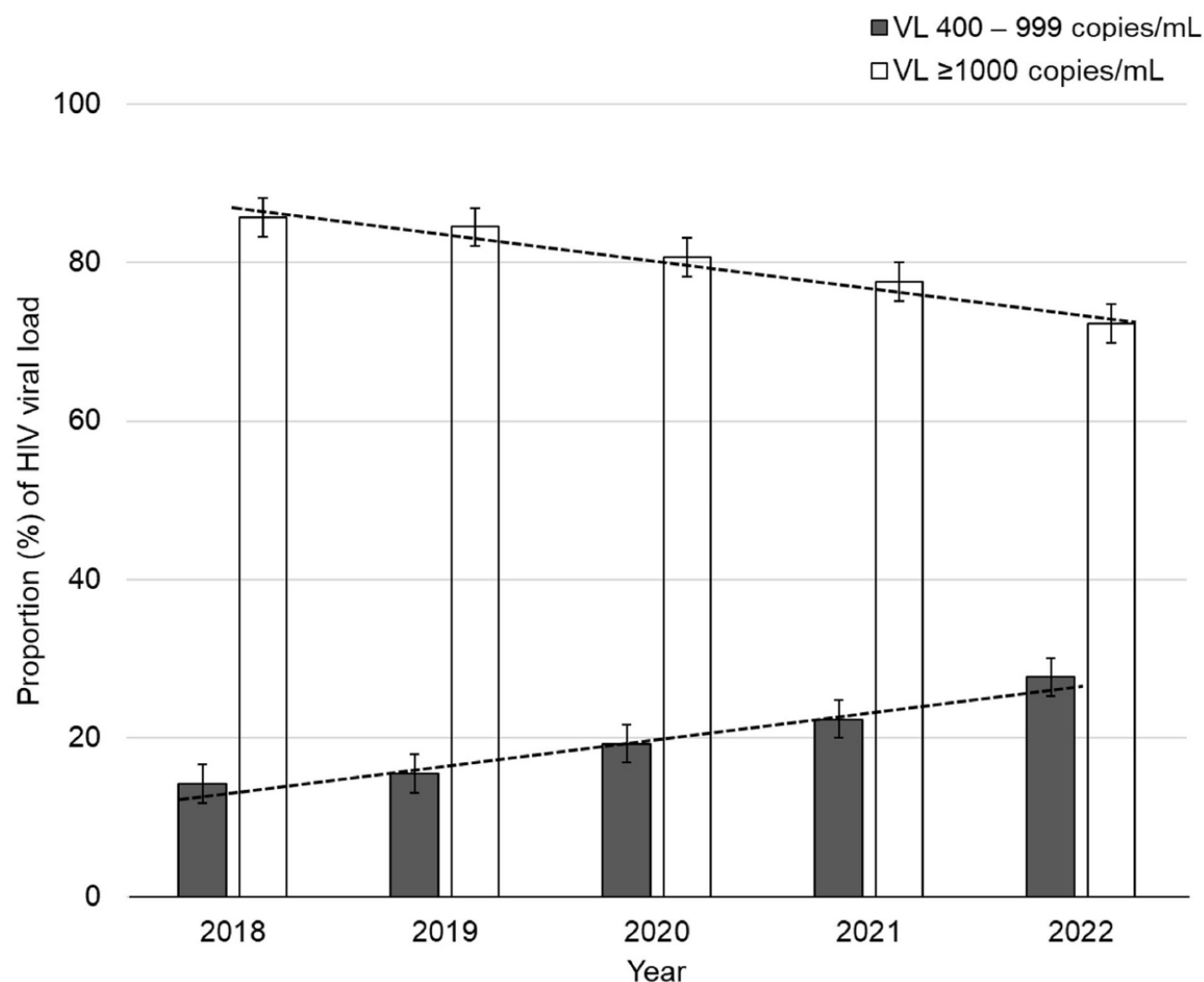


Fig 4. Trend analysis of detectable HIV viral loads by year in KwaZulu-Natal province, South Africa. mL, millilitre; VL, viral load.

<https://doi.org/10.1371/journal.pgph.0003224.g004>

Overall, 244 significant hotspot facilities demonstrated spatial clustering of high median $\log_{10}$ VLs, $p < 0.05$ Getis-Ord G_i^* statistic. The coastal King Cetshwayo district had persistent spatial clustering of high median $\log_{10}$ HIV VLs for facilities within urban and peri-urban sub-districts, from 2018 to 2022. During the transition period to DTG (2020 to 2022), 58 facilities were identified as hotspots for at least 2 consecutive years. These hotspot facilities were located within the northern districts of Amajuba and Zululand, the coastal districts of uMkhanyakude, King Cetshwayo, iLembe, eThekweni and its neighbouring district, uMgungundlovu. Of note, the coastal Ugu district, which did not have significant hotspots from 2019 to 2021, demonstrated emergent HIV VL hotspots in 2022 (S2 Table).

From 2018 to 2022, we compared database records from facilities within hot- and coldspots demonstrating spatial clustering of statistically significant high and low median $\log_{10}$ HIV VLs

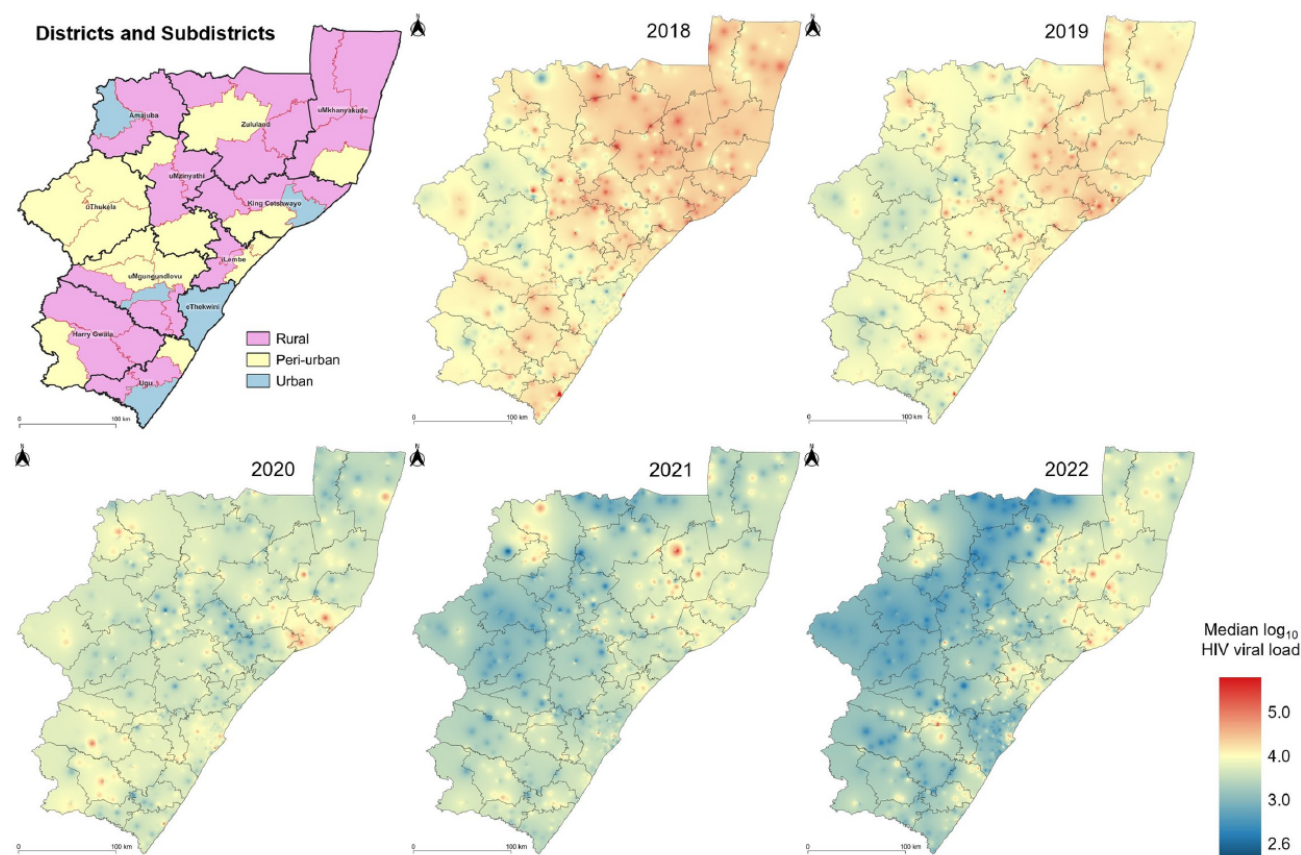


Fig 5. Decreasing HIV viral loads across subdistricts in KwaZulu-Natal province, South Africa. Inverse distance weighted interpolation maps from 2018 to 2022. Spectral colour change from red to blue reflects decrease in median $\log_{10}$ viral loads. Thick black outlines represent the borders of the 11 districts. Thin outlines represent the borders of the 44 subdistricts of KwaZulu-Natal. Republished from <https://pinea.app.carto.com/map/4d4c56c1-f82d-4409-b190-ea9ced309005> under a CC BY license, with permission from Carto Builder user Lilishia Gounder, original copyright 2024.

<https://doi.org/10.1371/journal.pgph.0003224.g005>

(S3 Table). The median age was 33 years (IQR 24–41 years) and 34 years (IQR 25–43 years) for the hot- and coldspot groups, respectively. There was a marginal difference in the proportion of males within the hot- and coldspot groups (35.2% versus 35.6%), and females within the hot- and coldspot groups (61.3% versus 61.8%). The median $\log_{10}$ VL was 4.140 $\log_{10}$ copies/mL and 3.558 $\log_{10}$ copies/mL for the hot- and coldspot groups, respectively. The proportion of inpatients was higher in hotspot facilities versus coldspots, throughout the study period. Hotspot facilities were mostly located within urban subdistricts and coldspot facilities were predominantly in peri-urban subdistricts as shown in S3 Table.

Discussion

This study demonstrated that geospatial mapping of VLs offers a very useful tool for targeted health systems strengthening activities to achieve the UNAIDS 95-95-95 HIV treatment goals. More importantly this study highlights the need to direct efforts to known communities with higher levels of viraemia on ART, to strengthen VL re-suppression efforts and HIV prevention activities and ultimately, to implement activities to curb ART resistance emergence. This is

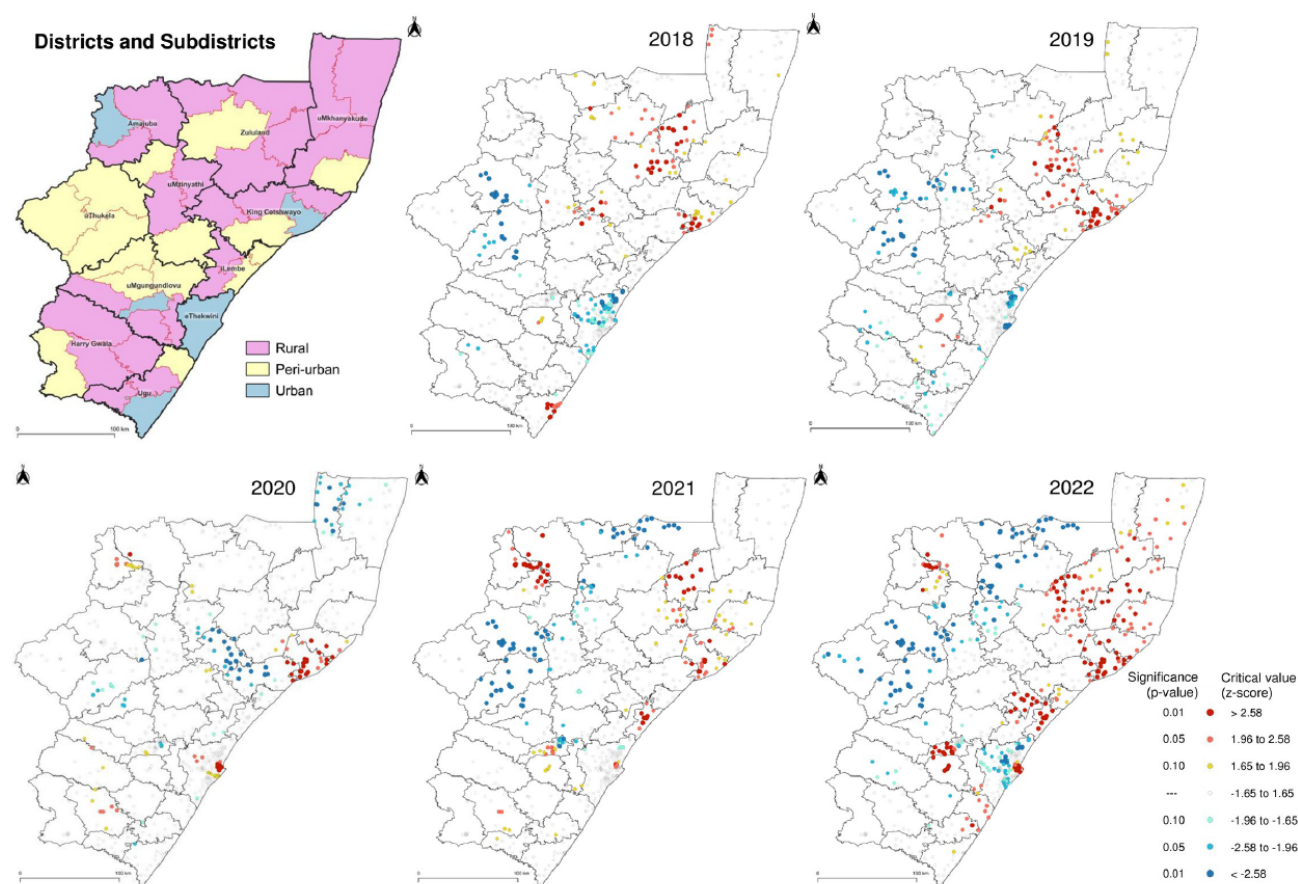


Fig 6. Hot- and coldspot analysis of HIV viral loads per facility in KwaZulu-Natal province, South Africa. Getis-Ord G_i^* hot- and coldspot statistical analysis of median $\log_{10}$ viral load mapped per facility. Red, orange and yellow circles reflect the statistical significance of primary, secondary and tertiary hotspots at 99%, 95% and 90% confidence intervals, respectively. Light to dark blue circles reflect the statistical significance of tertiary, secondary and primary coldspots at 90%, 95% and 99% confidence intervals, respectively. Thick and thin outlines represent the borders of districts and subdistricts, respectively. Republished from <https://pineappleapp.carto.com/map/4d4c56c1-f82d-4409-b190-ea9ced309005> under a CC BY license, with permission from Carto Builder user Lilishia Gounder, original copyright 2024.

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especially important in resource-limited HIV-hyperendemic settings where strategic data-driven information, or epidemic intelligence can help appropriate deployment of scarce resources for maximal impact in epidemic control. We have shown that spatiotemporal mapping can be used to visually monitor VLs during a change in regimen. Furthermore, we have shown that spatiotemporal mapping is important in monitoring trends of viral suppression as well as identifying hotspots and geographical groups that require intensification of HIV services.

In our study, the majority of VLs were from outpatients attending public sector facilities that provide primary or level 1 healthcare. These findings support the WHO strategy that aims to strengthen the interface between primary healthcare and HIV services [28], highlighting the importance of HIV VL monitoring within primary healthcare settings. Infrequent monitoring of VLs, results in delayed detection of ART failure and intervention, with resultant clinical deterioration and increased risk of HIV transmission [11]. Our study showed heterogeneity in the distribution of detectable VLs by level of urbanization across the 11 districts of KZN. At a

provincial level, we noted an overall downward trend in the proportion of VLs ≥ 1000 copies/mL between 2018 and 2022, which is in keeping with the steady downward trend in the proportion of VLs ≥ 1000 copies/mL observed at a national level [29].

We also observed an inverse upward trend in the proportion of low-level viraemia from 2% in 2018 to 4% in 2022, as more people who previously had high-level viraemia start to achieve viral suppression on more potent DTG regimens. An upward trend of low-level viraemia was also noted in Uganda, from 2% in 2016 to 8.6% in 2020 [30], amid first-line DTG rollout in 2018. The 2022 Ugandan HIV prevention and treatment guidelines now promote active monitoring of patients for low-level viraemia using non-suppression cut-offs of >200 copies/mL or >400 copies/mL for plasma and DBS, respectively [31]. In the South African setting, a previous study showed that low-level viraemia of 400–999 copies/mL was associated with a fivefold increased risk of virological failure [32], while another study suggested that patients with DTG resistance could present with delayed VL suppression or low-level viraemia [33]. Thus, to ensure the ongoing durability of DTG use in our setting, it would be pragmatic to actively monitor low-level viraemia in PLWH.

Using interpolation methods to geospatially map median VLs per facility location, we found that facilities in the rural northern districts of KZN as well as the peri-urban and urban coastal districts of KZN maintained relatively higher HIV VLs. Getis-Ord G_i^* hot- and cold-spot analysis showed spatial clustering of high median $\log_{10}$ HIV VLs i.e., hotspot facilities in the northern KZN districts of Amajuba, Zululand and uMkhanyakude for at least two consecutive years amid transition to DTG. Northern KZN has a high burden of HIV and/or sexually transmitted infections [3]. Additionally, the northern districts that share borders with neighbouring countries, have exposure to cross-border transport or truck routes and historically, migrants and mobile populations are at higher risk of HIV acquisition [34]. Furthermore, these populations generally come to know their HIV status long after they are infected, inadvertently play a significant role in the transmission of HIV [35–37], experience challenges with linking to or remaining in HIV treatment programmes and as a result, may develop viral non-suppression [37]. This is further supported by the decrease in viral suppression that we observed in Northern KZN (S1 Fig), highlighting the need to strengthen VL monitoring in this region.

The districts along the coastal belt that had emergent hotspot facilities in 2021 and 2022 were iLembe and Ugu, respectively, while the metropolitan eThekweni district which has access to the main Durban trade port, had emergent hotspot facilities from 2020 to 2022. These districts are known to have a high triple burden of HIV, tuberculosis and sexually transmitted infections [3], together with the neighbouring inland area of uMgungundlovu which demonstrated re-emerging hotspot facilities during 4 of the 5 years (Fig 6). King Cetshwayo, which has access to the Richard's Bay trade port, had spatial clustering of high median $\log_{10}$ HIV VLs from 2018 to 2022, specifically within peri-urban and urban subdistricts. Despite substantial progress in HIV testing and treatment coverage in King Cetshwayo district, previous studies have shown that a 'core-group' of individuals with unsuppressed VLs in this district are less likely to get tested for HIV and initiate ART [38–40]. Moreover, trade ports have been shown to be spaces of HIV vulnerability in southern Africa [41].

Emerging hotspots are of much concern given the positive impact DTG is expected to have on reducing population HIV VLs, due to its high genetic barrier to resistance [42, 43]. However, the emerging hotspots observed between 2020 and 2022 could be attributed to temporary disruptions caused by the COVID-19 pandemic in HIV treatment services, largely leading to ART interruptions and lack of treatment adherence [44]. In addition, HIV differentiated service delivery could play a critical role in supporting uninterrupted ART and reducing avoidable contact with healthcare facilities during pandemics such as that experienced with

COVID-19 [45]. However, before deploying interventions, there is a need to evaluate the current systems in place and establish sustainable strategies for improving or developing these systems. Several studies have indicated an urgent need to develop systems that can collect locally relevant data in a standardized manner, to create geospatial models that can be accurately applied to the HIV epidemic [46–48].

Geospatial and temporal mapping are an essential part of strengthening health systems, and may be particularly useful in resource-limited settings [19]. Evidence supports the application of geospatial analysis to the field of public health and the HIV epidemic [18, 49–52]. A multinational study in sub-Saharan Africa demonstrated the benefits of mapping HIV clusters as a strategy for identifying populations at high risk of HIV infection, while another study used the technology to identify correlations between migration patterns and risk of HIV acquisition [51, 52]. Other studies showed that GIS could be applied to modelling the usage and accessibility of healthcare facilities [18] as well as understanding the overlap between infectious and non-infectious diseases to provide multi-disease care targeted to specific populations in rural South Africa [53]. Although our study is a retrospective analysis, our database and geospatial methods could be explored further to build a system to geospatially monitor HIV VLs in real time, to inform health system interventions.

Our findings using geospatial and temporal analysis should be interpreted with consideration of the following limitations. This study is based on facilities, not on households, and it is presumed that individuals would visit the healthcare facility closest to their place of residence, which is not always the case. It has been shown, however, that two-thirds of South Africans live less than 2 km away from their nearest public clinic [54]. HIV VL testing practices may differ by facility or district, which could influence the results of this study. Also, a study conducted in one rural district in KZN suggested that community VL derived from population-based surveillance did not necessarily correlate with routine facility-based VL measurements [9]. We created a database of de-identified laboratory test data, not individual patient data, hence there was no accurate unique patient identifier to link patient data serially. The data represents only patients who had a VL test and may therefore underestimate VLs in this population, as those lost to follow-up or not adhering to their follow-up visit schedule may have a higher proportion of detectable VLs. Due to varying lower limits of detection among the different VL analysers used, VL specimens <400 copies/mL were excluded from the analyses where median log₁₀ values were required. Nevertheless, we used ratios of suppressed to detectable VLs to visualize changes in VL suppression rates over time.

Furthermore, demographic data was limited to laboratory-captured information and ART history was not captured. Although our results reveal a higher proportion of detectable HIV VL records from female patients, it is possible that this could be a bias of the demographics of the population accessing HIV care, and not necessarily an indication that more women are vir-aemic on ART. This in part could be associated with poor retention in HIV care among males as reported by the UNAIDS and as shown by studies from other African countries, including South Africa [55, 56]. Lastly, there is a need to prospectively assess longitudinal geospatial trends of VLs to assess whether the hotspots of VLs observed were the impact of the pandemic that settles over time as the health system recovers from the impact of COVID-19 pandemic, or whether this is a concerning trend that becomes sustained or worse over time.

Conclusions

In conclusion, the application of geospatial analysis and visualization of the HIV epidemic by mapping routinely collected programmatic VL data, highlights potential weaknesses in health-care facilities across KwaZulu-Natal province, South Africa. Identifying facilities with high

proportions of people with low- and high-level viraemia and loci of at-risk populations provides invaluable data to both clinicians and policymakers, emphasizing the need for ongoing HIV VL monitoring coupled with HIV drug resistance surveillance. Such geospatial analyses allow for geographically targeted interventions in the HIV treatment programme, including directed improvement of health services and training of health professionals at specific facilities within identified hotspots in KZN, South Africa. Therefore, despite the rollout of DTG, there is a need for targeted deployment of public health resources in HIV hyperendemic regions, with the goal of enhancing HIV viral re-suppression in PLWH and at a public health level, preventing further HIV transmission and HIV-related deaths.

Supporting information

S1 Fig. Ratio of detectable to suppressed HIV viral loads across subdistricts in KwaZulu-Natal province, South Africa. The ratio of detectable (≥ 400 copies/mL) to suppressed (< 400 copies/mL) HIV viral loads were mapped per facility using the inverse distance weighted interpolation method. Spectral colour change from blue to red reflects the increase in ratio of detectable to suppressed HIV viral loads. A ratio of 0.05 indicates 1 detectable viral load per 20 suppressed viral loads, while a ratio of 0.5 indicates 1 detectable viral load per 2 suppressed viral loads. Thick black outlines represent the borders of the 11 districts. Thin outlines represent the borders of the 44 subdistricts of KwaZulu-Natal. Republished from <https://pineappleapp.carto.com/map/4d4c56c1-f82d-4409-b190-ea9ced309005> under a CC BY license, with permission from Carto Builder user Lilishia Gounder, original copyright 2024.

(TIF)

S2 Fig. Violin plot of HIV viral loads in KwaZulu-Natal province, South Africa, for the period 2018 to 2022. mL, millilitre; VL, viral load. The violin plot for $\log_{10}$ viral loads ≥ 400 copies/millilitre sampled across KwaZulu-Natal subdistricts showed a significant downward trend by year, $p < 0.01$ Jonckheere–Terpstra test.

(TIF)

S3 Fig. Linear prediction of median $\log_{10}$ HIV viral loads per facility in KwaZulu-Natal province, South Africa, for the period 2018 to 2022. VL, viral load. The linear prediction model for $\log_{10}$ viral loads ≥ 400 copies/millilitre sampled across KwaZulu-Natal subdistricts showed a significant downward trend by year, $p < 0.01$ Jonckheere–Terpstra test.

(TIF)

S4 Fig. Decreasing HIV viral loads across subdistricts in KwaZulu-Natal province, South Africa, between 2018 and 2022. Choropleth maps show median $\log_{10}$ viral loads ≥ 400 copies/millilitre sampled across KwaZulu-Natal subdistricts. Thick black outlines represent the borders of the 11 districts. Thin outlines represent the borders of the 44 subdistricts of KwaZulu-Natal. Republished from <https://pineappleapp.carto.com/map/4d4c56c1-f82d-4409-b190-ea9ced309005> under a CC BY license, with permission from Carto Builder user Lilishia Gounder, original copyright 2024.

(TIF)

S5 Fig. HIV viral loads per facility before and during the transition to dolutegravir in KwaZulu-Natal province, South Africa. Inverse distance weighted interpolation maps showing changes in median $\log_{10}$ viral loads at the facility level. Map A represents the period before dolutegravir rollout i.e., 2018–2019 and map B represents the transition period to dolutegravir i.e., 2020–2022. Spectral colour change from red to blue reflects a decrease in median $\log_{10}$ viral loads. Republished from <https://pineappleapp.carto.com/map/4d4c56c1-f82d-4409-b190-ea9ced309005> under a CC BY license, with permission from Carto Builder user Lilishia Gounder, original copyright 2024.

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S6 Fig. Moran scatterplots of median $\log_{10}$ HIV viral loads per facility in KwaZulu-Natal province, South Africa, between 2018 and 2022. For cluster analysis, we used the median $\log_{10}$ HIV viral load per facility to calculate Moran's I statistic and p-values, and created Moran scatterplots for each year of the study period. Each plot represents an individual year: (A) 2018, (B) 2019, (C) 2020, (D) 2021 and (E) 2022. Moran's I statistic measures spatial autocorrelation and index values for Moran's I statistic range from -1 to +1. A positive index value indicates positive spatial autocorrelation.
(TIF)

S1 Table. Proportion of all HIV viral loads by year and district. mL, millilitre; VL, viral load. The proportion of VL records collected per facility is cumulatively represented as a proportion per district and grouped according to the district in which that facility is located.
(DOCX)

S2 Table. Proportion of HIV viral loads by district and year, from hotspot facilities demonstrating spatial clustering of high median $\log_{10}$ HIV viral loads. VL, viral load. ^a 244 unique hotspot facilities demonstrating spatial clustering of high median $\log_{10}$ HIV VLs were identified from 2018 to 2022 cumulatively. However, some facilities were repeatedly identified as hotspot facilities and were therefore included in the different analyses for each of those specific years. ^b Each unique viral load record was included in the analysis based on the status of the facility and the specific year in which it was collected. If facilities were identified as statistically significant hotspots based on $p < 0.05$ Getis-Ord G_i^* statistic, the viral load records linked to that facility and year were included in the respective analysis. ^c Hotspot facilities were identified in 10 of the 11 districts. Facilities from uThukela did not have spatial clustering of high median $\log_{10}$ HIV VLs during the study period. The proportion of VL records per hotspot facility is cumulatively represented as a proportion per district, grouped according to the district in which that facility is located.
(DOCX)

S3 Table. Comparison of facilities within hot- and coldspots from 2018 to 2022. IQR, interquartile range; mL, millilitre; VL, viral load. ^a Each unique viral load record was included in the analysis based on the status of the facility and the specific year in which it was collected. If facilities were identified as statistically significant hotspots or coldspots based on $p < 0.05$ Getis-Ord G_i^* statistic, the viral load records linked to that facility and year were included in the respective analysis. ^b Records from correctional facilities or frail care facilities. ^c Obtained from annual reports and integrated development plans for individual subdistricts.
(DOCX)

S1 Data. Minimal dataset.
(XLSX)

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2.1. Bridging Text

The manuscript in chapter 2 described the trends in HIV-1 VLs in KwaZulu-Natal from 2018 to 2022, amid the programmatic transition to DTG-based first-line ART. The results showed an overall decrease in elevated VLs (≥ 1000 copies/mL) over time, which was however accompanied with an increase in low-level viraemia (VLs ranging from 400 to 999 copies/mL). The chapter also highlights the use and role of geospatial population level monitoring of VLs. Notably, certain coastal and northern parts of KZN exhibited consistently elevated VLs and emerging hotspots, highlighting regions needing intensified HIV-1 care. Considering that detectable VLs are of significant concern in the development of HIVDR, we assessed patterns of HIV-1 drug resistance observed through geospatial analysis of routine diagnostic testing in KwaZulu-Natal, South Africa in chapter 3, and characterized HIV-1 drug resistance in ART-experienced children and adolescents through geospatial analysis of routine genotypic tests in KwaZulu-Natal, South Africa in chapter 4. The following chapter presents a study that was conducted to assess levels of HIVDR in adult ART-experienced patients in KZN, South Africa. The chapter is based on a manuscript that was published in the *Viruses Journal* in 2024. The manuscript supplementary materials are provided in Appendix 3.

Chapter 3: Patterns of HIV-1 drug resistance observed through geospatial analysis of routine diagnostic testing in KwaZulu-Natal, South Africa

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Article

Patterns of HIV-1 Drug Resistance Observed Through Geospatial Analysis of Routine Diagnostic Testing in KwaZulu-Natal, South Africa

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Abstract: HIV-1 drug resistance (HIVDR) impedes treatment and control of HIV-1, especially in high-prevalence settings such as KwaZulu-Natal (KZN) province, South Africa. This study merged routine HIV-1 genotypic resistance test (GRT) data with Geographic Information Systems coordinates to assess patterns and geographic distribution of HIVDR in KZN, among ART-experienced adults with virological failure. We curated 3133 GRT records generated between 1 January 2018 and 30 June 2022, which includes the early phase of dolutegravir (DTG) rollout, of which 2735 (87.30%) had HIVDR. Of the 2735, major protease, nucleoside, and non-nucleoside reverse transcriptase inhibitor mutations were detected in 41.24%, 84.97% and 88.08% of GRTs, respectively. Additional genotyping of HIV-1 *integrase* for 41/3133 (1.31%) GRTs showed that 17/41 (41.46%) had integrase strand transfer inhibitor resistance. Notably, of 26 patients on DTG with *integrase* genotyping, 9 (34.62%) had DTG-associated resistance mutations. Dual- or triple-class resistance was observed in four of every five GRTs. The odds of HIVDR increased significantly with age, with ≥60 years having 5 times higher odds of HIVDR compared to 18–29 years ($p = 0.001$). We identified geospatial differences in the burden of HIVDR, providing proof of concept that this could be used for data-driven public health decision making. Ongoing real-time HIVDR surveillance is essential for evaluating the outcomes of the updated South African HIV treatment programme.

Keywords: HIV-1; drug resistance; KwaZulu-Natal; South Africa; geospatial analysis; dolutegravir; genotypic susceptibility scores

1. Introduction

The HIV-1 epidemic in sub-Saharan Africa is associated with a heterogeneous geographical distribution, and specific “hotspots” have gained prominence for higher HIV-1 prevalence [1]. In South Africa, where HIV-1 subtype C is predominant, the province of KwaZulu-Natal (KZN) has the highest prevalence of HIV-1 and is often regarded as the epicentre of the epidemic [2,3]. In efforts to curb the epidemic globally, HIV-1 treatment programmes have adopted the UNAIDS 95-95-95 strategy, the third 95 being 95% of people living with HIV (PLWH) on antiretroviral therapy (ART) achieving viral suppression [4]. A major obstacle to the control of HIV-1 is the development and transmission of HIV-1 drug resistance (HIVDR), with an evident increase in levels of non-nucleoside reverse transcriptase inhibitor (NNRTI) pre-treatment drug resistance (PDR) locally and globally [5–10]. In response, South Africa adopted new HIV treatment guidelines, which included a fixed-dose combination containing dolutegravir (DTG), a potent integrase strand transfer inhibitor (INSTI) with a high genetic barrier to resistance, as the preferred first-line regimen for PLWH initiating ART [11,12].

Coupled with the transition to DTG is the effective monitoring of HIVDR and treatment outcomes [13], and the ADVANCE study highlighted this by demonstrating the impact of PDR on the efficacy of first-line regimens containing DTG [14,15]. HIV viral load (VL) testing remains standard of care for monitoring patients on ART. When two or more VLs are ≥ 1000 copies/millilitre (mL) taken ≥ 2 years after starting a drug regimen containing DTG or a protease inhibitor (PI), with adherence $>80\%$, the patient is considered to have virological failure [11]. In South Africa, as per clinical guidelines, HIVDR testing is reserved primarily for patients failing second- or third-line ART prior to switching HIV-1 treatment regimens. However, HIVDR testing for patients failing a first-line DTG-based regimen may be authorized by an expert (i.e., clinical virologist, third-line committee member, helpline consultant, or nominated provincial expert) on a case-by-case basis [11,16].

Within KZN, high HIV incidence rates persist in specific areas, reflecting the need for individualized interventions that are specific to the local epidemiology [5]. Previous work from our group using spatiotemporal mapping of HIV VLs across KZN province from 2018 to 2022, showed persistently higher VLs in northern and coastal regions of the province, despite an overall viral suppression rate of 86% [17]. Moreover, with the continuing evolution of drug resistance even after years on successful ART [18], it is imperative to have a high index of suspicion for HIVDR in older PLWH [19–21]. The problem of drug resistance has been described in our setting for other infectious diseases such as tuberculosis (TB), with several studies highlighting the spatial distribution of rifampicin-resistant TB within various regions of South Africa [22–25]. However, there is a paucity of scientific evidence for monitoring HIVDR through geospatial analysis in hyper-endemic areas, such as South Africa. To address the gaps in monitoring and analysis of HIVDR in KZN, we combined routine HIVDR genotypic data with Geographic Information Systems (GIS) coordinates to assess the geographic distribution and provide “real-world” estimates of patterns of HIVDR in KZN, South Africa.

2. Materials and Methods

2.1. Study Population and Design

This study was a retrospective analysis of routinely processed HIV genotypic resistance test (GRT) data acquired from the Central Data Warehouse (CDW) within the South African National Health Laboratory Service (NHLS). The NHLS is the biggest provider of diagnostic laboratory services in South Africa, and it delivers efficient and economical services to all healthcare facilities within the public sector, catering for nearly 80% of the overall

population [26]. Patient demographic data and linked laboratory test data are stored at the NHLS CDW and are essential for monitoring Department of Health programmes at the national and provincial level [27].

We obtained de-identified GRT data for PLWH ≥ 18 years of age who had attended a healthcare facility within the public sector in KZN province, South Africa (Figure 1). We did not include children (< 18 years of age) because paediatric ART regimens differ from adult ART regimens [28] and would require a separate statistical and geospatial analysis. The study timeframe of 1 January 2018 to 30 June 2022 represented a period during the rollout and transition to DTG in first-line ART regimens in South Africa and paralleled our previous study on geospatial mapping of HIV VLs in KZN province [17]. DTG was rolled out as first-line ART in South Africa in December 2019; however, ART initiations onto TLD (Tenofovir disoproxil fumarate-Lamivudine-Dolutegravir) were slow, due to the lockdowns experienced during the COVID-19 pandemic [29]. All GRT requests and specimens were received at the Department of Virology, which is located within the NHLS laboratory division of the Inkosi Albert Luthuli Central Hospital in Durban, KZN, South Africa. The Department of Virology is the sentinel testing site for HIV GRTs in KZN province. GRT requests were clinically indicated in patients who were on PI- and/or INSTI-based ART according to the standard of care in South Africa, with VLs ≥ 1000 HIV-1 RNA copies/mL of plasma.

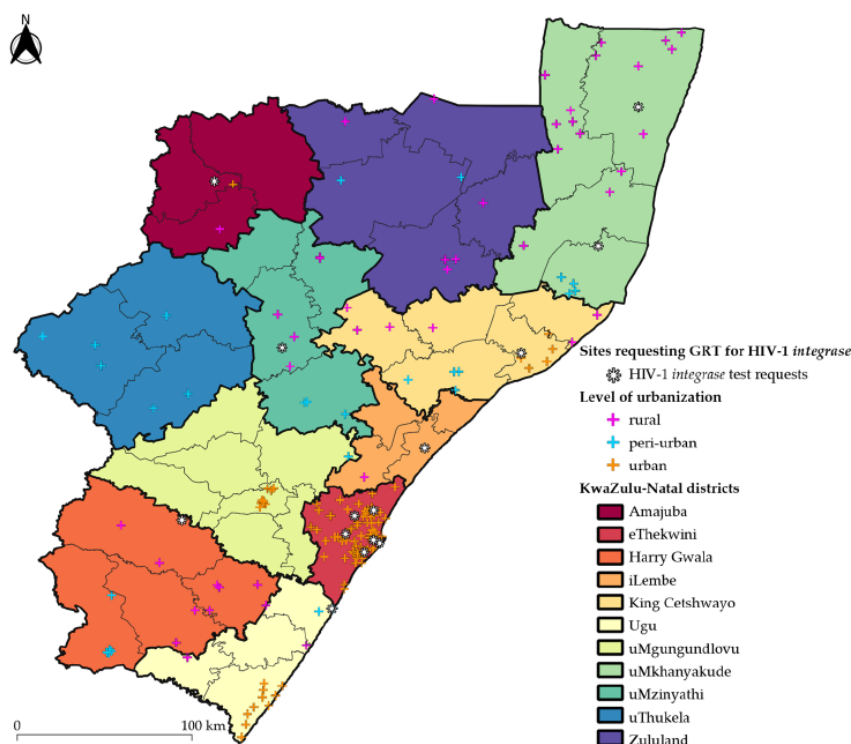


Figure 1. Public-sector healthcare facilities with HIV-1 genotypic resistance tests included by district in KwaZulu-Natal province, South Africa. Individual cross symbols pinpoint the Geographic Information Systems (GIS) coordinates of each healthcare facility that had requested a genotypic resistance test (GRT), the data of which had been included in the study. The asterisk denotes healthcare facilities that requested HIV-1 *integrase* testing. The cross symbols are coloured in pink, blue or orange based on whether the healthcare facility's GIS coordinates are within a rural, peri-urban or urban subdistrict, respectively. The thin and thick black outlines represent the borders of the subdistricts and districts, respectively. Each district is illustrated in a different colour. The basemap of KwaZulu-Natal province was republished under a CC BY license with permission obtained from Carto Builder user Lilishia Gounder, original copyright 2024. Available at: <https://pinea.app.carto.com/map/4d4c56c1-f82d-4409-b190-ea9ced309005> (accessed on 18 October 2024).

2.2. Routine Laboratory Procedures for HIV Genotyping

We extracted viral RNA from 1mL of patient plasma using a NucliSENS EasyMag (bioMérieux, Marcy l'Etoile, France) extraction system, according to the manufacturer's instructions. We performed reverse transcription and nested polymerase chain reaction (PCR) amplification of the *protease* (PR) and *reverse transcriptase* (RT) genes using the Applied Biosystems HIV-1 Genotyping Kit (ThermoFisher Scientific, Waltham, MA, USA). We reversed transcribed and amplified the *integrase* (IN) gene using Superscript III one-step RT-PCR system with HiFi Platinum Taq (ThermoFisher Scientific, Waltham, MA, USA) and conducted nested PCR using the Expand High Fidelity Plus PCR System (Merck Life Sciences, Rockville, MD, USA). We verified the successful amplification of an approximately 1050 base pair (bp) fragment for PR and RT genes, and a 1200 bp fragment for the IN gene and viewed the application products on 1% agarose gel.

We performed Sanger sequencing reaction preparations using Big-Dye XTerminator v3.1 kit (Applied Biosystems, Foster City, CA, USA) for PR, RT and IN genes, according to the manufacturer's instructions. We sequenced samples on an ABI 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using PR and RT sequencing primers from Applied Biosystems HIV-1 Genotyping Kit (ThermoFisher Scientific, Waltham, MA, USA), and in-house IN sequencing primers described previously [30,31]. We assessed sequence quality and generated consensus sequence alignments using Geneious Prime software 2021.1.1 (Biomatters Ltd., Auckland, New Zealand). We detected HIVDR mutations and determined antiretroviral (ARV) drug susceptibilities using the Stanford University HIV Drug Resistance Database v9.0. [32].

We determined PI drug susceptibilities for ritonavir-boosted atazanavir (ATV/r), darunavir (DRV/r), and lopinavir (LPV/r). Drug susceptibilities for nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), NNRTIs and INSTIs included lamivudine (3TC), abacavir (ABC), zidovudine (AZT), emtricitabine (FTC), tenofovir disoproxil fumarate (TDF), efavirenz (EFV), etravirine (ETR), nevirapine (NVP), raltegravir (RAL), and DTG. We defined HIVDR as any GRTs with the following ARV drug susceptibilities: potential low-level resistance (PLLR), low-level resistance (LLR), intermediate resistance (IR), or high-level resistance (HLR) to any PI, NRTI, NNRTI or INSTI available as per standard of care in South Africa.

2.3. Interactive HIV-1 Drug Resistance Database

We created an interactive database using HIVDR reports from PLWH ≥ 18 years of age with HIV GRT data generated during the period from 1 January 2018 to 30 June 2022, at healthcare facilities within the public sector in KZN province. We categorized GRT records into the following two groups: (i) HIVDR, resulting in reduced susceptibility to at least one ARV drug, and (ii) no HIVDR, resulting in full susceptibility to ARV drugs. We included age, sex, specimen collection date, district of specimen origin, healthcare facility name, healthcare facility type (i.e., outpatient or inpatient department), healthcare facility level of urbanization, HIVDR mutations detected, ARV drug susceptibility, current ARV drug regimen, and most recent HIV VL, as meta-data for each GRT database record. We categorized age into 3 groups: (i) young adults (18–29 years), (ii) adults (30–59 years), and (iii) elderly (≥ 60 years). We excluded GRT records where the patient's age was < 18 years or the patient's age was not included. Based on the healthcare facility location, we assigned global positioning system (GPS) coordinates to each GRT record.

We further assigned genotypic susceptibility scores (GSSs) to GRT records with complete regimen data. For each drug prescribed, a GSS value of 1 was assigned if no resistance or PLLR was determined, while 0.5 was assigned if resistance was LLR or IR, and 0 was assigned if HLR was determined. The sum of all the individual drug scores provided the total GSS for that regimen. Patients who had complete drug regimen data were grouped based on the number of active drugs prescribed: < 2 ; 2; and > 2 . A value of > 2 indicated an active regimen, whereas values of < 2 indicated drug resistance with reduced potency of the ARV regimen [33].

2.4. Statistical Analysis

We used chi-square tests and logistic regression analysis to assess associations between HIVDR and sex, year of specimen collection, ARV drug regimen, healthcare facility type, level of urbanization, age group, and HIV VLs. We used descriptive statistics to analyze patterns of drug class resistance, genotypic susceptibility scores and mutations, presented as proportions/percentages in graphs and tables. We performed all statistical analyses using Stata 18.0 software SE (StataCorp. 2023, College Station, TX, USA).

2.5. Geospatial Mapping of HIV Genotypic Resistance Test Records

Using healthcare facility-linked GPS coordinates from the curated HIV GRT database, we generated geospatial maps in QGIS 3.30 software [34], with each GRT record assigned to the healthcare facility of specimen collection. We created inverse distance weighted (IDW) interpolation maps to visualize the ARV drug susceptibility levels per healthcare facility. With the IDW interpolation method, unsampled areas closest to the healthcare facility show values comparable to the value measured at the healthcare facility location.

3. Results

We curated 3133 GRT records from 179 public-sector healthcare facilities across the 11 districts of KZN province (Supplementary Figure S1 and Table S1), of which 2735 (87.30%) had HIVDR. Additionally, HIV-1 *integrase* genotyping was requested in 41/3133 (1.31%) GRT records. Only 2 out of the 41 GRTs with *integrase* genotyping were fully susceptible to all ARV drugs. About 9 in every 10 GRTs were from patients on PI-based ART (2830/3133) with either LPV/r or ATV/r. Figure 2 shows a summary of GRT records that were obtained and analysed.

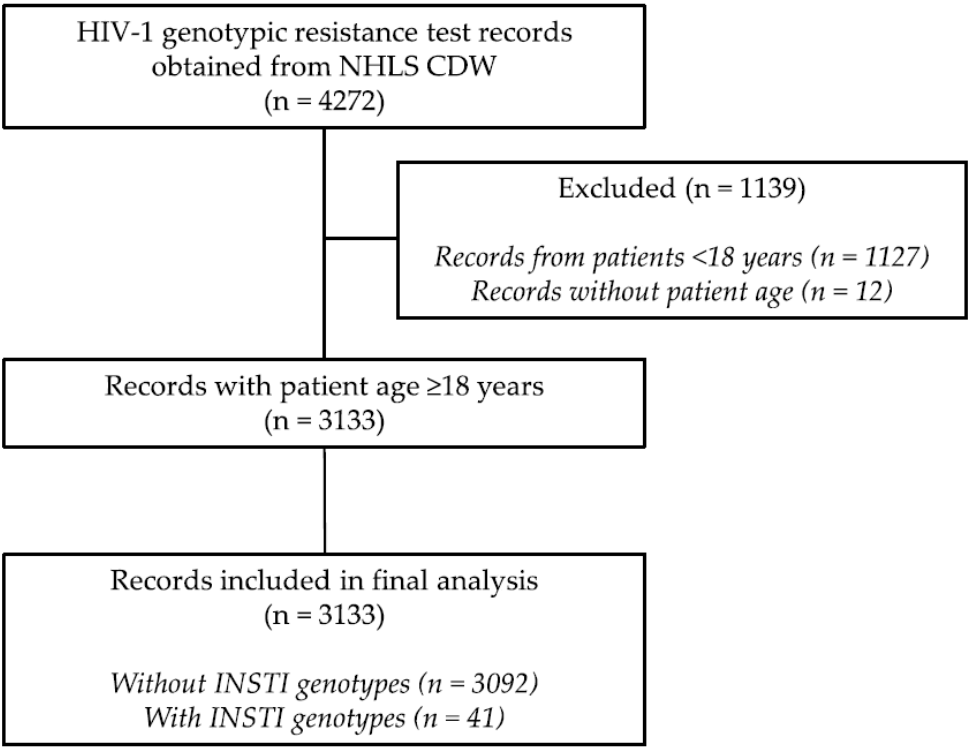


Figure 2. Flow diagram of HIV-1 genotypic resistance test records obtained and included in the final analysis. CDW, central data warehouse; INSTI, integrase strand transfer inhibitor; NHLS, National Health Laboratory Service.

More than two thirds (2202/3133; 70.28%) of GRTs were from healthcare facilities within urban subdistricts, with nearly similar proportions from peri-urban (480/3133; 15.32%) and rural (451/3133; 14.40%) subdistricts (Table 1).

Table 1. Characteristics of HIV-1 genotypic resistance test records included in this study.

Variable	All N = 3133 (100.00%)	No HIVDR N = 398 (12.70%)	HIVDR N = 2735 (87.30%)
Sex			
Male	1126 (35.94%)	130 (11.55%)	996 (88.45%)
Female	1982 (63.26%)	266 (13.42%)	1716 (86.58%)
Unknown	25 (0.80%)	2 (8.00%)	23 (92.00%)
Age in years, median (IQR)	39 (30–46)	36 (22–43)	39 (32–46)
18–29	750 (23.94%)	156 (20.80%)	594 (79.20%)
30–59	2284 (72.90%)	237 (10.38%)	2047 (89.62%)
≥60	99 (3.16%)	5 (5.05%)	94 (94.95%)
Collection year			
2018	597 (19.06%)	75 (12.56%)	522 (87.44%)
2019	675 (21.54%)	78 (11.56%)	597 (88.44%)
2020	763 (24.35%)	115 (15.07%)	648 (84.93%)
2021	666 (21.26%)	72 (10.81%)	594 (89.19%)
2022 ^a	432 (13.79%)	58 (13.43%)	374 (86.57%)
ARV drug regimen			
LPV/r or ATV/r -based	2830 (90.33%)	362 (12.79%)	2468 (87.21%)
DRV/r-based ^b	30 (0.96%)	1 (3.33%)	29 (96.67%)
DRV/r-based with RAL	9 (0.29%)	0 (0.00%)	9 (100%)
DRV/r-based with DTG	10 (0.32%)	1 (10.00%)	9 (90.00%)
RAL-based ^c	5 (0.16%)	1 (20.00%)	4 (80.00%)
DTG-based ^c	36 (1.15%)	7 (19.44%)	29 (80.56%)
NNRTI-based ^d	36 (1.15%)	5 (13.89%)	31 (86.11%)
Unknown	177 (5.65%)	21 (11.86%)	156 (88.14%)
HIV VL in log ₁₀ copies/mL, median (IQR) ^e	4.71 (4.10–5.28)	4.99 (4.33–5.45)	4.66 (4.07–5.25)
Healthcare facility type			
Outpatients	2950 (94.16%)	373 (12.64%)	2577 (87.36%)
Inpatients	183 (5.84%)	25 (13.66%)	158 (86.34%)
Level of urbanization ^f			
Rural subdistricts	451 (14.40%)	44 (9.76%)	407 (90.24%)
Peri-urban subdistricts	480 (15.32%)	55 (11.46%)	425 (88.54%)
Urban subdistricts	2202 (70.28%)	299 (13.58%)	1903 (86.42%)

ARV, antiretroviral; ATV/r, ritonavir-boosted atazanavir; copies/mL, copies/millilitre; DRV/r, ritonavir-boosted darunavir; DTG, dolutegravir; EFV, efavirenz; HIVDR, human immunodeficiency virus drug resistance; INSTI, integrase strand transfer inhibitor; IQR, interquartile range; LPV/r, ritonavir-boosted lopinavir; NNRTI, non-nucleoside reverse transcriptase inhibitor; RAL, raltegravir; VL, viral load. ^a Data only include period from 1 January 2022 to 30 June 2022. ^b Etravirine included in 4 regimens containing ritonavir-boosted darunavir. ^c Regimen did not include a protease inhibitor. ^d Etravirine included in 1 NNRTI-based regimen. ^e Most recent HIV VL missing for 104 records. ^f Categories obtained from integrated development plans and annual reports for individual subdistricts in KwaZulu-Natal.

Detection of HIVDR was 90.24%, 86.42% and 88.54% in facilities within rural, urban and peri-urban subdistricts, respectively. The GRTs that included HIV-1 *integrase* genotyping were predominantly (33/41; 80.49%) from healthcare facilities within urban subdistricts. There was a higher proportion of GRTs from females (1982/3133; 63.26%) as compared to males (1126/3133; 35.94%). Relative to those in the age group 18–29 years, the odds of HIVDR were more than 2 times higher among those aged 30–59 years (odds ratio (OR) = 2.27, 95% confidence interval (CI): 1.82–2.83, $p < 0.001$), and nearly 5 times higher among those aged ≥60 years (OR = 4.94, 95% CI: 1.97–12.35, $p = 0.001$). For every log₁₀ VL increase, there was a 31.28% reduction in the odds of HIVDR detection (OR = 0.687, 95%

CI: 0.60–0.79, $p < 0.001$). The odds of HIVDR detection did not significantly change by year of collection, ARV drug regimen, healthcare facility type or facility level of urbanization ($p > 0.05$) (Table 1).

3.1. Patterns of HIV-1 Drug Resistance

3.1.1. Drug Class Resistance

Of the 2735 (87.30%) with HIVDR detected, 583 (21.32%) had single-class resistance, 1170 (42.78%) had dual-class resistance, 973 (35.58%) had triple-class resistance, and 9 (0.33%) had HIVDR to four-drug classes (Figure 3), of which 3/9 were on DTG-based ART. The predominant pattern of resistance identified in approximately two-thirds of GRTs, was NRTI and NNRTI drug class resistance, either with PI resistance (35.36%) or without PI resistance (37.80%). The frequency of HIVDR mutations by drug class was as follows: 1128 (41.24%) with major PI-mutations, 2324 (84.97%) with NRTI mutations, and 2409 (88.08%) with NNRTI mutations. Only 41 GRTs had HIV-1 *integrase* resistance testing conducted, of which 39 had HIVDR mutations, with 17 (43.59%) having INSTI-specific mutations. Overall, there were no changes in levels of resistance observed over time. The patterns and proportions of drug class resistance observed per year are shown in Figure 3 and Supplementary Table S2.

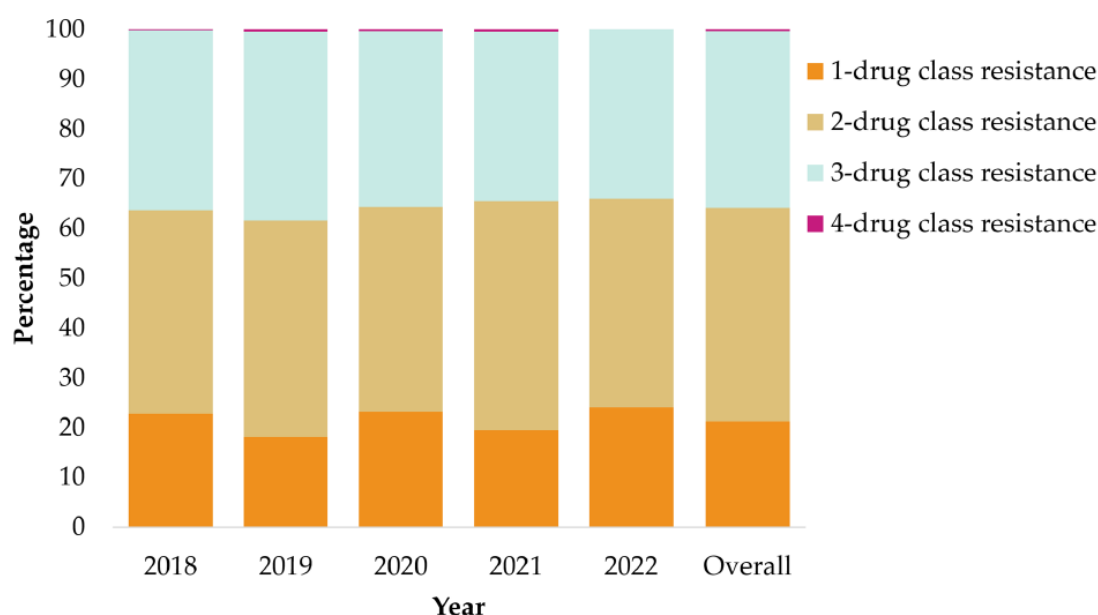


Figure 3. Patterns of antiretroviral drug class resistance observed in 2735 genotypes with HIVDR obtained from KwaZulu-Natal province, South Africa. Please note that 41 genotypes included HIV-1 *integrase* testing, of which only 9 met the definition for 4-drug class resistance.

3.1.2. Mutations Detected in Genotypic Resistance Test Records with HIV-1 Drug Resistance

The most frequently observed mutations by drug class were NRTI mutation M184VI (80.2%), NNRTI mutation K103NS (51.9%), and PI mutation M46IL (28.8%). Of the 39 *integrase* genotypes with HIVDR mutations, E138KAT and N155H (12.8%) were the most common INSTI mutations detected (Figure 4). Approximately half (1161/2324; 49.96%) of all GRTs with NRTI mutations had thymidine analogue mutations (TAMs). Overall, the most common TAMs observed were type-2 TAMs D67N (670/2735), K219QE (642/2735), and K70R (614/2735), and the type-1 TAM M41L (364/2735). The K65R mutation was detected in 6.22% (170/2735) of GRTs and 37.06% (63/170) of those with K65R had received TDF in their current regimen. Of 699 patients on TDF-based regimens, 295 had TAMs. Of those, we had data on prior ARV exposure for 270/295, and 210 of the 270 (77.77%) had documented prior drug exposure to thymidine analogues, i.e., AZT or stavudine.

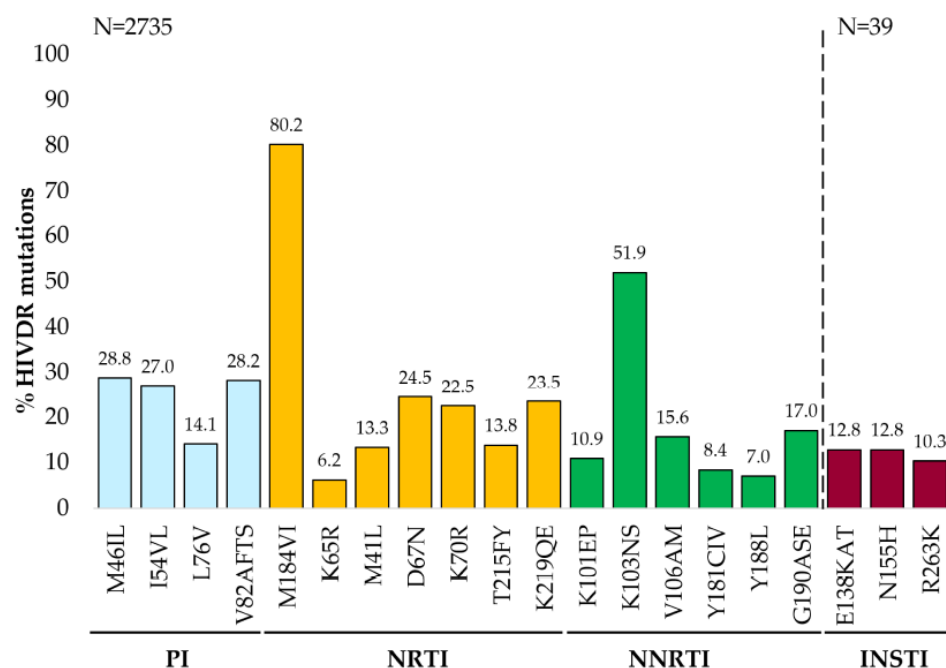


Figure 4. Specific mutations detected in 2735 genotypes with HIVDR obtained from KwaZulu-Natal province, South Africa. Mutations shown on the horizontal axis include “major” mutations observed in >6% of the genotypes with HIV-1 drug resistance, “major” as defined by Stanford HIV Drug Resistance Database or 2022 edition IAS–USA drug resistance mutations list [32,35]. HIVDR, HIV-1 drug resistance; INSTI, integrase strand transfer inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

Despite only 36 (1.15%) GRTs from patients on current NNRTI-based ART, approximately 8 in every 9 (2409/2735) GRTs had NNRTI-associated HIVDR mutations detected. In addition to K103NS, the G190ASE, V106AM, and K101EP mutations were each detected in more than 10% of GRT requests. The proportion of GRTs with intermediate to high-level ETR resistance was 20.99% (574/2735), of which 67.07% (385/574) had ETR-associated mutations, i.e., L100I, K101P, Y181CIV, G190E and M230L. Overall, the mutation E138AGKQ conferred resistance to ETR in 13.67% (374/2735) of HIVDR GRTs.

Of the 1128 GRTs with major PI mutations, 894 (79.26%) had intermediate to high-level resistance to LPV/r or ATV/r, with two-thirds (588/894) also conferring resistance to DRV/r. However, the majority (2182/2735; 79.78%) of HIVDR GRTs showed susceptible to potential low-level resistance to DRV/r. Twenty-six of thirty-nine INSTI GRTs were from patients on DTG, of which 9 (34.62%) had mutations that alone confer DTG resistance, namely, H51Y, G118R, E138KAT, G140A, S147G, Q148R, N155H, and R263K (Supplementary Table S3). Details of mutations detected in all GRTs with HIVDR were categorized by drug class [32,35] and are listed in Supplementary Table S4.

3.1.3. Genotypic Susceptibility Scores

For the purposes of genotypic susceptibility score (GSS) analysis, we included only GRTs that had complete regimen data available (i.e., 2934/3133), regardless of whether they had HIVDR or not. Of the 2934 GRTs, 46 were from patients receiving DTG in their current ART regimen (Table 2). All current regimens included XTC, which is either 3TC or FTC. Patients on EFV-based ART with TDF had the lowest predicted GSSs, with approximately 6 in every 7 having a GSS of <2. Other regimens with lower predicted GSSs included being on a RAL regimen with TDF, a DRV/r regimen with TDF, an ABC regimen with a boosted PI, and an ABC regimen with DTG. Generally, 3-drug regimens with DTG had poor GSSs, with most having a GSS ≤ 2 (Table 2). The highest GSSs (>2) were predicted in regimens

that included at least four drugs, with TDF and AZT, plus DTG and/or DRV/r. Table 2 shows a summary of the regimens that are grouped based on their NRTI drugs, which included TDF-based, AZT-based, TDF plus AZT-based, and ABC-based ART.

Table 2. Genotypic susceptibility scores for antiretroviral drug regimens used in South African public health sector.

Current Regimen *	N = 2934	Genotypic Susceptibility Score for Regimen		
		<2	2	>2
TDF-based	n = 649			
TDF + XTC + EFV	20	17 (85%)	0	3 (15%)
TDF + XTC + boosted ATV/LPV	574	255 (44.42%)	111 (19.34%)	208 (36.24%)
TDF + XTC + boosted DRV	19	12 (63.16%)	4 (21.05%)	3 (15.79%)
TDF + XTC + RAL	4	3 (75%)	0	1 (25%)
TDF + XTC + DTG	19	8 (42.10%)	2 (10.53%)	9 (47.37%)
TDF + XTC + RAL + boosted DRV	6	3 (50%)	0	3 (50%)
TDF + XTC + DTG + boosted DRV	6	1 (16.67%)	2 (33.33%)	3 (50%)
TDF + XTC + DTG + boosted DRV + ETR	1	0	0	1 (100%)
AZT-based	n = 1927			
AZT + XTC + boosted ATV/LPV	1911	761 (39.82%)	663 (34.69%)	487 (25.49%)
AZT + XTC + boosted DRV	4	0	1 (25%)	3 (75%)
AZT + XTC + DTG	10	4 (40%)	2 (20%)	4 (40%)
AZT + XTC + DTG + boosted DRV	1	0	0	1 (100%)
AZT + XTC + DTG + boosted DRV + ETR	1	0	0	1 (100%)
TDF- and AZT-based	n = 45			
AZT + XTC + boosted ATV/LPV + TDF	37	4 (10.81%)	3 (8.11%)	30 (81.08%)
AZT + XTC + boosted DRV + TDF	6	0	0	6 (100%)
AZT + XTC + DTG + TDF	1	0	0	1 (100%)
AZT + XTC + DTG + boosted DRV + TDF	1	0	0	1 (100%)
ABC-based	n = 313			
ABC + XTC + boosted ATV/LPV	307	199 (64.82%)	3 (0.98%)	105 (34.20%)
ABC + XTC + DTG	6	4 (66.67%)	0	2 (33.33%)

ABC, abacavir; ATV, atazanavir; AZT, zidovudine; DRV, darunavir; DTG, dolutegravir; EFV, efavirenz; ETR, etravirine; LPV, lopinavir; RAL, raltegravir; TDF, tenofovir disoproxil fumarate; XTC, lamivudine/emtricitabine. * Genotypic tests were conducted at the time of antiretroviral treatment failure. Integrase strand transfer inhibitors are assumed to have an individual genotypic susceptibility score (GSS) of 1 if HIV-1 *integrase* testing was not already included in the genotypic resistance test record. Boosted refers to ritonavir-boosted regimens. For ATV/LPV, the drug with the lower penalty score in Stanford University HIV Drug Resistance Database was used.

3.2. Geospatial Analysis of HIV-1 Drug Resistance

ARV drug susceptibility levels were mapped by facility location across KZN province to assess the impact on select drugs of interest in South Africa, as shown in Figure 5 and Supplementary Figure S2. Generally, most districts had susceptible to low-level resistance to TDF and ETR, with slightly higher levels of resistance in northern KZN. Low to intermediate levels of LPV/r resistance were also noted in northern KZN, with susceptible to potential low-level resistance to DRV/r, a drug of choice in people failing DTG-based ART (Supplementary Figure S2). All districts showed intermediate to high levels of resistance to EFV, NVP, and XTC, despite most GRTs (93.16%) being obtained from patients on non-NNRTI-based regimens. Again, northern KZN showed the highest burden of EFV, NVP, and XTC resistance across the province (Figure 5, Supplementary Figure S2). The geospatial analysis of DTG susceptibility levels was limited, due to the small number (41/3133) of HIV-1 *integrase* genotypes requested. Low- to high-level DTG resistance was noted in two rural districts, based only on two DTG-resistant genotypes from either district.

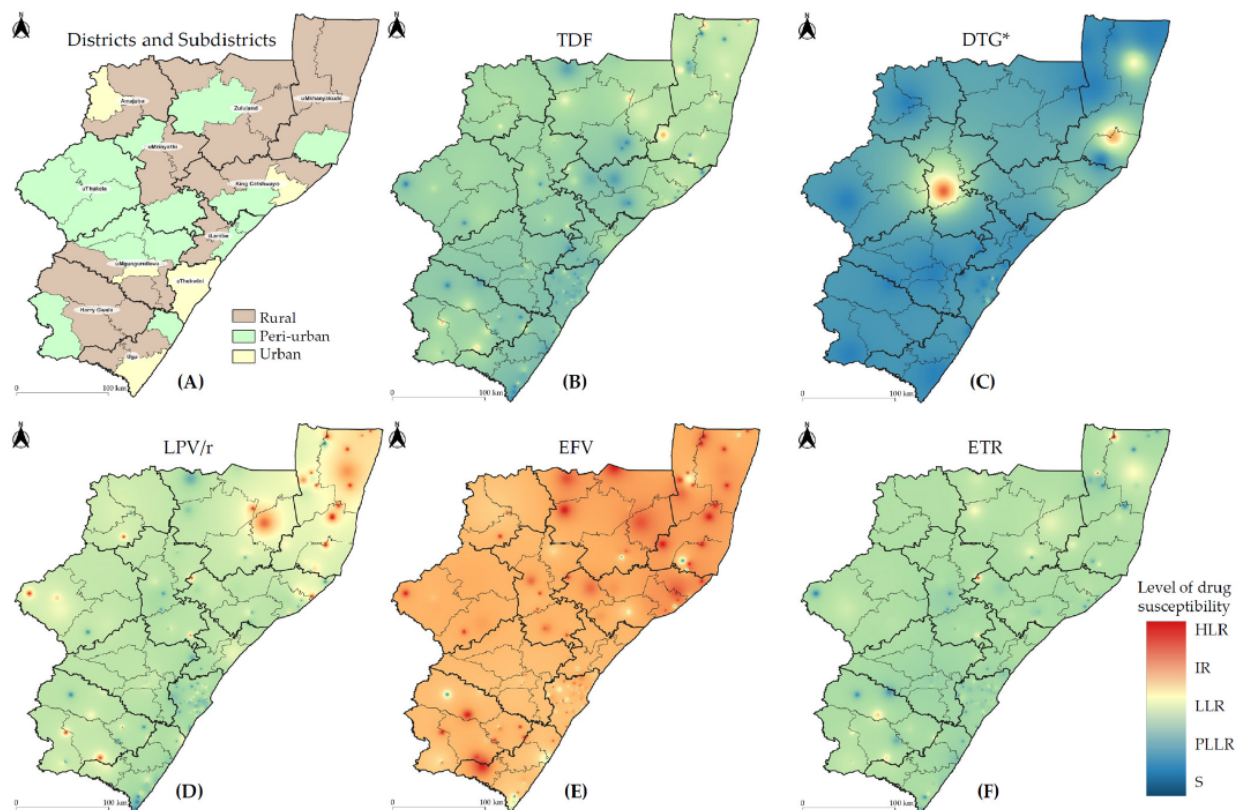


Figure 5. Antiretroviral drug susceptibility levels across KwaZulu-Natal province, South Africa. Districts and subdistricts are categorized as follows: (A) level of urbanization. Inverse distance weighted interpolation maps cumulatively reflect the drug susceptibilities for the following: (B) TDF, tenofovir; (C) DTG, dolutegravir; (D) LPV/r, lopinavir with boosted ritonavir; (E) EFV, efavirenz; (F) ETR, etravirine. Spectral colour change from blue to red reflects the drug susceptibility level as follows: S, susceptible; PLLR, potential low-level resistance; LLR, low-level resistance; IR, intermediate resistance; HLR, high-level resistance. The thin and thick black outlines represent the borders of the 44 subdistricts and 11 districts of KwaZulu-Natal (KZN) province, respectively. The basemap of KZN province was republished under a CC BY license with permission obtained from Carto Builder user Lilishia Gounder, original copyright 2024. Available at: <https://pinea.app.carto.com/map/4d4c56c1-f82d-4409-b190-ea9ced309005> (accessed on 18 October 2024). * Please note that HIV-1 *integrase* testing was performed for 41 genotypes; the remaining 3092 genotypes that did not have *integrase* test requests were assumed to be susceptible to DTG for the purposes of creating the DTG interpolation map.

4. Discussion

In this retrospective analysis of more than 3000 routine HIV genotypic data from KZN province, South Africa, we observed high proportions of HIVDR among ART-experienced adults with virological failure, consistent with previous studies [36,37]. This was mainly driven by *reverse transcriptase* mutations, with approximately 1 in every 3 GRTs having dual-class NRTI and NNRTI resistance. The majority of GRTs (90%) were from patients currently receiving a PI-based regimen, but only 36% (1128/3133) had PI resistance, an attribute of PI's high genetic barrier to resistance [38,39], and suggesting that virological failure on LPV/r-based regimens is driven by poor adherence related to drug tolerability. Of those on DTG-based ART with an INSTI genotype, ~35% had DTG-associated resistance, raising concerns about the durability of DTG in highly treatment-experienced patients. In addition, geospatial analysis showed higher levels of resistance to EFV and other drugs,

particularly in northern rural KZN, a known HIV hyper-endemic region [3,5], highlighting the need for intensified HIV-1 treatment monitoring through regular VL testing, adherence support interventions, ongoing drug resistance surveillance, and strengthening of health systems. This study serves as a proof of concept that geospatial analysis could potentially be used for data-driven public health decision making.

South Africa rolled out DTG in the National HIV treatment programme in December 2019 [11]. Amid this transition, our study found persistently increased levels of NNRTI and NRTI resistance in patients with virological failure, similar to other South African studies [5–7,36,40]. However, in this study, of 26 patients on DTG with an INSTI genotype, ~35% (9/26) had DTG-associated resistance mutations, which is among the highest reported to date. The DTG-RESIST study among viraemic adults on DTG-based ART observed ~5% DTG resistance [41,42]. More cross-sectional studies from low-to-middle-income countries are showing levels of DTG resistance ranging from ~4 to 20% among people with virological failure on DTG-based ART, exceeding levels reported in clinical trials, even among highly treated individuals, as observed by the World Health Organization [42,43]. In this study, the most common mutation observed in the nine patients with DTG-associated resistance was E138KAT (4/9), which alone confers potential low-level resistance to DTG and low-level resistance to cabotegravir (CAB) [32]. Other mutations included G118R (3/9) and R263K (3/9), the most common DTG resistance mutations in INSTI-naïve individuals with virological failure [44], conferring high- and intermediate levels of resistance to DTG, respectively [32]. We also observed the occurrence of multiple INSTI resistance mutations in one individual (T66A, E138K, Y143R, with accessory mutations Q95K, T97A and S147), which together cause low- to high-level resistance to all FDA-approved INSTI drugs [32]. Although our limited geospatial analysis for DTG indicates high viral susceptibility levels across the KZN province, the accumulation of mutations in those with INSTI resistance signify an impending problem and suggests the need for heightened surveillance of DTG-specific mutations to avoid jeopardizing future DTG-based ART, long-acting CAB-based treatment, and prevention strategies.

Approximately 89% of all HIVDR GRTs had NNRTI-associated mutations detected, despite only 1% on current NNRTI-based regimens, showing the persistence of NNRTI mutations. One in every five GRTs had intermediate to high-level resistance to ETR, a second-generation NNRTI, of which 67% had mutations that confer resistance to both first- and second-generation NNRTIs, i.e., L100I, K101P, Y181CIV, G190E and M230L. The NNRTI mutation E138AGKQ is considered a polymorphism in HIV-1 subtype C, but it conferred resistance to ETR in 13.67% (374/2735) of HIVDR GRTs, warranting further evaluation of the impact of *reverse transcriptase* mutations at position 138 on ETR treatment [45,46]. Despite EFV being largely compromised, geospatial analysis in this study shows relatively lower levels of resistance to ETR across KZN, warranting its continued use in future regimens.

The temporal trends of HIVDR were similar, with dual- or triple-class resistance observed in four out of every five patients. The increased risk of virological failure driven by the presence of dual-class resistance has been described previously [47]. Half of all GRTs with NRTI mutations detected had at least one TAM. The proportion of TAMs identified in GRTs from patients on TDF-based regimens with documented exposure to thymidine analogues was 77.77% (210/270), suggestive of accumulation of TAMs during prior ART exposure. By inference, proportions of atypical TAMs were ~22.22%; however, this may not be an accurate reflection since we do not know whether the prior drug exposure data for these patients were complete. Other studies from sub-Saharan Africa reported prevalences ranging from 16 to 41% for atypical TAMs [37,48], and considering their potential to reduce susceptibility to AZT and TDF, there is a need to better understand the occurrence of atypical TAMs in TDF-treated patients. The K65R mutation, which increases viral susceptibility to AZT and reduces viral susceptibility to TDF, was present in over 6% of GRTs, as shown in the geospatial analysis where most regions in KZN show susceptible to low levels of TDF resistance. Cumulatively, 699 GRTs were from patients on a regimen

containing TDF, and of those 43.78% (306/699) had a GSS of <2. Other studies have reported higher proportions of K65R mutations in the South African setting [37,49].

The value of geospatial data visualization techniques in improving understanding of the spatial distribution of HIV and resistance mutation prevalence has been previously described [5]. However, this study used geospatial data visualization at a much more granular level and based on community surveillance. In our study, geospatial analysis of drug susceptibility levels mapped across KZN demonstrated that northern KZN had the highest burden of EFV, NVP, and XTC resistance, albeit high levels of resistance evident in most parts of the province. Low to intermediate levels of LPV/r and DTG resistance were also noted in northern KZN, highlighting a need for targeted programmatic interventions in this area of the province. Although two-thirds of GRTs were from patients who had attended healthcare facilities in urban subdistricts, more HIVDR was detected in rural areas. Rural areas have fewer resources available and less consistent monitoring of HIV as compared to urban settings [50], and other studies in rural settings have shown increasing proportions of HIVDR [51]. Taken together, these data suggest the need for vigilant HIVDR monitoring in patients experiencing virological failure on first-line TDF + XTC + DTG, with timely HIVDR genotyping and switching of ART, among second-line failures.

Although more GRTs were obtained from females, the levels of HIVDR were similar among males and females. The absence of HIVDR and the presence of high VLs in those aged 18–59 years implies non-adherence, as evident in other studies with similar age groups [50,52], and interventions must be prioritized in this population to curb the progression to HIVDR. Other studies have described the relationship between HIVDR and older age, even highlighting the role of elderly PLWH in the transmission of PDR [19–21]. While it is possible that older patients were more treatment-experienced and more likely to be exposed to drug interactions with ART from treatments prescribed for other co-morbidities, these data were not available for inclusion in this analysis.

Our findings should be interpreted with consideration of the following limitations. We did not account for GRT testing practices per facility, nor contextualize our geospatial analysis within the local population of PLWH, nor ART coverage per district. Future research should aim to integrate HIV VL and GRT data, ideally within the context of ART coverage, to provide more detailed insights that inform data-driven public health decision making. The HIVDR database was based on de-identified GRTs, so we could not determine whether a patient had more than one GRT performed, and we could not link serial GRTs, if any. There were missing data for certain database records, because some patient data were not recorded on test request forms completed at sample collection. Previous ARV drug regimen data were not consistently documented, so, where available, we described prior thymidine analogue exposure for patients currently on TDF-based regimens with TAMs. Moreover, duration of ART regimen was not electronically captured; therefore, we could not contextualize the persistence of HIVDR mutations within a specific timeframe. Bearing in mind that <2% of patients were on INSTI-based regimens, appropriate population-level analyses of the impact of specific mutations were limited. If HIV-1 *integrase* testing was not requested and therefore not performed, we presumed the GRT to be susceptible to INSTI drugs. We made this presumption for the purposes of creating the DTG interpolation map, determining the number of drug-class resistance patterns and calculating the GSS scores. It has been shown that DTG resistance is rare in the general population of PLWH, especially among ART-naïve individuals [53]. Our geospatial analysis was based on facility location, not households, based on the presumption that patients sought healthcare services at facilities closest to their homes. The clinic where the GRT was collected might not be the site where the individual receives most HIV care, especially if there is an up-referral to advanced clinical care facilities for patients with virological failure. However, a previous study from South Africa demonstrated that about 67% of individuals live less than 2 kilometres away from their nearest primary healthcare facility [54].

Considering that this study was performed during the transition to DTG-based first-line regimens in South Africa, there is a need to assess the current prevalence of HIVDR

mutations, particularly those associated with DTG resistance, to identify areas in need of directed interventions. In conclusion, our study demonstrated that the trends of HIVDR have remained static among treatment-experienced adults failing ART in KZN. Therefore, ongoing real-time HIVDR surveillance with geospatial mapping is essential for evaluating the outcomes of the updated HIV treatment programmes, while identifying areas with higher HIVDR, and providing directed interventions at a patient and public health level.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/v16101634/s1>, Figure S1: Proportion of HIV-1 genotypic resistance tests per subdistrict in KwaZulu-Natal, South Africa; Figure S2: Interpolation maps of antiretroviral drug susceptibility levels across KwaZulu-Natal province, South Africa; Table S1: HIV-1 genotypic resistance tests categorized by the districts and subdistricts of KwaZulu-Natal province, South Africa; Table S2: Patterns of antiretroviral drug class resistance found in 3133 genotypic resistance tests from patients in KwaZulu-Natal province, South Africa; Table S3: Characteristics of nine patients on dolutegravir-based treatment with dolutegravir resistance in KwaZulu-Natal, South Africa; Table S4: HIV-1 drug resistance mutations detected in genotypic resistance tests from patients in KwaZulu-Natal, South Africa.

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Informed Consent Statement: This study did not obtain informed consent from individuals due to the use of retrospective anonymized HIVDR data. The research ethics council that approved this study waived the need for participant consent.

Data Availability Statement: The original contributions presented in the study are included in the article and Supplementary Materials, and further inquiries can be directed to the corresponding author.

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3.1. Bridging Text

The manuscript in chapter 3 describes a study of the patterns of HIVDR among adults attending public sector healthcare facilities in KwaZulu-Natal. The period from 2018 to 2022 marks a systematic transition to DTG-based first-line ART, during which it became essential to heighten HIVDR surveillance for DTG-related resistance mutations. In this context, the study revealed that around 35% (9/26) of persons on DTG-based ART had mutations conferring resistance to DTG, as determined by *integrase* genotyping. Geospatial analysis revealed geographic disparities in the burden of HIVDR, with northern KZN having the highest levels of resistance to NNRTIs, alongside low to intermediate levels of boosted PI and DTG resistance. Overall, this study highlighted that 80% of GRTs from adults exhibited dual- or triple-drug class resistance to HIV-1. These findings inspired further analysis of HIVDR patterns in children and adolescents experiencing virological failure (VLs ≥ 1000 copies/mL while receiving care at public healthcare facilities in KwaZulu-Natal. Therefore, chapter 4 presents a study that was conducted to determine the patterns and frequencies of HIVDR in children and adolescents aged 0 to 17 years in KZN, South Africa. The chapter has been prepared as a manuscript for submission to the Journal of the Pediatric Infectious Diseases Society. The manuscript supplementary material are provided in Appendix 4.

Chapter 4: Characterization of HIV-1 drug resistance in ART-experienced children and adolescents through geospatial analysis of routine genotypic tests in KwaZulu-Natal, South Africa

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Characterization of HIV-1 drug resistance in ART-experienced children and adolescents through geospatial analysis of routine genotypic tests in KwaZulu-Natal, South Africa

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Running head: HIV-1 drug resistance among children in KZN, South Africa

Summary:

- Data from South Africa demonstrated that younger children failing antiretroviral therapy had higher HIV-1 drug resistance (HIVDR) levels compared to adolescents.
- Geospatial analysis of ARV drug susceptibility levels in children showed higher HIVDR to lamivudine, nevirapine and efavirenz across KwaZulu-Natal.

Abstract:

Background HIV-1 drug resistance (HIVDR) complicates antiretroviral therapy (ART) management in children and adolescents living with HIV (CALWH), particularly in high-prevalence areas such as KwaZulu-Natal (KZN), South Africa.

Methods This study described trends and geospatial changes in HIVDR among ART-experienced CALWH in KZN by integrating routine HIV-1 genotypic resistance test (GRT) data with facility-linked Geographic Information Systems (GIS) coordinates.

Results Of 914 HIV-1 GRT records obtained between January 2018 and June 2022, from 0–17-year-old CALWH, 80.96% (740/914) had HIVDR. Of the 740 with HIVDR, 609 (82.30%) had non-nucleoside reverse transcriptase inhibitor (NNRTI) mutations, 525 (70.95%) had nucleoside reverse transcriptase inhibitor (NRTI) mutations and 181 (24.46%) had major protease inhibitor (PI) mutations. Approximately 1 in every 6 GRTs with HIVDR (129/740; 17.43%) had triple-class NRTI, NNRTI and PI-resistance mutations. When analyzed by age category, ~9 in every 10 GRT records from CALWH aged 0-9 years (237/257, 92.22%) had HIVDR, compared to 76.56% (503/657) aged 10-17 years, (OR=3.63, 95% CI: 2.22-5.93, $p<0.001$). GIS mapping showed low to intermediate levels of resistance to abacavir and intermediate to high levels of resistance to lamivudine, nevirapine and efavirenz.

Conclusions Routine HIV-1 GRT records integrated with GIS analysis demonstrated higher HIVDR levels among younger children with notable geographic variation in antiretroviral drug susceptibility levels across KZN, emphasizing the potential of geospatial analysis for data-driven public health decision-making. Continuous real-time monitoring of HIVDR is crucial to assess the impact of the revised HIV-1 treatment guidelines for CALWH in South Africa.

Key words: HIV-1 drug resistance, geospatial, children, adolescents, South Africa

Introduction

Despite the progress made in antiretroviral therapy (ART) and the improvements in therapeutic outcomes for people living with HIV, HIV-1 drug resistance (HIVDR) constitutes a significant global challenge to sustained lifelong therapy [1]. Children and adolescents living with HIV (CALWH) in resource-limited settings face an increased risk of developing HIVDR due to limited child-friendly drug formulations, adherence challenges, lack of weight gain, dose adjustments, maternally transmitted HIVDR and potential prior exposure to non-nucleoside reverse transcriptase inhibitor (NNRTI)-based ART for prevention of vertical transmission (PVT) [2–5]. In 2013, the World Health Organization (WHO) recommended ritonavir-boosted lopinavir (LPV/r)-based ART as the preferred regimen for CALWH aged <3 years, regardless of PVT drug exposure history [6].

South Africa, home to the largest pediatric HIV-1 epidemic globally [7], implemented guidelines in 2013 that recommended abacavir (ABC), lamivudine (3TC), and LPV/r as the first-line ART regimen for children aged 0-3 years, with efavirenz (EFV) replacing LPV/r for CALWH >3 years of age [8]. Progressively, tenofovir disoproxil fumarate (TDF) replaced ABC in treatment regimens for CALWH aged >10 years and ≥ 30 kilograms (kgs), while all NNRTI-based regimens were transitioned to LPV/r or dolutegravir (DTG) prior to and during the rollout of DTG in December 2019 [3,9]. Subsequently, both WHO and South African national guidelines recommend a DTG-based regimen in all children over 1 month of age and ≥ 3 kgs [10,11]. Despite availability of more robust regimen in ART programs, progress towards the UNAIDS 95-95-95 targets for CALWH remains stagnant, with only 63% of eligible children aged 0-14 years receiving ART in South Africa, and just 54% of treated children from Eastern and Southern Africa attaining virological suppression in 2023 [12–14].

Viral load (VL) non-suppression among CALWH is closely linked to suboptimal adherence, a major factor in treatment failure and the emergence of HIVDR [15–17]. A systematic review conducted in 2024, encompassing 28539 CALWH from 52 countries, indicated that the global prevalence of HIVDR was 74.16% among ART-experienced children and 26.31% among ART-naïve children, with the highest prevalence of 37.80% [95% confidence interval (CI) 26.24-50.08] observed in Southern Africa [16]. South Africa holds approximately 10% of the global population of CALWH [13]. Despite this, baseline genotypic resistance tests (GRTs) are not routinely recommended for CALWH in national guidelines, unless the child is perinatally infected with the mother failing a protease-inhibitor (PI)- or DTG-based regimen

[11]. Given the high prevalence of HIVDR among pediatrics in South Africa [3], there is a critical need for effective near-real time geospatial HIVDR surveillance in CALWH. This would help to monitor HIVDR in treatment-experienced CALWH across the country. Therefore, this study combined data from routine GRT data with Geographic Information Systems (GIS) data to survey and investigate the geospatial variations of HIVDR among CALWH in the hyperendemic province of KwaZulu-Natal (KZN), South Africa.

Materials and Methods

Study population and design

This retrospective study analyzed routine HIV-1 GRT data from the National Health Laboratory Service (NHLS) Central Data Warehouse (CDW), spanning the period from 1 January 2018 to 30 June 2022. The study period encompasses the pre-transition and transition phases to DTG as part of first-line ART regimens in South Africa, aligning with our previous research on geospatial mapping of HIV-1 VLs [18] among children and adults, and HIVDR among adults [19]. The NHLS is South Africa's largest provider of diagnostic laboratory services, serving ~80% of the population, and playing a critical role in supporting the Department of Health's oversight of national and provincial programmes [20]. The South African programmatic indication for GRTs is virological failure, defined as two or more VLs ≥ 1000 copies/milliliter (mL) taken ≥ 2 years after initiating DTG- or PI/r-based regimens, with $>80\%$ patient adherence to ART [11]. However, previous guidelines from 2015 and 2020 recommended GRTs after consultation with a specialist, for CALWH with increased VL on PI or DTG-based regimens (regardless of duration) with concurrent rifampicin-containing TB therapy, as well as newly diagnosed children <2 years of age whose mother received PI-based ART during pregnancy/lactation [5,9]. In this study, viral RNA extracted from patient plasma specimens with VLs ≥ 1000 copies/mL were genotyped and analyzed for the HIV-1 *protease* (PR), *reverse transcriptase* (RT) and *integrase* (IN) genes, as described previously [19].

We analyzed HIV-1 genotypic data from children (0-9 years of age) and adolescents (10-17 years of age) [10], who received healthcare at public-sector facilities in KZN. We excluded GRT records obtained from individuals ≥ 18 years of age (i.e., adults). We used data from individuals <18 years to develop an interactive database for geospatial analysis and data visualization. We identified HIVDR mutations and assessed antiretroviral (ARV) drug susceptibilities using the Stanford University HIV Drug Resistance Database v9.6 [21]. We assessed drug susceptibilities for nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs),

(NRTIs), NNRTIs and PIs, and defined HIVDR as any GRT showing reduced drug susceptibility (i.e., potential low-level resistance (PLLR) to high-level resistance (HLR)) to any ARV drug available in standard of care regimens in South Africa [19]. Given that only a few GRTs (n=8) included IN genotyping, we did not conduct geospatial analysis of the integrase strand transfer inhibitor (INSTI)-resistance mutations further. Figure 1 presents a summary of the GRT records that were retrieved and analyzed.

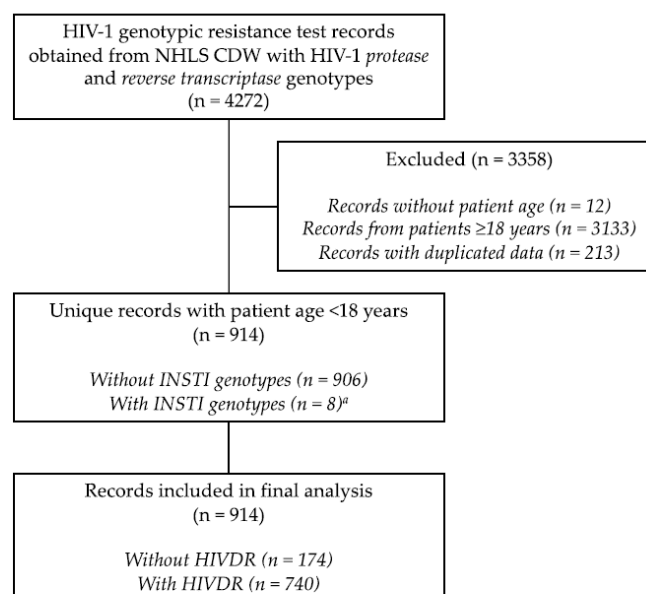


Figure 1. Flowchart of HIV-1 genotypic resistance test records retrieved and included in final analysis for children and adolescents receiving HIV-1 care at public sector healthcare facilities in KwaZulu-Natal, South Africa. CDW, central data warehouse; HIVDR, HIV-1 drug resistance; INSTI, integrase strand transfer inhibitor; NHLS, National Health Laboratory Service.

^a Excluded specifically from geospatial analysis due to a small number of INSTI genotypes (n=8)

Using the GRT records, we created a HIVDR database categorized according to the absence or presence of HIVDR, i.e., decreased susceptibility to ≥ 1 ARV drug. We included the following meta-data for each GRT record: patient age, sex, date and location of specimen collection (i.e., healthcare facility name, district and subdistrict), type of healthcare facility (i.e., outpatient or inpatient department), most recent VL, current ART regimen, HIVDR mutations detected and ARV drug susceptibility. We further categorized age into 4 groups: (i) 0-3 years, (ii) 4-9 years, (iii) 10-14 years and (iv) 15-17 years, based on the regimens that were used for children in South Africa during the study period [5,9]. For GRT records with complete details of all current ARV drugs in the regimen, we assigned genotypic susceptibility scores (GSSs) for individual

drugs, i.e., a GSS of 1 for no resistance or PLLR, 0.5 for low-level resistance (LLR) or intermediate resistance (IR), and 0 for HLR. The total GSS for each record was calculated by adding the individual drug scores and a total score >2 indicated an active regimen, while a total score <2 suggested reduced potency due to HIVDR [22].

For each GRT record, we assigned the global positioning system (GPS) coordinates of the respective healthcare facility at which the GRT specimen was collected, and used the coordinates to create geospatial maps in QGIS 3.30 software [23]. We produced inverse distance weighted (IDW) interpolation maps to illustrate the ARV drug susceptibility levels per facility and to identify spatiotemporal variations in HIVDR levels across KZN. The IDW interpolation approach assumes that unsampled areas near a facility have drug susceptibility values similar to those observed at the facility's location, as described previously [19].

Statistical analysis

We used descriptive statistics to assess patterns of HIVDR, GSSs and mutations, and presented the results as proportions and percentages in graphs and tables. Logistic regression analysis and chi-square tests were used to evaluate the associations between HIVDR and patient GRT variables, which included year in which specimen was collected, sex, age group, VLs, ART regimen and type of healthcare facility. We conducted all statistical analyses in Stata 18.0 software SE (StataCorp. 2023, College Station, TX, USA).

Ethical considerations

We obtained ethics approval from the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (reference number: BREC/00003120/2021) on 10 September 2021. We received further permission from the NHLS Academic Affairs and Research Management System for the use of routinely processed retrospective de-identified HIVDR records for research purposes. As this study used retrospective anonymized HIVDR data, the research ethics committee that approved the study waived the consent requirement.

Results

Of 914 GRT records obtained from 114 public-sector facilities, all individuals had HIV-1 subtype C infection with 80.96% (740/914) exhibiting HIVDR. There was no significant difference in proportion of HIVDR between sexes, with a median age of 13 years (Interquartile range (IQR): 8-16). Notably, the proportion of HIVDR was significantly higher in children

compared to adolescents (92.22% versus 76.56% respectively), $p<0.05$. When compared to adolescents aged 15-17 years, the likelihood of HIVDR was nearly 3 times higher in children aged 0-4 years (odds ratio (OR)=2.94, 95% confidence interval (CI): 1.54–5.60, $p=0.001$), and over 4 times higher in children aged 5-9 years (OR=4.15, 95% CI: 1.94–8.89, $p<0.001$). There were no significant changes observed in HIVDR across the study years (Table 1).

Table 1. Characteristics of HIV-1 genotypic resistance tests from children and adolescents in KwaZulu-Natal, South Africa

Variable	All GRTs N=914 (100.00%)	No HIVDR N=174 (19.04%)	HIVDR N=740 (80.96%)	p-value
Year of collection				
2018	205 (22.43%)	40 (19.51%)	165 (80.49%)	0.96
2019	201 (21.99%)	37 (18.41%)	164 (81.59%)	
2020	223 (24.40%)	45 (20.18%)	178 (79.82%)	
2021	178 (19.47%)	31 (17.42%)	147 (82.58%)	
2022 ^a	107 (11.71%)	21 (19.63%)	86 (80.37%)	
Sex				
Female	451 (49.34%)	91 (20.18%)	360 (79.82%)	0.27
Male	445 (48.69%)	82 (18.43%)	363 (81.57%)	
Unknown	18 (1.97%)	1 (5.56%)	17 (94.44%)	
Age in years				
15-17	339 (37.09%)	76 (22.42%)	263 (77.58%)	0.524
10-14	318 (34.79%)	78 (24.53%)	240 (75.47%)	
5-9	123 (13.46%)	8 (6.50%)	115 (93.50%)	
<5	134 (14.66%)	12 (8.96%)	122 (91.04%)	
VL in log ₁₀ copies/mL, median (IQR) ^b				
	4.80 (4.26-5.31)	4.91 (4.59-5.20)	4.71 (4.18-5.33)	0.058
Type of healthcare facility				
Inpatients	97 (10.61%)	17 (17.53%)	80 (82.47%)	0.16
Outpatients	817 (89.39%)	157 (19.22%)	660 (80.78%)	

GRT, genotypic resistance test; HIVDR, HIV-1 drug resistance; IQR, interquartile range; VL, viral load

^a The data correspond to the first half of the year, as the study period concluded on 30 June 2022.

^b The most recent viral load was not documented for 36 records.

P-values in bold indicate statistically significant results.

Patterns of HIV-1 drug resistance

Among the 740 individuals with confirmed HIVDR, 609 (82.30%) had NNRTI mutations, 525 (70.95%) had NRTI mutations, and 181 (24.46%) had major PI mutations. By ARV drug class, the most frequently detected mutations were NRTI mutation M184VI (66.49%), NNRTI mutation K103NS (48.51%), and PI mutation I54VL (19.32%). The mutation L74V, which confers IR resistance to ABC, was detected in 13.38% (99/740) of GRTs as shown in Figure 2. Approximately 7% (50/740) of HIVDR GRTs with NNRTI-resistance were from children <3 years of age with no past or current NNRTI drug exposure. Only 8 GRTs included HIV-1 IN genotyping, 7 of which had HIVDR and of those, 3 (37.50%) had resistance mutations to INSTIs i.e., E138K+G118R+T66I+M50I, Y143R+T97A and H51Y, respectively. Mutations identified in GRTs exhibiting HIVDR were classified according to drug class using the Stanford HIV Drug Resistance Database and 2022 IAS–USA drug resistance mutations list [21,24] and are presented in Supplementary Table S1.

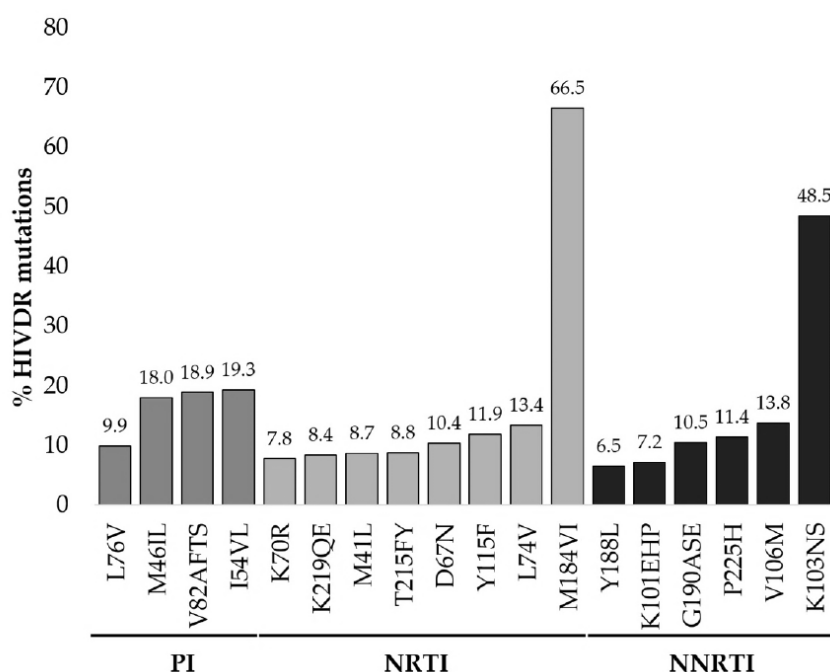


Figure 2. Analysis of specific mutations by drug class identified in 740 HIV-1 drug-resistant genotypes from children and adolescents attending public sector healthcare facilities in KwaZulu-Natal, South Africa. The horizontal axis displays "major" mutations identified in over 6% of HIV-1 drug-resistant genotypes, as classified by the Stanford HIV Drug Resistance Database or the 2022 IAS–USA list of drug resistance mutations [21,24]. HIVDR, HIV-1 drug resistance; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside/nucleotide reverse transcriptase inhibitor; PI, protease inhibitor.

Thymidine analogue mutations (TAMs) were identified in 25.94% (158/609) of individuals with NNRTI resistance, with 51.90% (82/158) and 24.05% (38/158) exhibiting TAM2 and TAM1 mutations, respectively, and 24.05% (38/158) demonstrating both TAM1 and TAM2 mutations. The predominant TAMs identified were the type-2 TAMs D67N (77/158), K219QE (62/158), and K70R (58/158), along with the type-1 TAM M41L (64/158). Within the context of patient ART regimens, 55 out of 394 (13.96%) individuals on non-thymidine NRTI regimens had TAMs. Data on previous ARV exposure was available for 36 of these 55 patients, of which 24 (66.67%) had a documented history of receiving thymidine analogues, either zidovudine (AZT) or stavudine. Notably, the K65R mutation was identified in only 10 (1.35%) out of 740 GRTs with HIVDR.

Of the 740 with HIVDR, 293 (39.59%) GRTs had single-class resistance (mainly NNRTI resistance), whilst 317 (42.84%) had dual-class resistance, 129 (17.43%) had triple-class resistance, with only 1 (0.14%) having four-drug class resistance. When stratified according to age, ~3 out of every 4 children aged 5-9 years had HIVDR to at least one ARV drug class (86/155; 74.78%), while the proportions among those aged 0-4, 10-14 and 15-17 years were 72.13% (88/122), 59.16% (142/240) and 49.81% (131/263), respectively. The overall proportion of triple-class resistance reduced from 25.45% in 2018 to 15.12% in 2022. Temporal changes in drug class resistance among CALWH are shown in Supplementary Figure S1.

Genotypic susceptibility

The majority of GRTs were from individuals on PI/r-based ART (802/914; 87.75%) comprising LPV/r or ritonavir-boosted atazanavir (ATV/r). When comparing regimens containing AZT (n=434) and TDF (n=26) to ABC-based regimens (n=391), there was a 23.48% reduction in the odds of HIVDR in patients on TDF-based regimens (OR = 0.77, 95% CI: 0.30–1.97, $p>0.58$). GSSs were predicted only in GRTs with complete regimen data (i.e., 851 out of 914) regardless of whether HIVDR was present. Patients on a regimen containing ABC with EFV or PI/r, and those on an AZT regimen with EFV, had the lowest predicted GSSs. Lower GSSs in patients on EFV containing regimens are largely due to NNRTIs low genetic barrier to resistance [25]. The highest GSSs were predicted among those on ABC with AZT and PI/r (Table 2). Only 8 GRTs were from CALWH on INSTIs, 6 of which were on DTG-based ART and of these, 66.67% (4/6) had GSSs >2. Details of HIVDR proportions and GSSs for all other regimens are shown in Supplementary Table S2.

Table 2. HIVDR proportions and genotypic susceptibility scores for commonly used antiretroviral regimens in children and adolescents receiving HIV-1 care at public health facilities in KwaZulu-Natal, South Africa between 2018 and 2022

ART regimen ^a	All GRTs	No HIVDR	HIVDR	Genotypic susceptibility score (%)		
	N=851 (%)	N=165 (%)	N=686 (%)	<2	2	>2
ABC-based						
ABC+PI/r	339 (39.84)	59 (17.40)	280 (82.60)	203 (59.88)	1 (0.29)	135 (39.82)
ABC+EFV	28 (3.29)	5 (17.86)	23 (82.14)	21 (75.00)	1 (3.57)	6 (21.43)
AZT-based						
AZT+PI/r	419 (49.24)	85 (20.29)	334 (79.71)	87 (20.76)	119 (28.4)	213 (50.84)
AZT+EFV	8 (0.94)	0 (0.00)	8 (100.00)	7 (87.5)	1 (12.5)	0 (0.00)
TDF-based						
TDF+PI/r	19 (2.23)	4 (21.05)	15 (78.95)	8 (42.11)	0 (0.00)	11 (57.89)

ABC, abacavir; ART, antiretroviral therapy; AZT, zidovudine; EFV, efavirenz; GRT, genotypic resistance test; HIVDR, HIV-1 drug resistance; PI/r, boosted protease inhibitor; TDF, tenofovir disoproxil fumarate; XTC, emtricitabine/lamivudine

^a All regimens include XTC which is either emtricitabine or lamivudine. PI/r refers to ritonavir-boosted atazanavir or lopinavir and the PI with the lowest identified penalty score was used [21]. GRTs were done at time of antiretroviral treatment failure. The regimens listed in this table were those administered to at least 1% of the children and adolescents with complete ART regimen data.

Geospatial Analysis

GIS mapping using IDW interpolation revealed general uniformity in drug susceptibilities across the 11 districts of KZN province, with a few notable variations seen in individual drugs (Figure 3). Overall, the majority of districts ranged from susceptible to PLLR to TDF, AZT and ritonavir-boosted darunavir (DRV/r), with LLR observed to boosted PI drugs (namely LPV/r and ATV/r). Furthermore, most districts showed LLR to IR to ABC, with localized areas of HLR observed in districts of northern KZN. Notably, there was IR to HLR to the commonly used XTC and the NNRTI drugs, EFV and nevirapine (NVP), despite only 4.16% (38/914) of CALWH having prior exposure to NNRTI-based regimens. The IDW interpolation maps of ARV drug susceptibility levels for TDF, AZT, ABC, XTC, EFV, NVP, PI/r (i.e., LPV/r or ATV/r) and DRV/r are shown in Figure 3.

Discussion

This retrospective study of 914 routine HIV GRTs from children and adolescents in KwaZulu-Natal, South Africa, showed that HIVDR was 3 to 4 times higher in GRT records from children aged 0-9 years when compared to adolescents aged 15-17 years. Comparable results were seen from routine GRTs processed between November 2017 and December 2018, indicating a higher prevalence of PI-resistance mutations in children compared to adolescents (43% versus 26%) [26] in KZN. The higher HIVDR rate could be due to persistent viraemia on a failing regimen from delayed diagnosis of virological failure and consequently, delay in HIV-1 GRT requests. Alternatively, children with virological failure may be referred to specialists who are less likely to request a GRT if the patient is non-adherent. Furthermore, as children transition to adolescence, adherence challenges emerge hence GRTs may indicate the presence of wildtype virus with no HIVDR. HIVDR was predominantly caused by NNRTI mutations, with almost 80% of GRTs exhibiting NNRTI resistance, despite less than 5% of individuals being on an NNRTI-based treatment regimen. Several South African studies have highlighted the increasing prevalence of HIVDR in CALWH, primarily due to maternally transferred NNRTI-associated resistance, emphasizing the urgent need for continued monitoring of this phenomenon as maternal ART guidelines transition to a DTG-based regimen [3,16,27,28]. Overall, the temporal trends of dual- and triple-class resistance were identified in 3 out of every 5 children, which is concerning, given the limited number of ARV drug classes available for optimization of second- and third-line ART in South Africa. Notwithstanding the shift to more potent TDF- and DTG-based regimens from 2018 to 2022, the prevalence of resistance remained unchanged.

Moreover, geospatial analysis revealed that most districts across KZN showed LLR to PI/r drugs despite 9 out of 10 CALWH receiving PI/r-based regimens, consistent with the well-established high genetic barrier to resistance associated with PIs. Across all districts of KZN, there was LLR to IR to ABC, commonly used in first-line ART regimens for children aged 0-9 years. Notably dispersed localities in northern KZN districts had HLR to ABC, suggesting the need for targeted programmatic interventions in this region which is historically known for HIV-1 hyperendemicity [29–31]. These geospatial analyses maps demonstrated variations in ARV drug susceptibility levels, emphasizing the need for enhanced HIV-1 treatment monitoring in CALWH. This includes the need for timely HIV-1 GRT and ART regimen switching, especially for patients on ARV drugs showing reduced viral susceptibility. This

work in CALWH, along with our previous research on HIVDR among adults in KZN [19], demonstrates how geospatial analysis may be used to inform data-driven public health decision-making.

The predominant NRTI mutation identified was M184VI, present in two-thirds of all GRTs, followed by L74V, detected in 13.4% of GRTs and causing IR to ABC. Two-thirds of CALWH (24/36) with confirmed prior exposure to thymidine analogues, had TAMs, suggesting the persistence of these mutations from previous ART exposure. This implies that in GRTs from patients on non-thymidine analogues, with no known exposure to thymidine analogues (i.e., atypical TAMs), the prevalence of TAMs was around 33.33%. However, this may not fully reflect atypical TAMs given the uncertainty regarding the completeness of previous drug exposure data for these CALWH. Given that TAMs can reduce susceptibility to TDF and AZT, it is necessary to further explore the significance of atypical TAMs in CALWH undergoing TDF-based therapy. Only ~1% of GRTs had the K65R mutation, as geospatially demonstrated in the TDF interpolation map with susceptible levels to PLLR homogeneously reflected across KZN. Nonetheless, of 27 GRTs from CALWH on TDF-containing regimens, 37.0% (10/27) had a GSS of <2, indicative of HIVDR with reduced regimen potency.

The NNRTI mutation, K103NS was found in over half of all GRTs, resulting in HLR to EFV and NVP. Similarly, high levels of NNRTI-resistance between 65% and 95% were noted in a study among CALWH under 16 years of age [3] and the proportion among children under 18 months was 53% [2]. Our analysis revealed that 7% of HIVDR GRTs exhibiting NNRTI resistance came from children under 3 years of age who had no prior or present exposure to NNRTI drugs as part of their regimen. This data implies transmitted resistance with persistent NNRTI mutations in the absence of drug pressure, as demonstrated in early studies where K103N persisted for years in women who received single-dose NVP for PVT [32,33]. The most frequently detected PI-specific mutation was I54V (19.1%), which causes LLR to LPV/r while preserving full susceptibility to DRV/r, demonstrating the viability of second-generation PIs when customizing future treatment options for CALWH with HIVDR.

Despite the rollout of DTG in December 2019, there has been a lag in transitioning CALWH to DTG-containing regimens in South Africa. In this study, only 8 GRTs were from CALWH on DTG-based ART, with 62.50% showing GSSs ≥ 2 , indicating sustained efficacy of the regimen. The National treatment guidelines were revised in 2023 to include dispersible DTG

tablets for improved dosing in pediatric patients [11], however, the availability of these dispersible tablets remains limited. While there is ample evidence that DTG is a potent INSTI with a high genetic barrier to resistance [34,35], it is essential that the NRTIs combined with DTG are fully active, to prevent functional DTG monotherapy, and preserve its long-term use in HIV-1 treatment programmes for CALWH. GRT data provides clear evidence on ARV drug susceptibility levels [21], contributing to improved ART outcomes among CALWH [36]. Given that two out of every five ART-naïve and four out of every five ART-experienced CALWH in Southern Africa have HIVDR [16], it is imperative that GRTs are made more accessible to CALWH with viraemia.

Similar to our previous geospatial studies [18,19], our findings should be interpreted with regard to the following limitations. Data regarding previous ARV drug regimens and duration on ART were not consistently documented, and we did not merge serial GRTs, if present, within the HIVDR database. Our geospatial analyses used GIS data of facilities rather than household locations, based on the assumption that CALWH obtained a GRT at the facility closest to their home. We did not consider facility GRT testing practices, nor contextualize our geographical analysis within the local CALWH population, nor account for ART coverage by district/subdistrict or facility. Given that only 8 CALWH were on DTG-based regimens, we were not able to geospatially analyze drug susceptibility levels for DTG. Therefore, similar studies are needed, ideally addressing the limitations identified in this study, to better understand the burden of HIVDR and inform ART decision making. Lastly, future research could include integration of maps with PVT and pediatric HIVDR data, to find linked HIVDR problems among antenatal and infant populations in certain geographic regions.

Conclusions

By integrating routine HIV-1 GRT records with GIS analysis, we identified significantly higher levels of HIVDR among younger children. This underscores the urgent need for policies that support near-real time surveillance of HIVDR, to enable targeted treatment interventions to prevent HIVDR mutations and timely ART switching for CALWH experiencing virological failure. Moreover, the use of dispersible DTG-based regimens for CALWH should be expedited to improve overall ART coverage and achieve better virological suppression, by using more a palatable and effective ART regimen. Geospatial mapping revealed geographic variations in antiretroviral drug susceptibility levels across KZN, highlighting the potential use

of geospatial analysis in HIVDR surveillance to support data-driven public health decision-making.

List of abbreviations

3TC: lamivudine, ABC: abacavir, ART: antiretroviral therapy, ARV: antiretroviral, ATV/r: ritonavir-boosted atazanavir, AZT: zidovudine, CALWH: children and adolescents living with HIV, CDW: Central Data Warehouse, CI: confidence interval, DRV/r: ritonavir-boosted darunavir, DTG: dolutegravir, EFV: efavirenz, GIS: Geographic Information Systems, GPS: global positioning system, GRTs: genotypic resistance tests, GSSs: genotypic susceptibility scores, HIVDR: HIV-1 drug resistance, HLR: high-level resistance, IDW: inverse distance weighted, IN: integrase, INSTI: integrase strand transfer inhibitor, IQR: Interquartile range, IR: intermediate resistance, kgs: kilograms, KZN: KwaZulu-Natal, LLR: low-level resistance, LPV/r: ritonavir-boosted lopinavir, mL: milliliter, NHLS: National Health Laboratory Service, NNRTI: non-nucleoside reverse transcriptase inhibitor, NRTIs: nucleoside/nucleotide reverse transcriptase inhibitors, NVP: nevirapine, OR: odds ratio, PI: protease-inhibitor, PI/r: ritonavir-boosted lopinavir/atazanavir, PLLR: potential low-level resistance, PR: protease, PVT: prevention of vertical transmission, RT: reverse transcriptase, TAMs: thymidine analogue mutations, TDF: tenofovir disoproxil fumarate, VL: viral load, WHO: World Health Organization, XTC: lamivudine/emtricitabine

Author Contributions

Conceptualization, L.G., R.P., K.G. and B.C.; methodology, L.G., M.A. and B.C.; software, L.G.; validation, L.G., M.A. and B.C.; formal analysis, L.G. and B.C.; investigation, L.G.; resources, L.G. and B.C.; data curation, L.G.; writing—original draft preparation, L.G. and B.C.; writing—review and editing, M.A., A.K., K.G., R.P., and K.N.; visualization, L.G.; supervision, B.C.; project administration, L.G. and B.C.; funding acquisition, L.G., K.N. and B.C. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

The original contributions presented in the study are included in the article and Supplementary Materials, and further inquiries can be directed to the corresponding author.

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Conflicts of Interest

The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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Chapter 5: Synthesis of research findings

5.1. Key Themes

South Africa currently has the largest HIV treatment programme worldwide, consisting of 5.9 million individuals on ART in 2023, i.e., 1 out of every 5 PLWH on ART globally [1]. The South African treatment program has advanced significantly, with the transition to more effective first-line DTG-containing regimens initiated in 2019 [2]. Despite a deceleration in DTG-rollout during the COVID-19 pandemic, transferring all eligible PLWH to DTG-based ART continues to be a primary health goal in South Africa [3]. Our narrative review of the literature in Chapter 1 indicated that, while South Africa has met the initial target of the UNAIDS 95-95-95 with 95% of PLWH aware of their status [4], the second target concerning ART coverage remains unmet, particularly among the paediatric population, where ART coverage is as low as 42% in certain regions of the country [5]. Where coverage has been achieved the end goal is virological suppression, which is the third unmet target for South Africa with only 71% of PLWH on ART achieving virological suppression [1,4]. To improve the end goal of PLWH remaining on life-long ART with virological suppression, the South African treatment guidelines were revised in 2023 to ensure that all PLWH on ART with a viral load of ≥ 50 copies/mL were systematically evaluated for adherence and comprehensively screened for co-infections, among other considerations [6]. In this way, patients with LLV were closely monitored, while patients with virological failure were switched promptly without prolonging their time on ineffective ART which could lead to development of HIVDR mutations.

Novel monitoring approaches are crucial in evaluating outcomes of treatment programmes and geospatial analysis is one such approach. Although the geospatial approach has been used for evaluating HIV-1 prevalence, this tool has not been as widely used in monitoring treatment response to the HIV-1 epidemic in South Africa. The NHLS is a crucial element of new implementation programmes however it is an underutilized resource for monitoring ART outcomes in South Africa [7]. Although the NHLS has developed a surveillance approach which integrates public sector patient-level CD4 and VL test result data from the CDW with data from the health information system [8], these data dashboards lack hotspot visualization and have not incorporated HIVDR data, which is a critical area, not only in HIV care, but also in the overall context of antimicrobial resistance. Moreover, the paucity of data regarding HIVDR presents a significant obstacle. While mathematical models can help synthesize available evidence, a key difficulty in making accurate predictions is the absence of comprehensive longitudinal data on the impact of DTG resistance on clinical outcomes [9]. For example, the Thembisa mathematical model was designed to answer policy questions relating to HIV treatment within the South African HIV-1 epidemic as well as contextualize progress made towards the UNAIDS 95-95-95 targets. It also provides downloadable, pre-categorized demographic statistics that can be further analysed using geospatial methods. However, the predefined categories limit the types of analyses that can be performed [10].

Data from routine testing are invaluable in that they are a continuous source of big data, both historically and in real-time, representing the typical cohorts of patients encountered in a public healthcare setting. When routinely tested data from VL and HIVDR testing are repurposed for geospatial analysis in near-real time, as demonstrated in Chapters 2, 3 and 4, the result is a dynamic monitoring tool that can provide valuable insights into the treatment landscape of the HIV-1 epidemic in South Africa. Geospatial analysis of repurposed routine HIVDR genotypic data to determine HIVDR hotspots is a novel approach. By consolidating patient healthcare information, it has the potential to provide valuable insights for policymakers and guide targeted interventions.

The visualization of detectable VLs through geospatial and temporal mapping, presented in Chapter 2, demonstrated that while there is an overall downward trend in the proportion of high-level viraemia, there were hyperendemic areas, particularly in northern KZN and along the coastal belt, with emerging hotspots even after DTG rollout. The temporary disruption of HIV treatment services during the COVID-19 pandemic, may have contributed to ART interruption, treatment non-adherence and ultimately loss of viral suppression [11]. However, northern KZN has historically been recognized as a hyperendemic region for HIV-1, exposed to marginalized cross-border migrant communities with a greater risk of HIV-1 acquisition [12], while coastal districts have access to trade ports, areas of HIV vulnerability in southern Africa [13]. Over time, we observed an inverse rising trend in the percentage of LLV, increasing from 2% in 2018 to 4% in 2022, as more individuals with previously high-level viraemia began to attain viral suppression with increasingly effective DTG regimens [14]. Identifying facilities with significant proportions of individuals exhibiting low- and high-level viraemia, as well as locales of at-risk populations, including those with risk-taking behaviour, yields critical data for health care providers and policymakers, underscoring the need for expedient VL monitoring, such as point-of care VL testing, alongside HIVDR surveillance.

Novel approaches to HIVDR surveillance, presented in Chapters 3 and 4, utilized big data from routinely processed HIV-1 GRTs to explore geospatial data visualization at a more detailed level, for adults (≥ 18 years) and children (0-17 years), respectively, using community surveillance. The geospatial analyses of drug susceptibility levels in KZN revealed significant levels of HIVDR across the province, with northern KZN having the highest prevalence of EFV, NVP and XTC resistance. The findings clearly indicated consistently elevated levels of NRTI and NNRTI resistance, with over 80% of all GRTs demonstrating resistance to NNRTIs, despite $<5\%$ being on NNRTI-based regimens. The most frequently observed NRTI mutation was M184VI, detected in $>65\%$ of all GRTs, and for NNRTIs, K103NS was observed in almost half of all GRTs, conferring high-level resistance to EFV and NVP. Similarly, high levels of NNRTI-resistance were observed in a study among adults failing mostly LPV/r-based ART, where the prevalence of NNRTI-associated mutations was 63.1%, despite a median duration of ~ 3.5 years on PI-based ART [15] while the prevalence of NNRTI-resistance among children aged <16 years ranged from 65%-95%, [16] and 53% specifically among children <18 months of age [17]. Notably, our study found that 7% of HIVDR GRTs with NNRTI-resistance were from children <3 years of age with no past or current NNRTI drug exposure. These data strongly suggest persistence of NNRTI

mutations in the absence of drug pressure, as shown in early studies where K103N persisted for years in women receiving single-dose NVP for the prevention of mother-to-child transmission [18,19].

The prevalence of TAMs detected in GRTs from patients on non-thymidine NRTI regimens with confirmed exposure to thymidine analogues was 77.77% (210/270) in adults and 66.67% (24/36) in children, indicating a persistence of TAMs from previous ART exposure. In GRTs from patients on non-thymidine NRTI regimens with no confirmed exposure to thymidine analogues (i.e., atypical TAMs), the prevalence of TAMs was around 22.22% for adults and 33.33% for children. However, this may not be an accurate representation of atypical TAMs due to the lack of clarity regarding the completeness of past medication exposure data for these individuals. Nonetheless, research from sub-Saharan Africa indicated prevalence rates of atypical TAMs between 16% and 41% [20,21]. Given their potential for lowering susceptibility to AZT and TDF, it is imperative to improve understanding of atypical TAMs in patients undergoing TDF treatment. The K65R mutation which confers resistance to TDF, was present in approximately 6% and 1% of GRTs from adults and children, respectively. Cumulatively, 699 GRTs were from adults and 27 GRTs were from children on TDF-containing regimens and of those 43.8% (306/699) adults and 37.0% (10/27) children had a GSS of <2, indicative of drug resistance with reduced potency of the ARV regimen.

The most common PI-specific mutations observed were M46IL in adults (28.8%) and I54V in children (19.1%), causing low-level resistance to LPV/r, while maintaining full susceptibility to DRV/r, showing better potency of PI drugs. For adults, we investigated the presence of DTG-specific mutations and found that, of 26 patients taking DTG that had HIV-1 *integrase* genotyping, around 35% (9/26) had DTG-associated resistance mutations, representing one of the highest rates reported to date. Among children, only eight GRTs included HIV-1 *integrase* genotyping of which 3 had HIVDR and two (2/8, 25%) had DTG-specific mutations. The predominant mutation identified in the nine adults exhibiting DTG-associated resistance was E138KAT (4/9), which alone confers low-level resistance to DTG and cabotegravir (CAB). Of the two children with DTG-specific mutations, one child on AZT/3TC/DRVr/RAL had E138KAT+G118R+T66I mutations resulting in high-level resistance to DTG, while the other child on AZT/FTC/DTG had a H51Y mutation, which alone causes potential low-level resistance to DTG. The accumulation of mutations associated with INSTI resistance indicates an impending problem and underscores the necessity for increased monitoring of DTG-specific mutations to prevent compromising future DTG-based ART and long-acting CAB-based therapies. Notably, when paediatric GRTs were analysed by age category, ~9 in every 10 children between the ages of 0-9 years (237/257, 92.22%) had HIVDR, compared to 76.56% (503/657) between the ages of 10-17 years, (OR = 3.63, 95% CI: 2.22-5.93, $p < 0.001$), warranting enhanced treatment monitoring in younger children on ART. Overall, the temporal trends of dual- and triple-class resistance were observed in 3 out of every 5 children (Figure 9a), and 4 out of every 5 adults (Figure 9b).

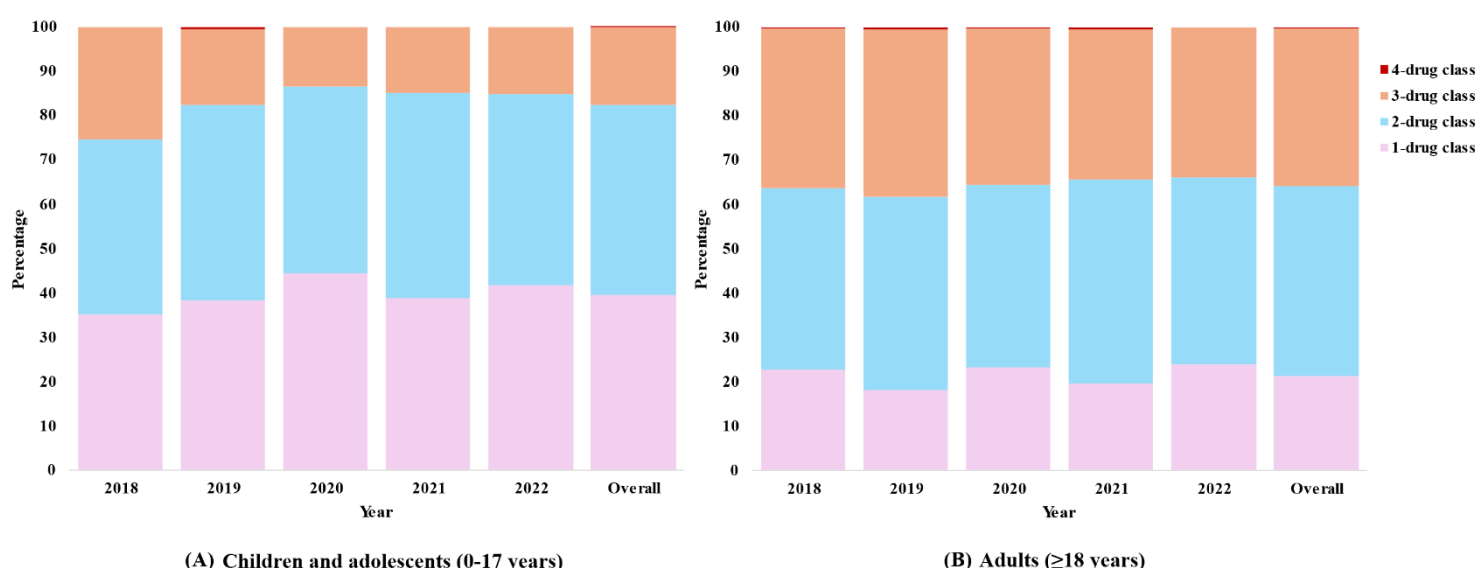


Figure 9. Patterns of antiretroviral drug class resistance among children, adolescents and adults.

(A) 740 genotypic resistance tests with confirmed HIV drug resistance from children and adolescents, of which 8 had *integrase* genotyping, 2 of which met the criteria for 4-drug class resistance.

(B) 2735 genotypic resistance tests with confirmed HIV drug resistance from adults, of which 41 had *integrase* genotyping, 9 of which met the criteria for 4-drug class resistance.

The findings of multi-drug class resistance in more than half of patients with GRT-confirmed HIVDR is alarming, especially with the limited number of ARV drug classes available for optimization of second- and third-line ART. After the failure of first-line ART, the susceptibility of each ARV drug, as determined by GRT, should be assessed individually to customize subsequent treatment options and examine the feasibility of recycling ARV drugs [22]. As much as one can argue that DTG has a high genetic barrier to resistance [23], DTG monotherapy fosters the emergence of INSTI-resistant strains, albeit only after prolonged exposure. Although this scenario is improbable when integrated with other nucleosides in a fixed-dose combination or with nucleotides administered once daily that have prolonged intracellular half-lives [24], it is essential that the NRTIs combined with DTG are fully active, to preserve the long-term use of DTG in HIV-1 treatment programmes.

5.2. Recommendations for policy

The data presented in this study showed that geospatial and temporal mapping have the potential to enhance HIV-1 treatment decisions, with great potential to be a robust monitoring tool for predicting (through interpolation methods) localities with high-level viraemia in resource-constrained environments where VL testing may not be readily available. GIS-linked health data can be used to model the utilization and accessibility of healthcare facilities, as well as to analyse the overlap of infectious and non-infectious diseases, hence

facilitating life-long multi-disease chronic care tailored to specific populations. Based on these geo-localized analyses, policymakers can for example, make better informed decisions regarding deployment of healthcare workers to rural versus urban primary healthcare facilities, or expand specialized infectious diseases services within areas of persistent high-level viraemia, especially since many of these areas are known to be hyperendemic for other sexually transmitted diseases as well as tuberculosis. Identifying regions with a triple burden of communicable diseases necessitates policies that provide timely, targeted interventions and impactful treatment campaigns (e.g., U = U; Undetectable = Untransmittable), to reduce new infections in at-risk populations through treatment optimization in those with existing diseases. Furthermore, pinpointing geographic regions with high-level viraemia serves as a proxy indicator for HIVDR emergence, highlighting the need to develop policies with focused HIVDR monitoring in addition to nationwide HIVDR surveillance systems.

Ongoing real-time HIVDR surveillance is essential to assess the impact of the revised HIV-1 treatment programme in South Africa. By integrating routine HIV-1 GRT records with GIS analysis, we identified relatively higher levels of HIVDR among younger children, highlighting the dire need for policies which support the near-real time surveillance of HIVDR in children, with clear criteria for timeous switching of ART in children with virological failure. Moreover, the use of dispersible DTG-based regimens for children should be accelerated to enhance overall ART coverage and achieve better virological suppression through the utilization of effective ARV medications. Given the significant geographic disparities in the HIVDR burden throughout KZN, particularly in northern KZN where elevated levels of HIVDR to ARVs such as ABC are observed in children and reduced susceptibility to EFV, NVP, and XTC are observed in adults, the national policy shift from EFV-based first-line ART regimens to DTG-based first-line ART regimens is scientifically justified, necessitating the urgent upscale of DTG based-ART in these high priority geographic areas.

Data policies should be more inclusive of the potential use of geospatial analysis to inform data-driven public health decision-making. Our studies have demonstrated the value of developing HIV-1 databases using repurposed big data derived from routinely processed HIVDR testing. These databases can be integrated with GIS and data dashboards to evaluate the geographic spread of HIVDR. The interest in record linkage methodologies among health researchers has surged due to the rise of Big Data generated through normal interactions rather than for research purposes, improving access to the computing power necessary for linking these data [25]. During the COVID-19 pandemic, the linkage of prevalence databases to GIS to generate interactive web-based dashboards enabled the global internet community to track new infections in near-real time [26], proving that the infrastructures and resources already exist to further develop this technology for monitoring ART outcomes and geospatially surveying HIVDR. Furthermore, the development of data dashboards facilitates the communication of data patterns and are pertinent to disease surveillance, enabling the rapid dissemination, comprehension, and interpretation of databases [27]. Policymakers must allocate resources for the development of database and dashboard interface components within the HIV treatment program, as

these technologies will facilitate the standardization and integration of healthcare services across public and private sector healthcare facilities.

5.3. Recommendations for future research

Considering that the geospatial analysis of VLs investigated facilities rather than households, the assumption made was that individuals will seek out the nearest healthcare facility to their dwelling, which is not always true. These differences in testing practices could have impacted our geospatial mapping outcomes and should be taken into consideration in future studies. Moreover, community VLs obtained from population-based surveillance may not consistently align with normal facility-based VL results. Consequently, future studies should incorporate geospatial analysis of VL data at community level by using household-linked GIS data. Moreover, it is essential to prospectively evaluate longitudinal geospatial trends in VLs to assess whether the observed hotspots are a consequence of the COVID-19 pandemic, which will potentially decrease as health systems recover, or if they represent a pattern that may persist or increase over time.

For geospatial analysis of HIVDR, given that GRT testing practices by facility were not incorporated, nor contextualized within the local population of PLWH, nor ART coverage by district, subdistrict or facility, future research should endeavour to integrate VL and GRT data, preferably within the framework of ART coverage, to yield more nuanced insights that facilitate data-driven public health decision-making. Future HIVDR database research should also incorporate algorithms to correlate sequential GRTs, if applicable, and laboratory information systems should be designed to capture ART duration for all previous ARV drugs. These studies were conducted during the shift to DTG-based first-line regimens in South Africa, and further evaluation of the current incidence of HIVDR mutations is needed, especially those linked to DTG resistance, to pinpoint geolocations requiring targeted treatment interventions. Furthermore, future research could include integration of maps with both adult and paediatric HIVDR data, to find areas most in need of intervention, as well as find linked HIVDR problems in certain geographic regions e.g. antenatal and infant HIVDR. Moreover, capacity development in paediatric HIV care, which impacts HIVDR-related outcomes in younger children, should be further strengthened.

In conclusion, there are significant knowledge gaps in the interpretation of LLV in conjunction with high-level viraemia, and there is a lack of near-real-time surveillance of HIVDR across South Africa. Repurposed stored routine VL and HIVDR GRT data for geospatial analysis could prove to be an invaluable monitoring and evaluation tool in generating "real-world" estimates of virological suppression and HIVDR within its geospatial context. Geospatial disparities exist in the burden of HIVDR, and demonstrating this could facilitate data-driven public health decision-making. Continuous real-time HIVDR surveillance remains crucial for assessing the efficacy of the revised South African HIV-1 treatment program.

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Appendices

Appendix 1: Biomedical Research Ethics Committee Approval



10 September 2021

Dr Lilishia Gounder (201293117)
School of Lab Med & Medical Sc
Medical School

Dear Dr Gounder,

Protocol reference number: BREC/00003120/2021
Project title: Monitoring and analysis of HIV drug resistance in KwaZulu-Natal, South Africa
Degree: PhD

EXPEDITED APPLICATION: APPROVAL LETTER

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application.

The conditions have been met and the study is given full ethics approval and may begin as from 10 September 2021. Please ensure that outstanding site permissions are obtained and forwarded to BREC for approval before commencing research at a site.

Note to PI: New certificates are not GCP certificates; they are research ethics certificates but they are acceptable for this study.

This approval is subject to national and UKZN lockdown regulations, see (http://research.ukzn.ac.za/Libraries/BREC/BREC_Amended_Lockdown_Level_3_Guidelines.sflb.ashx). Based on feedback from some sites, we urge PIs to show sensitivity and exercise appropriate consideration at sites where personnel and service users appear stressed or overloaded.

This approval is valid for one year from 10 September 2021. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2020) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at <http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx>.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be noted by a full Committee at its next meeting taking place on 12 October 2021.

Yours sincerely,


Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

Biomedical Research Ethics Committee
Chair: Professor D R Wassenaar
UKZN Research Ethics Office Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
Email: BREC@ukzn.ac.za
Website: <http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx>

Founding Campuses:  Edgewood  Howard College  Medical School  Pietermaritzburg  Westville

INSPIRING GREATNESS

27 October 2022

Dr Lilishia Gounder (201293117)
School of Laboratory Medicine & Medical Science
Medical School

Dear Dr Gounder,

Protocol reference number: BREC/00003120/2021

Project title: Monitoring and analysis of HIV drug resistance in KwaZulu-Natal, South Africa

Degree: PhD

RECERTIFICATION APPLICATION APPROVAL NOTICE

Approved: 10 September 2022

Expiration of Ethical Approval: 09 September 2023

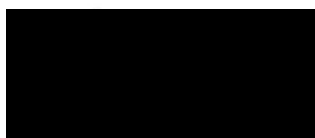
I wish to advise you that your application for Recertification received on 26 September 2022 for the above protocol has been **noted and approved** by a sub-committee of the Biomedical Research Ethics Committee (BREC) for another approval period. The start and end dates of this period are indicated above.

Your response to BREC queries dated 30 September has been noted.

If any modifications or adverse events occur in the project before your next scheduled review, you must submit them to BREC for review. Except in emergency situations, no change to the protocol may be implemented until you have received written BREC approval for the change.

The committee will be notified of the above approval at its next meeting to be held on 08 November 2022.

Yours sincerely



.....
Ms A Marimuthu
(for) Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

18 October 2023

Dr Lilishia Gounder (201293117)
School of Laboratory Medicine & Medical Science
Medical School

Dear Dr Gounder,

Protocol reference number: BREC/00003120/2021

Project title: Monitoring and analysis of HIV drug resistance in KwaZulu-Natal, South Africa

Degree: PhD

RECERTIFICATION APPLICATION APPROVAL NOTICE

Approved: 10 September 2023

Expiration of Ethical Approval: 09 September 2024

I wish to advise you that your application for Recertification for the above protocol has been **noted and approved** by a sub-committee of the Biomedical Research Ethics Committee (BREC) for another approval period. The start and end dates of this period are indicated above.

If any modifications or adverse events occur in the project before your next scheduled review, you must submit them to BREC for review. Except in emergency situations, no change to the protocol may be implemented until you have received written BREC approval for the change.

The committee will be notified of the above approval at its next meeting to be held on 14 November 2023.

Yours sincerely



.....
Ms A Marimuthu
(for) Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

11 September 2024

Dr Lilishia Gounder (201293117)
School of Laboratory Medicine & Medical Science
Medical School

Dear Dr Gounder,

Protocol reference number: BREC/00003120/2021

Project title: Monitoring and analysis of HIV drug resistance in KwaZulu-Natal, South Africa

Degree: PhD

RECERTIFICATION APPLICATION APPROVAL NOTICE

Approved: 10 September 2024

Expiration of Ethical Approval: 09 September 2025

I wish to advise you that your application for Recertification for the above protocol has been **noted and approved** by a sub-committee of the Biomedical Research Ethics Committee (BREC) for another approval period. The start and end dates of this period are indicated above.

If any modifications or adverse events occur in the project before your next scheduled review, you must submit them to BREC for review. Except in emergency situations, no change to the protocol may be implemented until you have received written BREC approval for the change.

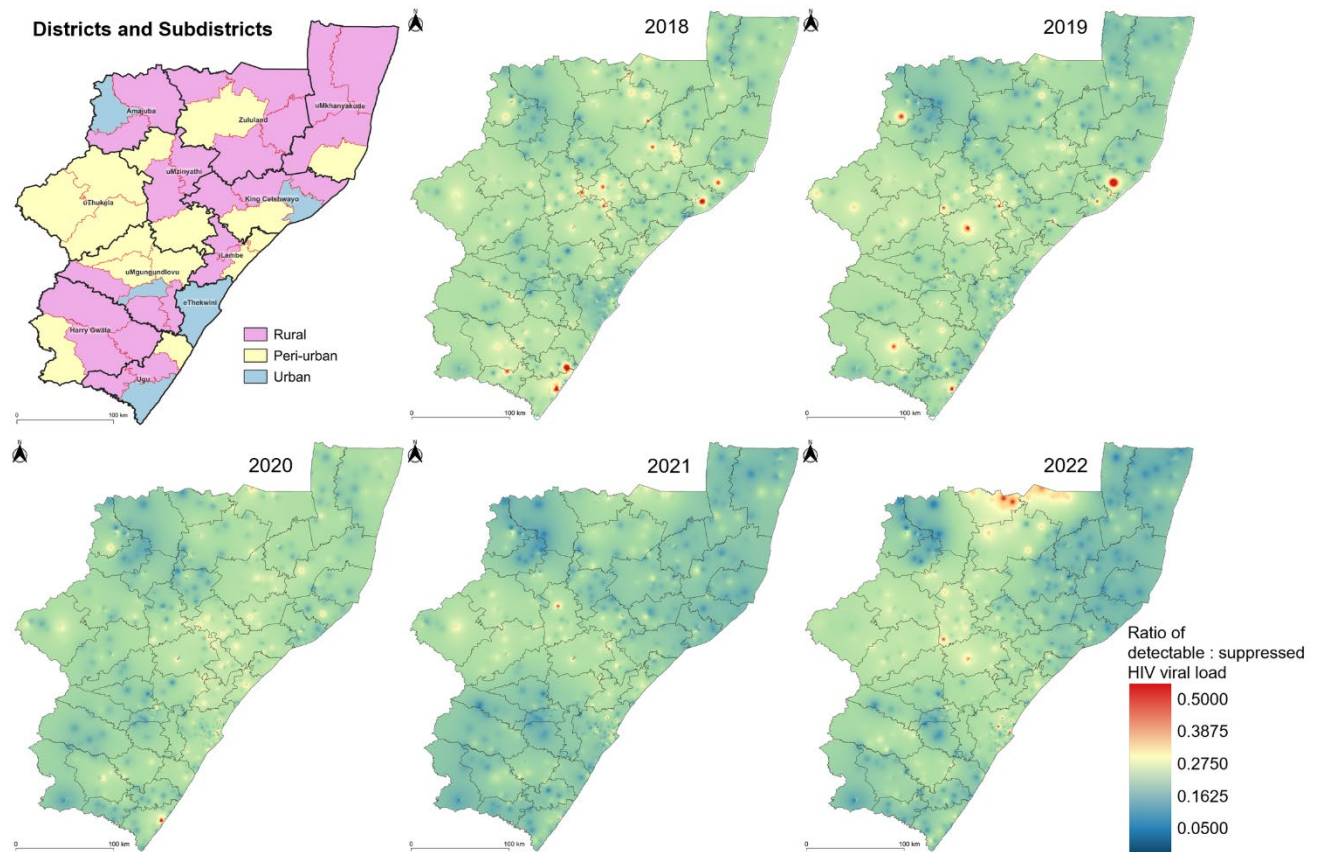
The committee will be notified of the above approval at its next meeting to be held on 08 October 2024.

Yours sincerely



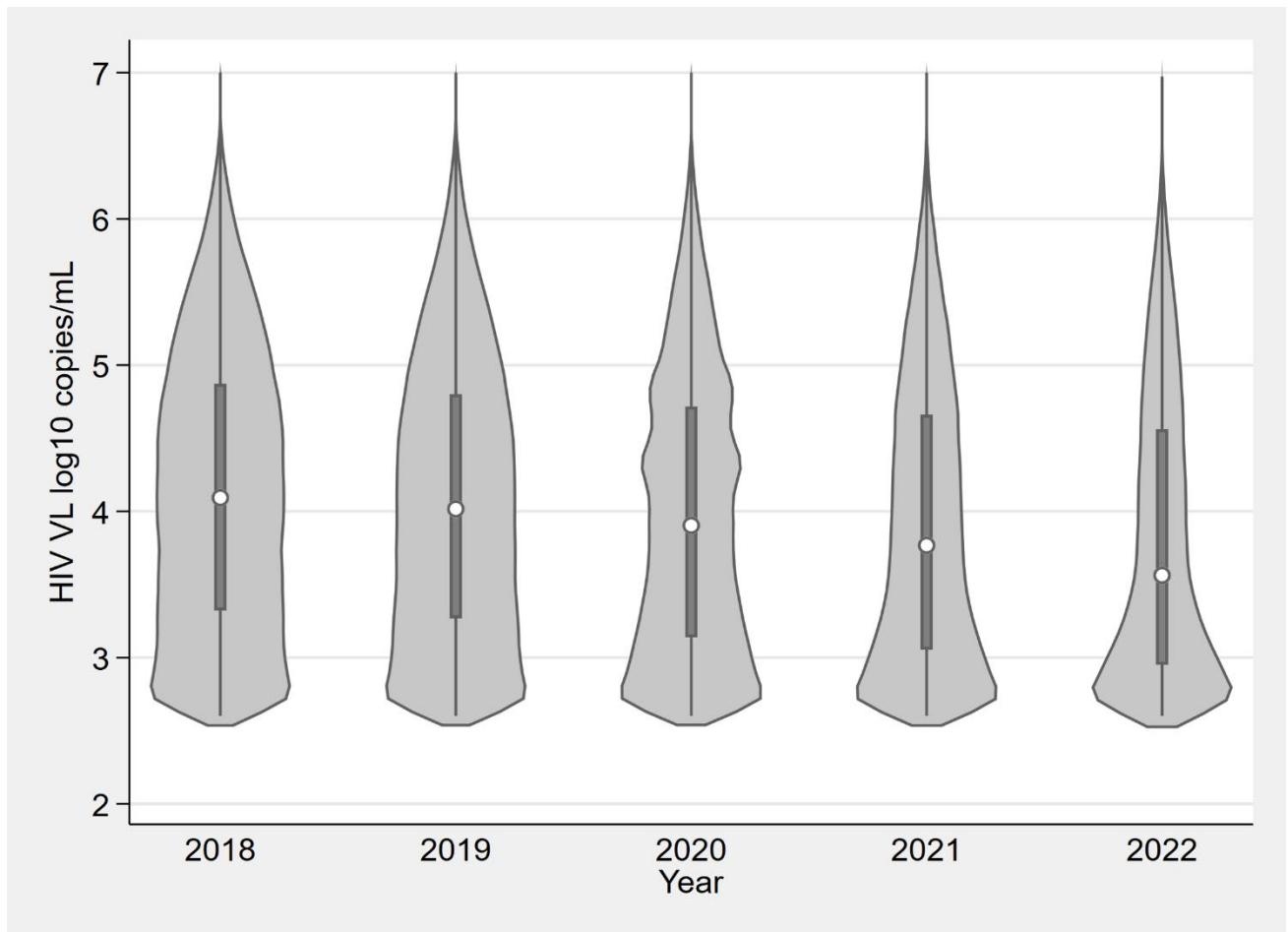
.....
Ms A Marimuthu
(for) Prof S Singh
Chair: Biomedical Research Ethics Committee

Appendix 2: Supplementary material to manuscript entitled “Geospatial and temporal mapping of detectable HIV-1 viral loads amid dolutegravir rollout in KwaZulu-Natal, South Africa”



S1 Fig. Ratio of detectable to suppressed HIV viral loads across subdistricts in KwaZulu-Natal province, South Africa.

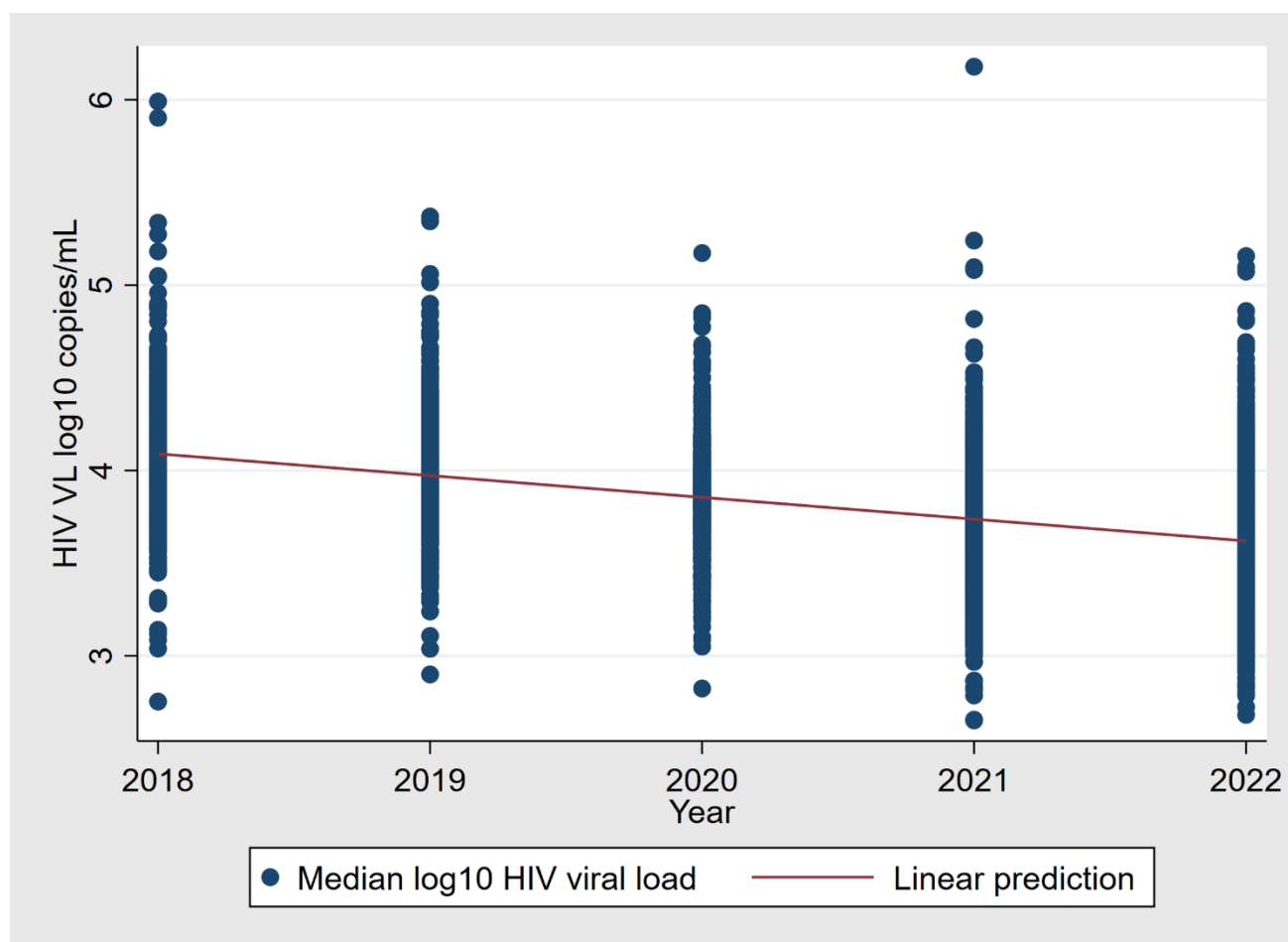
The ratio of detectable (≥ 400 copies/mL) to suppressed (< 400 copies/mL) HIV viral loads were mapped per facility using the inverse distance weighted interpolation method. Spectral colour change from blue to red reflects the increase in ratio of detectable to suppressed HIV viral loads. A ratio of 0,05 indicates 1 detectable viral load per 20 suppressed viral loads, while a ratio of 0,5 indicates 1 detectable viral load per 2 suppressed viral loads. Thick black outlines represent the borders of the 11 districts. Thin outlines represent the borders of the 44 subdistricts of KwaZulu-Natal. Republished from <https://pinea.app.carto.com/map/4d4c56c1-f82d-4409-b190-ea9ced309005> under a CC BY license, with permission from Carto Builder user Lilishia Gounder, original copyright 2024. <https://doi.org/10.1371/journal.pgph.0003224.s001>



S2 Fig. Violin plot of HIV viral loads in KwaZulu-Natal province, South Africa, for the period 2018 to 2022.

mL, millilitre; VL, viral load. The violin plot for log₁₀ viral loads ≥ 400 copies/millilitre sampled across KwaZulu-Natal subdistricts showed a significant downward trend by year, $p < 0.01$ Jonckheere–Terpstra test.

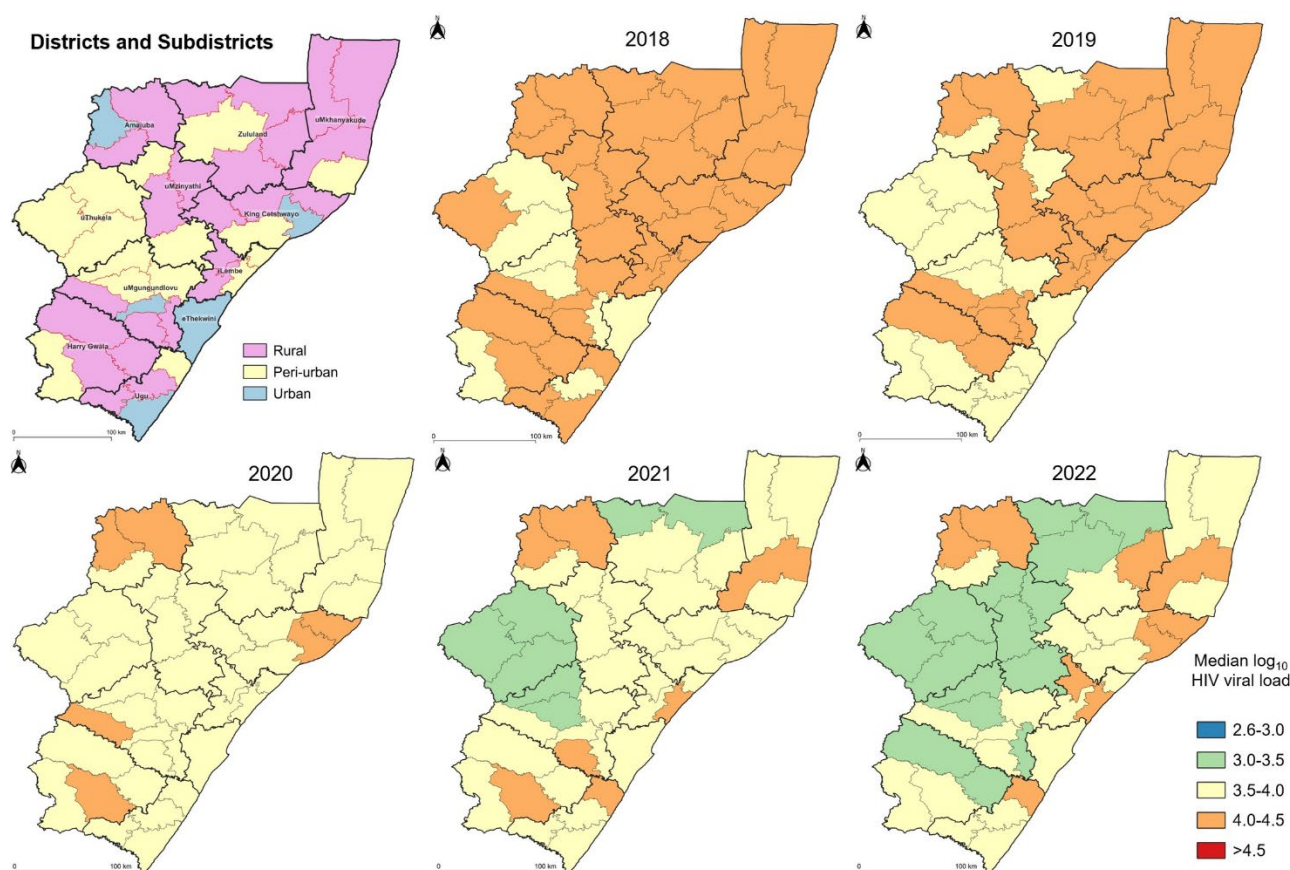
<https://doi.org/10.1371/journal.pgph.0003224.s002>



S3 Fig. Linear prediction of median log10 HIV viral loads per facility in KwaZulu-Natal province, South Africa, for the period 2018 to 2022.

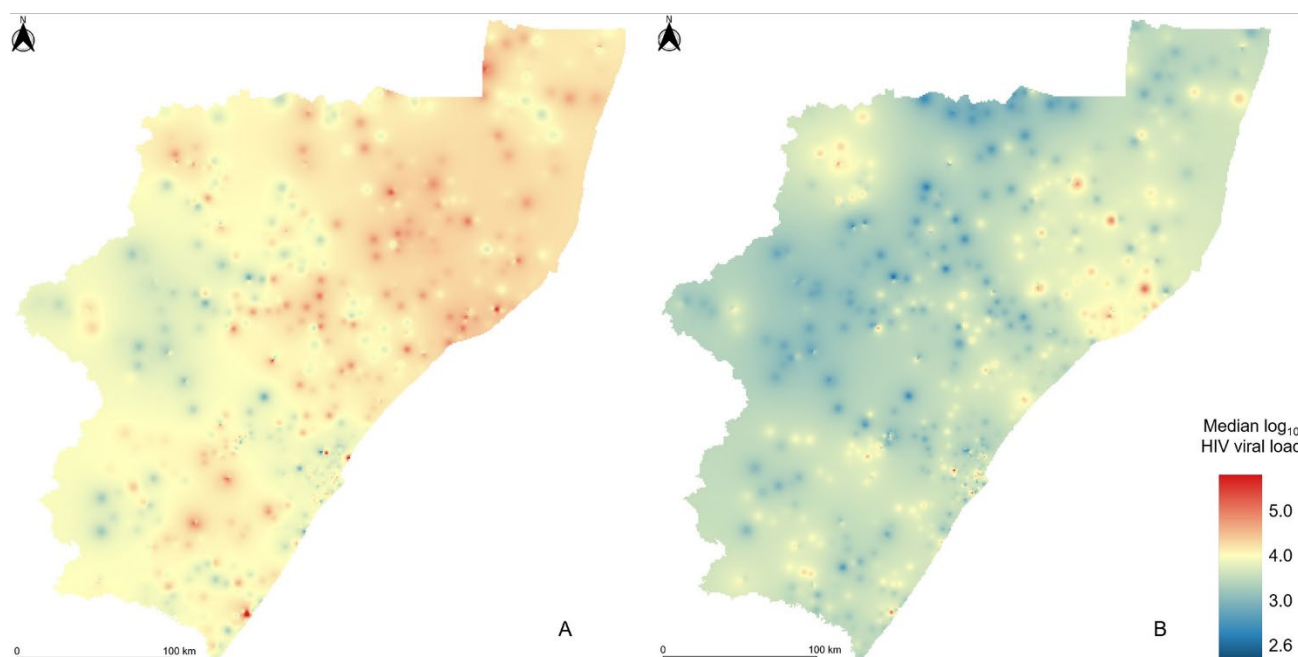
VL, viral load. The linear prediction model for log10 viral loads ≥ 400 copies/millilitre sampled across KwaZulu-Natal subdistricts showed a significant downward trend by year, $p < 0.01$ Jonckheere–Terpstra test.

<https://doi.org/10.1371/journal.pgph.0003224.s003>



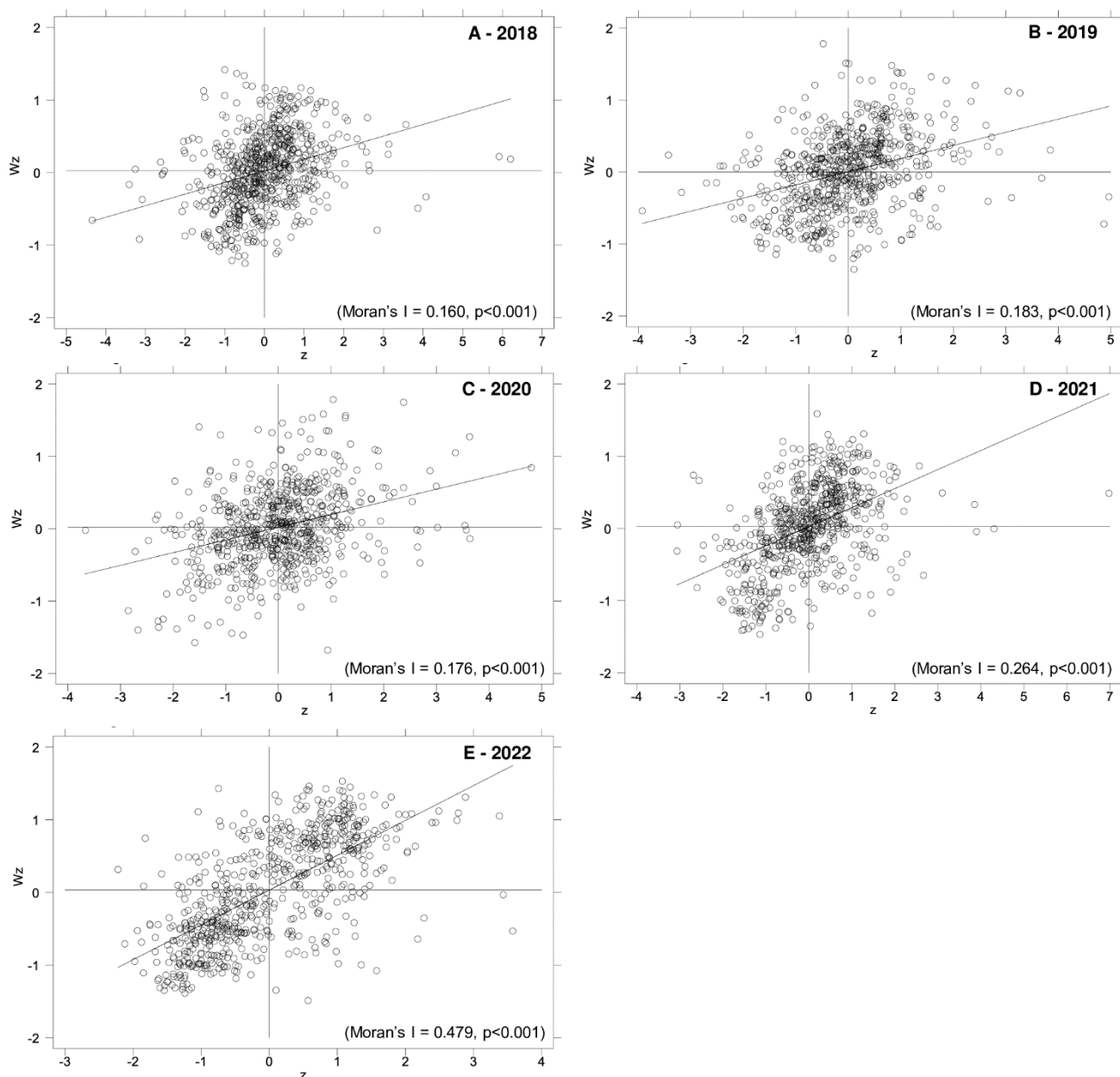
S4 Fig. Decreasing HIV viral loads across subdistricts in KwaZulu-Natal province, South Africa, between 2018 and 2022.

Choropleth maps show median $\log_{10}$ viral loads ≥ 400 copies/millilitre sampled across KwaZulu-Natal subdistricts. Thick black outlines represent the borders of the 11 districts. Thin outlines represent the borders of the 44 subdistricts of KwaZulu-Natal. Republished from <https://pineappleapp.carto.com/map/4d4c56c1-f82d-4409-b190-ea9ced309005> under a CC BY license, with permission from Carto Builder user Lilishia Gounder, original copyright 2024. <https://doi.org/10.1371/journal.pgph.0003224.s004>



S5 Fig. HIV viral loads per facility before and during the transition to dolutegravir in KwaZulu-Natal province, South Africa.

Inverse distance weighted interpolation maps showing changes in median log₁₀ viral loads at the facility level. Map A represents the period before dolutegravir rollout i.e., 2018–2019 and map B represents the transition period to dolutegravir i.e., 2020–2022. Spectral colour change from red to blue reflects a decrease in median log₁₀ viral loads. Republished from <https://pinea.app.carto.com/map/4d4c56c1-f82d-4409-b190-ea9ced309005> under a CC BY license, with permission from Carto Builder user Lilishia Gounder, original copyright 2024. <https://doi.org/10.1371/journal.pgph.0003224.s005>



S6 Fig. Moran scatterplots of median log₁₀ HIV viral loads per facility in KwaZulu-Natal province, South Africa, between 2018 and 2022.

For cluster analysis, we used the median log₁₀ HIV viral load per facility to calculate Moran's I statistic and p-values, and created Moran scatterplots for each year of the study period. Each plot represents an individual year: (A) 2018, (B) 2019, (C) 2020, (D) 2021 and (E) 2022. Moran's I statistic measures spatial autocorrelation and index values for Moran's I statistic range from -1 to +1. A positive index value indicates positive spatial autocorrelation. <https://doi.org/10.1371/journal.pgph.0003224.s006>

S1 Table. Proportion of all HIV viral loads by year and district.

District	VL in copies/mL	2018		2019		2020		2021		2022	
		≥400	<400	≥400	<400	≥400	<400	≥400	<400	≥400	<400
Amajuba	n (%)	7 061 (12.32)	50 258 (87.68)	7 575 (10.95)	61 618 (89.05)	8 111 (11.10)	64 930 (88.90)	7 175 (9.72)	66 632 (90.28)	3 558 (9.28)	34 777 (90.72)
eThekweni	n (%)	59 501 (11.89)	440 837 (88.11)	64 588 (12.24)	463 188 (87.76)	80 485 (14.36)	479 908 (85.64)	69 768 (12.26)	499 531 (87.74)	43 922 (14.17)	266 131 (85.83)
Harry Gwala	n (%)	9 845 (15.99)	51 724 (84.01)	11 161 (15.97)	58 743 (84.03)	10 137 (13.19)	66 719 (86.81)	9 655 (12.77)	65 977 (87.23)	5 706 (14.15)	34 610 (85.85)
iLembe	n (%)	14 249 (16.33)	73 000 (83.67)	14 801 (15.48)	80 810 (84.52)	16 710 (17.37)	79 505 (82.63)	15 639 (15.29)	86 663 (84.71)	7 589 (14.27)	45 605 (85.73)
King Cetshwayo	n (%)	21 198 (13.90)	131 285 (86.10)	21 983 (13.76)	137 835 (86.24)	24 758 (14.27)	148 728 (85.73)	19 448 (11.45)	150 341 (88.55)	10 445 (11.45)	80 809 (88.55)
Ugu	n (%)	18 054 (15.09)	101 626 (84.91)	16 617 (13.43)	107 158 (86.57)	17 036 (12.90)	115 077 (87.10)	15 513 (11.89)	114 999 (88.11)	7 996 (11.81)	59 699 (88.19)
uMgungundlovu	n (%)	21 222 (13.47)	136 376 (86.53)	25 838 (14.69)	150 055 (85.31)	24 394 (12.93)	164 223 (87.07)	22 662 (11.84)	168 766 (88.16)	13 332 (12.88)	90 140 (87.12)
uMkhanyakude	n (%)	15 115 (13.30)	98 534 (86.70)	15 055 (12.65)	103 930 (87.35)	17 541 (13.93)	108 381 (86.07)	14 149 (11.46)	109 353 (88.54)	6 831 (10.11)	60 765 (89.89)
uMzinyathi	n (%)	9 711 (15.30)	53 756 (84.70)	10 046 (14.70)	58 311 (85.30)	9 827 (13.80)	61 387 (86.20)	10 448 (14.52)	61 515 (85.48)	6 721 (17.07)	32 646 (82.93)
uThukela	n (%)	15 708 (16.51)	79 448 (83.49)	18 935 (17.48)	89 386 (82.52)	15 831 (13.82)	98 682 (86.18)	18 869 (15.43)	103 423 (84.57)	12 120 (16.70)	60 438 (83.30)
Zululand	n (%)	16 860 (15.35)	92 948 (84.65)	17 068 (13.94)	105 330 (86.06)	20 633 (15.08)	116 164 (84.92)	19 122 (13.75)	119 952 (86.25)	12 849 (16.26)	66 175 (83.74)

mL, millilitre; VL, viral load

The proportion of VL records collected per facility is cumulatively represented as a proportion per district and grouped according to the district in which that facility is located.

S2 Table. Proportion of HIV viral loads by district and year, from hotspot facilities demonstrating spatial clustering of high median log₁₀ HIV viral loads.

	2018	2019	2020	2021	2022
Number of facilities ^a , (number of VL records) ^b	69 (18 045)	69 (19 460)	55 (21 826)	81 (27 263)	175 (28 912)
District n, (%) ^c					
Amajuba	0 (0.00)	0 (0.00)	2 321 (10.63)	6 784 (24.88)	2 376 (8.22)
eThekwini	0 (0.00)	0 (0.00)	7 096 (32.51)	660 (2.42)	4 746 (16.42)
Harry Gwala	0 (0.00)	246 (1.26)	284 (1.30)	230 (0.84)	0 (0.00)
iLembe	0 (0.00)	0 (0.00)	0 (0.00)	6 370 (23.37)	4 986 (17.25)
King Cetshwayo	4 323 (23.96)	13 796 (70.89)	12 125 (55.55)	7 163 (26.27)	6 265 (21.67)
Ugu	5 854 (32.44)	0 (0.00)	0 (0.00)	0 (0.00)	985 (3.41)
uMgungundlovu	427 (2.37)	1 088 (5.59)	0 (0.00)	1 839 (6.75)	2 813 (9.73)
uMkhanyakude	294 (1.63)	0 (0.00)	0 (0.00)	954 (3.50)	3 946 (13.65)
uMzinyathi	230 (1.27)	190 (0.98)	0 (0.00)	0 (0.00)	0 (0.00)
Zululand	6 917 (38.33)	4 140 (21.27)	0 (0.00)	3 263 (11.97)	2 795 (9.67)

VL, viral load

^a 244 unique hotspot facilities demonstrating spatial clustering of high median log₁₀ HIV VLs were identified from 2018 to 2022 cumulatively. However, some facilities were repeatedly identified as hotspot facilities and were therefore included in the different analyses for each of those specific years.

^b Each unique viral load record was included in the analysis based on the status of the facility and the specific year in which it was collected. If facilities were identified as statistically significant hotspots based on $p < 0.05$ Getis-Ord Gi* statistic, the viral load records linked to that facility and year were included in the respective analysis.

^c Hotspot facilities were identified in 10 of the 11 districts. Facilities from uThukela did not have spatial clustering of high median log₁₀ HIV VLs during the study period. The proportion of VL records per hotspot facility is cumulatively represented as a proportion per district, grouped according to the district in which that facility is located.

S3 Table. Comparison of facilities within hot- and coldspots from 2018 to 2022

Variable	2018		2019		2020		2021		2022	
	Hotspot	Coldspot	Hotspot	Coldspot	Hotspot	Coldspot	Hotspot	Coldspot	Hotspot	Coldspot
Number of facilities, (number of VL records) ^a	69 (18 045)	63 (28 710)	69 (19 460)	78 (30 789)	55 (21 826)	49 (11 951)	81 (27 263)	79 (29 776)	175 (28 912)	127 (32 224)
Sex										
Male	6 333 (35.10%)	10 543 (36.72%)	7 136 (36.67%)	10 748 (34.91%)	7 501 (34.37%)	4 340 (36.31%)	9 499 (34.84%)	10 459 (35.13%)	10 161 (35.14%)	11 397 (35.37%)
Female	11 249 (62.34%)	17 505 (60.97%)	11 653 (59.88%)	19 338 (62.81%)	13 618 (62.39%)	7 390 (61.84%)	16 583 (60.83%)	18 301 (61.46%)	17 709 (61.25%)	19 902 (61.76%)
Unknown	463 (2.57%)	662 (2.31%)	671 (3.45%)	703 (2.28%)	707 (3.24%)	221 (1.85%)	1 181 (4.33 %)	1 016 (3.41%)	1 042 (3.60%)	925 (2.87%)
Age in years, median (IQR)										
<5	313 (1.73%)	481 (1.68%)	250 (1.28%)	393 (1.28%)	416 (1.91%)	122 (1.02%)	509 (1.87%)	362 (1.22%)	483 (1.67%)	308 (0.96%)
5-14	1 556 (8.62%)	2 201 (7.67%)	1 319 (6.78%)	2 048 (6.65%)	1 202 (5.51%)	1 151 (9.63%)	1 734 (6.36%)	2 299 (7.72%)	1 813 (6.27%)	1 894 (5.88%)
15-24	3 168 (17.56%)	4 541 (15.82%)	3 320 (17.06%)	4 648 (15.10%)	3 298 (15.11%)	2 210 (18.49%)	4 786 (17.55%)	4 591 (15.42%)	4 914 (17.00%)	4 095 (12.71%)
25-49	11 404 (63.20%)	18 730 (65.24%)	12 571 (64.60%)	20 348 (66.09%)	14 797 (67.80%)	6 897 (57.71%)	17 109 (62.76%)	18 169 (61.02%)	18 185 (62.90%)	20 482 (63.56%)
>50	1 402 (7.77%)	2 588 (9.01%)	1 783 (9.16%)	3 090 (10.04%)	1 823 (8.35%)	1 499 (12.54%)	2 692 (9.87%)	4 043 (13.58%)	3 208 (11.10%)	5 160 (16.01%)
Unknown	202 (1.12%)	169 (0.59%)	217 (1.12%)	262 (0.85%)	290 (1.33%)	72 (0.60%)	433 (1.59%)	312 (1.05%)	309 (1.07%)	285 (0.88%)
VL log₁₀ copies/mL, median (IQR)										
	4.267 (3.455- 5.041)	3.883 (3.188- 4.673)	4.250 (3.436- 5.009)	3.818 (3.143- 4.612)	4.090 (3.276- 4.849)	3.627 (3.017- 4.566)	4.072 (3.258- 4.894)	3.368 (2.904- 4.324)	4.08 (3.267- 4.919)	3.196 (2.848- 4.086)
Healthcare department										
Outpatients	16 556 (91.75%)	26 526 (92.39%)	17 750 (91.21%)	29 135 (94.63%)	19 249 (88.19%)	11 076 (92.68%)	23 309 (85.50%)	27 445 (92.17%)	25 221 (87.23%)	30 609 (94.99%)
Inpatients	1 451 (8.04%)	2 163 (7.53%)	1 654 (8.50%)	1 612 (5.24%)	2 470 (11.32%)	855 (7.15%)	3 869 (14.19%)	2 260 (7.59%)	3 661 (12.66%)	1 540 (4.78%)
Other ^b	38 (0.21%)	21 (0.07%)	56 (0.29%)	42 (0.14%)	107 (0.49%)	20 (0.17%)	85 (0.31%)	71 (0.24%)	30 (0.10%)	75 (0.23%)
Level of urbanization^c										
Rural subdistricts	7 702 (42.68%)	394 (1.37%)	8 351 (42.91%)	2 172 (7.05%)	1 974 (9.04%)	5 953 (49.81%)	5 302 (19.45%)	6 956 (23.36%)	8 341 (28.85%)	6 973 (21.64%)
Peri-urban subdistricts	1 298 (7.19%)	12 520 (43.61%)	809 (4.16%)	16 386 (53.22%)	567 (2.60%)	5 998 (50.19%)	7 771 (28.50%)	18 660 (62.67%)	6 430 (22.24%)	16 603 (51.52%)
Urban subdistricts	9 045 (50.12%)	15 796 (55.02%)	10 300 (52.93%)	12 231 (39.73%)	19 285 (88.36%)	0 (0.00%)	14 190 (52.05%)	4 160 (13.97%)	14 141 (48.91%)	8 648 (26.84%)

IQR, interquartile range; mL, millilitre; VL, viral load

^a Each unique viral load record was included in the analysis based on the status of the facility and the specific year in which it was collected. If facilities were identified as statistically significant hotspots or coldspots based on $p < 0.05$ Getis-Ord Gi* statistic, the viral load records linked to that facility and year were included in the respective analysis.

^b Records from correctional facilities or frail care facilities.

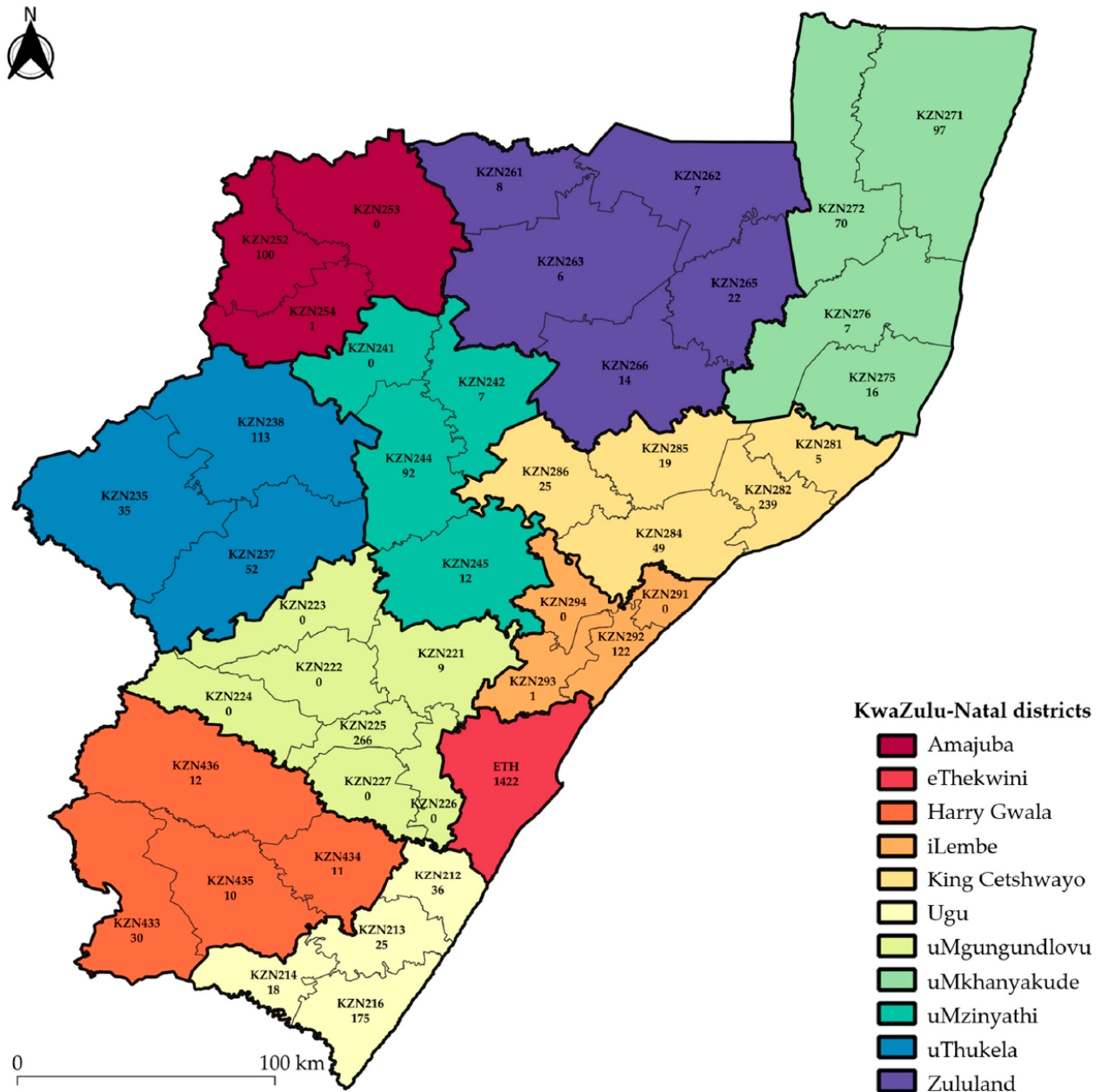
^c Obtained from annual reports and integrated development plans for individual subdistricts.

S1 Data. Minimal dataset.

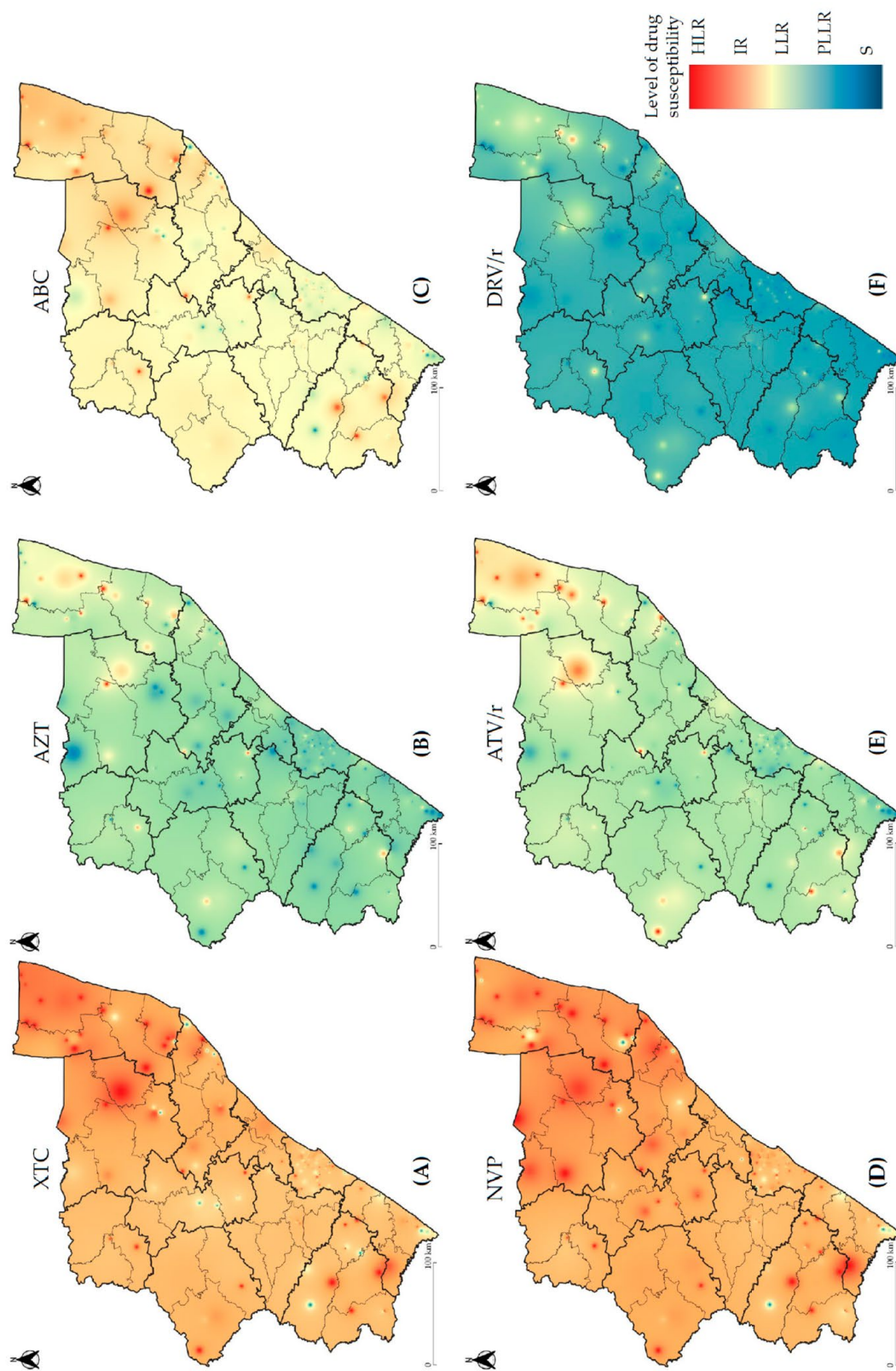
<https://doi.org/10.1371/journal.pgph.0003224.s010>

hcf_code	hcf_location_district	hcf_location_subdistrict	year	hcf_level_urbanization	hcf_level_care	median_age_per_hcf	median_log10_HIVVL_per_hcf	total_females_per_hcf	total_cases_per_hcf	cases_HIVVL_<400	cases_HIVVL_400-999	cases_HIVVL_≥1000
1	uThukela	Alfred Duma	2018	peri-urban	PHC/CHC/District Hospital	37	3,6201	1485	2306	2043	76	209
1	uThukela	Alfred Duma	2019	peri-urban	PHC/CHC/District Hospital	37	3,85	1597	2396	2043	74	279
1	uThukela	Alfred Duma	2020	peri-urban	PHC/CHC/District Hospital	38	3,91665	2493	2493	2167	54	272
1	uThukela	Alfred Duma	2021	peri-urban	PHC/CHC/District Hospital	38	3,2457	1874	3045	2544	151	350
1	uThukela	Alfred Duma	2022	peri-urban	PHC/CHC/District Hospital	39	3,2273	1195	1917	1573	108	236
2	eThekweni	eThekweni	2018	urban	PHC/CHC/District Hospital	35	3,97035	1408	2209	1933	53	223
2	eThekweni	eThekweni	2019	urban	PHC/CHC/District Hospital	36	3,849	1628	2452	2183	45	224
2	eThekweni	eThekweni	2020	urban	PHC/CHC/District Hospital	37	3,8413	1887	2901	2462	93	346
2	eThekweni	eThekweni	2021	urban	PHC/CHC/District Hospital	37	3,8404	1972	2995	2668	70	257
2	eThekweni	eThekweni	2022	urban	PHC/CHC/District Hospital	38	3,75515	1019	1537	1361	44	132
3	eThekweni	Regional Hospital	2018	urban	Regional Hospital	36	4,0462	6516	9654	7885	245	1524
3	eThekweni	Regional Hospital	2019	urban	Regional Hospital	36	4,0815	6391	9539	7767	245	1527
3	eThekweni	Regional Hospital	2020	urban	Regional Hospital	36	4,1481	7050	10144	8163	281	1700
3	eThekweni	Regional Hospital	2021	urban	Regional Hospital	36	4,0764	7154	10531	8662	283	1586
3	eThekweni	Regional Hospital	2022	urban	Regional Hospital	37	4,2438	4308	6336	5163	158	1015
4	uThukela	Inkosi Langalibalele	2018	peri-urban	PHC/CHC/District Hospital	37	3,9316	1883	1664	1664	36	183
4	uThukela	Inkosi Langalibalele	2019	peri-urban	PHC/CHC/District Hospital	37	3,6972	1584	2117	1817	60	240
4	uThukela	Inkosi Langalibalele	2020	peri-urban	PHC/CHC/District Hospital	37	3,7126	2219	2935	2532	90	313
4	uThukela	Inkosi Langalibalele	2021	peri-urban	PHC/CHC/District Hospital	37	3,31665	2087	2816	2360	153	303
4	uThukela	Inkosi Langalibalele	2022	peri-urban	PHC/CHC/District Hospital	38	3,2148	1297	1799	1530	97	172
5	Zululand	uPhongolo	2018	rural	PHC/CHC/District Hospital	34	4,1204	808	1089	917	30	142
5	Zululand	uPhongolo	2019	rural	PHC/CHC/District Hospital	35	3,8492	894	1218	1049	30	139
5	Zululand	uPhongolo	2020	rural	PHC/CHC/District Hospital	36	3,8543	913	1271	1098	40	133
5	Zululand	uPhongolo	2021	rural	PHC/CHC/District Hospital	36	3,195	1051	1454	1221	79	153
5	Zululand	uPhongolo	2022	rural	PHC/CHC/District Hospital	37	3,0704	647	894	681	96	117
6	Anajuba	Newcastle	2018	urban	PHC/CHC/District Hospital	38	3,8192	113	342	320	4	18
6	Anajuba	Newcastle	2019	urban	PHC/CHC/District Hospital	39	4,5281	118	355	330	5	20
6	Anajuba	Newcastle	2020	urban	PHC/CHC/District Hospital	40	4,4493	377	457	440	7	10
6	Anajuba	Newcastle	2021	urban	PHC/CHC/District Hospital	41	3,8546	68	239	232	3	4
6	Anajuba	Newcastle	2022	urban	PHC/CHC/District Hospital	41	3,294	320	440	390	4	46
7	uMzinyathi	Umvoti	2018	peri-urban	PHC/CHC/District Hospital	36	3,87545	320	425	386	10	29
7	uMzinyathi	Umvoti	2019	peri-urban	PHC/CHC/District Hospital	37	3,7058	328	425	415	12	32
7	uMzinyathi	Umvoti	2020	peri-urban	PHC/CHC/District Hospital	38	3,87965	342	459	408	20	37
7	uMzinyathi	Umvoti	2021	peri-urban	PHC/CHC/District Hospital	38	3,1202	330	465	408	20	37
7	uMzinyathi	Umvoti	2022	peri-urban	PHC/CHC/District Hospital	39	3,1762	246	320	282	14	24
8	Itembe	Maphumulo	2018	rural	PHC/CHC/District Hospital	36	4,2624	389	512	431	13	68
8	Itembe	Maphumulo	2019	rural	PHC/CHC/District Hospital	36,5	3,9339	410	561	470	11	80
8	Itembe	Maphumulo	2020	rural	PHC/CHC/District Hospital	38	3,96945	503	672	526	33	113
8	Itembe	Maphumulo	2021	rural	PHC/CHC/District Hospital	37	4,01885	524	683	567	23	93
8	Itembe	Maphumulo	2022	rural	PHC/CHC/District Hospital	37	3,9912	275	375	312	10	53
9	eThekweni	eThekweni	2018	urban	PHC/CHC/District Hospital	36	3,9289	1758	2518	2236	43	239
9	eThekweni	eThekweni	2019	urban	PHC/CHC/District Hospital	37	3,9795	2319	3266	2938	45	283
9	eThekweni	eThekweni	2020	urban	PHC/CHC/District Hospital	37	4,0128	2474	3516	2987	88	441
9	eThekweni	eThekweni	2021	urban	PHC/CHC/District Hospital	38	3,8092	2262	3351	2970	71	310
9	eThekweni	eThekweni	2022	urban	PHC/CHC/District Hospital	39	3,25	1448	2031	1755	104	172
10	eThekweni	eThekweni	2018	urban	PHC/CHC/District Hospital	35	3,88965	3347	4872	4404	74	394
10	eThekweni	eThekweni	2019	urban	PHC/CHC/District Hospital	35	3,6456	3727	5272	4703	113	456
10	eThekweni	eThekweni	2020	urban	PHC/CHC/District Hospital	35	3,8821	3803	5424	4718	135	571
10	eThekweni	eThekweni	2021	urban	PHC/CHC/District Hospital	36	3,442	4056	5772	5080	202	490
10	eThekweni	eThekweni	2022	urban	PHC/CHC/District Hospital	37	3,2671	2278	3425	2890	189	346
11	eThekweni	eThekweni	2020	urban	PHC/CHC/District Hospital	35		1	1	1	0	0
11	eThekweni	eThekweni	2021	urban	PHC/CHC/District Hospital	31	2,9666	6	6	5	1	0
12	uMzinyathi	Umvoti	2018	peri-urban	PHC/CHC/District Hospital	36	3,9063	1333	1847	1538	58	251
12	uMzinyathi	Umvoti	2019	peri-urban	PHC/CHC/District Hospital	36	3,8825	1419	1950	1629	64	257
12	uMzinyathi	Umvoti	2020	peri-urban	PHC/CHC/District Hospital	37	3,7415	1422	1934	1650	61	223
12	uMzinyathi	Umvoti	2021	peri-urban	PHC/CHC/District Hospital	37	3,6067	1479	1965	1648	84	233
12	uMzinyathi	Umvoti	2022	peri-urban	PHC/CHC/District Hospital	39	3,42105	793	1060	866	55	139
13	uThukela	Okhahlamba	2018	peri-urban	PHC/CHC/District Hospital	37	3,8549	1040	1456	1273	30	153
13	uThukela	Okhahlamba	2019	peri-urban	PHC/CHC/District Hospital	37	3,7686	1346	1909	1619	62	228
13	uThukela	Okhahlamba	2020	peri-urban	PHC/CHC/District Hospital	37	3,8549	1524	2143	1902	56	185
13	uThukela	Okhahlamba	2021	peri-urban	PHC/CHC/District Hospital	38	3,48385	1463	2102	1882	68	152
13	uThukela	Okhahlamba	2022	peri-urban	PHC/CHC/District Hospital	39	3,2005	891	1311	1098	79	134
14	uMgungundlovu	uMshwathi	2018	peri-urban	PHC/CHC/District Hospital	35	4,0211	4190	5955	4658	239	1058
14	uMgungundlovu	uMshwathi	2019	peri-urban	PHC/CHC/District Hospital	36	4,0086	4811	6680	5363	213	1104
14	uMgungundlovu	uMshwathi	2020	peri-urban	PHC/CHC/District Hospital	37	4,0257	4327	6182	5022	204	956
14	uMgungundlovu	uMshwathi	2021	peri-urban	PHC/CHC/District Hospital	37	3,8833	4922	6811	5710	209	892

Appendix 3: Supplementary material to manuscript entitled “Patterns of HIV-1 drug resistance observed through geospatial analysis of routine diagnostic testing in KwaZulu-Natal, South Africa”



Supplementary Figure S1. Proportion of HIV-1 genotypic resistance tests per subdistrict in KwaZulu-Natal, South Africa. The sub-district name together with the total number of genotypes received from that respective subdistrict, is shown in black text on the map. The thin and thick black outlines represent the borders of the subdistricts and districts, respectively. Each district is illustrated in a different colour. The basemap of KwaZulu-Natal province was republished under a CC BY license with permission obtained from Carto Builder user Lilishia Gounder, original copyright 2024. Available at: <https://pinea.app.carto.com/map/4d4c56c1-f82d-4409-b190-ea9ced309005>.



Supplementary Figure S2. Interpolation maps of antiretroviral drug susceptibility levels across KwaZulu-Natal province, South Africa. Inverse distance weighted interpolation maps cumulatively reflect the drug susceptibilities for: (A) XTC, lamivudine or emtricitabine; (B) AZT, zidovudine; (C) ABC, abacavir; (D) NVP, nevirapine; (E) ATV/r, atazanavir with boosted ritonavir and (F) DRV/r, darunavir with boosted ritonavir. Spectral colour change from blue to red reflects the drug susceptibility level as follows: S, susceptible; PLLR, potential low-level resistance; LLR, low-level resistance; IR, intermediate resistance; HLR, high-level resistance. The thin and thick black outlines represent the borders of the 44 subdistricts and 11 districts of KwaZulu-Natal (KZN) province, respectively. The basemap of KZN province was republished under a CC BY license with permission obtained from Carto Builder user Lilishia Gounder, original copyright 2024. Available at: <https://pineapple.app.carto.com/map/4d4c56c1-f82d-4409-b190-ea9ced309005>.

Supplementary Table S1. HIV-1 genotypic resistance tests categorized by the districts and subdistricts of KwaZulu-Natal province, South Africa.

District	Subdistrict	Total no. of GRTs	GRTs without HIVDR	GRTs with HIVDR
eThekweni	ETH	1422 (45.39%)	204 (14.35%)	1218 (85.65%)
Ugu	KZN212	36 (1.15%)	5 (13.89%)	31 (86.11%)
Ugu	KZN213	25 (0.80%)	5 (20.00%)	20 (80.00%)
Ugu	KZN214	18 (0.57%)	0 (0.00%)	18 (100.00%)
Ugu	KZN216	175 (5.59%)	27 (15.43%)	148 (84.57%)
uMgungundlovu	KZN221	9 (0.29%)	2 (22.22%)	7 (77.78%)
uMgungundlovu	KZN225	266 (8.49%)	32 (12.03%)	234 (87.97%)
uThukela	KZN235	35 (1.12%)	4 (11.43%)	31 (88.57%)
uThukela	KZN237	52 (1.66%)	8 (15.38%)	44 (84.62%)
uThukela	KZN238	113 (3.61%)	13 (11.50%)	100 (88.50%)
uMzinyathi	KZN242	7 (0.22%)	2 (28.57%)	5 (71.43%)
uMzinyathi	KZN244	92 (2.94%)	12 (13.04%)	80 (86.96%)
uMzinyathi	KZN245	12 (0.38%)	1 (8.33%)	11 (91.67%)
Amajuba	KZN252	100 (3.19%)	16 (16.00%)	84 (84.00%)
Amajuba	KZN254	1 (0.03%)	0 (0.00%)	1 (100.00%)
Zululand	KZN261	8 (0.26%)	0 (0.00%)	8 (100.00%)
Zululand	KZN262	7 (0.22%)	0 (0.00%)	7 (100.00%)
Zululand	KZN263	6 (0.19%)	0 (0.00%)	6 (100.00%)
Zululand	KZN265	22 (0.70%)	0 (0.00%)	22 (100.00%)
Zululand	KZN266	14 (0.45%)	1 (7.14%)	13 (92.86%)
uMkhanyakude	KZN271	97 (3.10%)	4 (4.12%)	93 (95.88%)
uMkhanyakude	KZN272	70 (2.23%)	10 (14.29%)	60 (85.71%)
uMkhanyakude	KZN275	16 (0.51%)	1 (6.25%)	15 (93.75%)
uMkhanyakude	KZN276	7 (0.22%)	0 (0.00%)	7 (100.00%)
King Cetshwayo	KZN281	5 (0.16%)	0 (0.00%)	5 (100.00%)
King Cetshwayo	KZN282	239 (7.63%)	20 (8.37%)	219 (91.63%)
King Cetshwayo	KZN284	49 (1.56%)	4 (8.16%)	45 (91.84%)
King Cetshwayo	KZN285	19 (0.61%)	2 (10.53%)	17 (89.47%)
King Cetshwayo	KZN286	25 (0.80%)	3 (12.00%)	22 (88.00%)
iLembe	KZN292	122 (3.89%)	12 (9.84%)	110 (90.16%)
iLembe	KZN293	1 (0.03%)	0 (0.00%)	1 (100.00%)
Harry Gwala	KZN433	30 (0.96%)	5 (16.67%)	25 (83.33%)
Harry Gwala	KZN434	11 (0.35%)	1 (9.09%)	10 (90.91%)
Harry Gwala	KZN435	10 (0.32%)	1 (10.00%)	9 (90.00%)
Harry Gwala	KZN436	12 (0.38%)	3 (25.00%)	9 (75.00%)

GRT, genotypic resistance test; HIVDR, human immunodeficiency virus drug resistance; no., number

Supplementary Table S2. Patterns of antiretroviral drug class resistance found in 3133 genotypic resistance tests from patients in KwaZulu-Natal province, South Africa.

Drug-class resistance patterns		All N=3133	2018 N=597	2019 N=675	2020 N=763	2021 N=666	2022 ^a N=432
		# GRTs; % (95% CI)	# GRTs; % (95% CI)	# GRTs; % (95% CI)	# GRTs; % (95% CI)	# GRTs; % (95% CI)	# GRTs; % (95% CI)
No resistance	PI/NRTI/NNRTI**	396; 12.64 (11.50-13.85)	75; 12.56 (10.01-15.49)	78; 11.56 (9.24-14.21)	115; 15.07 (12.61-17.81)	71; 10.66 (8.42-13.26)	57; 13.19 (10.15-16.75)
	PI/NRTI/NNRTI/INSTI	2; 0.06 (0.01-0.23)	0; 0.00 (0.00-0.62)*	0; 0.00 (0.00-0.55)*	0; 0.00 (0.00-0.48)*	1; 0.15 (0.00-0.83)	1; 0.23 (0.01-1.28)
Single-class resistance	INSTI	1; 0.03 (0.00-0.18)	0; 0.00 (0.00-0.62)*	0; 0.00 (0.00-0.55)*	0; 0.00 (0.00-0.48)*	0; 0.00 (0.00-0.55)*	1; 0.23 (0.01-1.28)
	PI**	17; 0.54 (0.32-0.87)	7; 1.17 (0.47-2.40)	1; 0.15 (0.00-0.82)	3; 0.39 (0.08-1.14)	2; 0.30 (0.04-1.08)	4; 0.93 (0.25-2.35)
	NRTI**	180; 5.75 (4.96-6.62)	32; 5.36 (3.69-7.48)	36; 5.33 (3.76-7.31)	45; 5.90 (4.33-7.81)	35; 5.26 (3.69-7.23)	32; 7.41 (5.12-10.30)
	NNRTI	6; 0.19 (0.07-0.42)	1; 0.17 (0.00-0.93)	0; 0.00 (0.00-0.55)*	1; 0.13 (0.00-0.73)	2; 0.30 (0.04-1.08)	2; 0.46 (0.06-1.66)
	NNRTI**	379; 12.10 (10.98-13.29)	79; 13.23 (10.62-16.22)	71; 10.52 (8.31-13.08)	101; 13.24 (10.91-15.85)	77; 11.56 (9.23-14.24)	51; 11.81 (8.92-15.23)
Dual-class resistance	NRTI & INSTI	1; 0.03 (0.00-0.18)	0; 0.00 (0.00-0.62)*	0; 0.00 (0.00-0.55)*	0; 0.00 (0.00-0.48)*	0; 0.00 (0.00-0.55)*	1; 0.23 (0.01-1.28)
	PI & NRTI	1; 0.03 (0.00-0.18)	0; 0.00 (0.00-0.62)*	0; 0.00 (0.00-0.55)*	0; 0.00 (0.00-0.48)*	1; 0.15 (0.00-0.83)	0; 0.00 (0.00-0.85)*
	PI & NRTI**	126; 4.02 (3.36-4.77)	24; 4.02 (2.59-5.92)	29; 4.30 (2.90-6.11)	20; 2.62 (1.61-4.02)	34; 5.11 (3.56-7.06)	19; 4.40 (2.67-6.78)
	PI & NNRTI**	8; 0.26 (0.11-0.50)	1; 0.17 (0.00-0.93)	2; 0.30 (0.04-1.07)	2; 0.26 (0.03-0.94)	3; 0.45 (0.09-1.31)	0; 0.00 (0.00-0.85)*
	NRTI & NNRTI	7; 0.22 (0.09-0.46)	0; 0.00 (0.00-0.62)*	0; 0.00 (0.00-0.55)*	3; 0.39 (0.08-1.14)	2; 0.30 (0.04-1.08)	2; 0.46 (0.06-1.66)
	NRTI & NNRTI**	1027; 32.78 (31.14-34.46)	188; 31.49 (27.78-35.39)	229; 33.93 (30.36-37.64)	242; 31.72 (28.43-35.15)	233; 34.98 (31.36-38.74)	135; 31.25 (26.90-35.85)
Triple-class resistance	NRTI, NNRTI & INSTI	6; 0.19 (0.07-0.42)	0; 0.00 (0.00-0.62)*	0; 0.00 (0.00-0.55)*	1; 0.13 (0.00-0.73)	3; 0.45 (0.09-1.31)	2; 0.46 (0.06-1.66)
	PI, NRTI & NNRTI	8; 0.26 (0.11-0.50)	1; 0.17 (0.00-0.93)	3; 0.44 (0.09-1.29)	0; 0.00 (0.00-0.48)*	4; 0.60 (0.16-1.53)	0; 0.00 (0.00-0.85)*
	PI, NRTI & NNRTI**	959; 30.61 (29.00-32.26)	188; 31.49 (27.78-35.39)	223; 33.04 (29.50-36.73)	228; 29.88 (26.65-33.27)	195; 29.28 (25.85-32.90)	125; 28.94 (24.70-33.46)
All-class resistance							
	PI, NRTI, NNRTI & INSTI	9; 0.29 (0.13-0.54)	1; 0.17 (0.00-0.93)	3; 0.44 (0.09-1.29)	2; 0.26 (0.03-0.94)	3; 0.45 (0.09-1.31)	0; 0.00 (0.00-0.85)*

#, number of; CI, confidence interval; GRT, genotypic resistance test; INSTI, integrase strand transfer inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

^a Data only include period from 1 January 2022 to 30 June 2022.

* 1-sided, 97.5% confidence interval.

** GRTs did not include HIV-1 *integrase* testing.

Supplementary Table S3. Characteristics of nine patients on dolutegravir-based treatment with dolutegravir resistance in KwaZulu-Natal, South Africa.

ID	Age	Sex	Log ₁₀ VL	Year ^a	Regimen (with XTC) ^b	GSS	Mutations by drug class			
							INSTI	PI	NRTI	NNRTI
1	37	Female	5.49	2018	TDF+DRV/r+DTG	1.5	<i>T66A</i> <i>Q95K</i> <i>T97A</i> <i>E138K*</i> <i>Y143R</i> <i>S147G</i>	<i>M46I</i> <i>I50V</i> <i>F53L</i> <i>V82A</i>	<i>M41L</i> <i>D67G</i> <i>M184V</i> <i>T215Y</i> <i>K219N</i>	<i>Y181C</i> <i>H221Y</i>
2	40	Female	NA	2021	AZT+ETR+DRV/r+DTG	3.5	<i>N155H</i> <i>G163R</i> <i>D232N</i>	<i>V32I</i> <i>M46I</i> <i>I54V</i> <i>L76V</i> <i>V82A</i> <i>I84V</i>	<i>D67N</i> <i>K70Q</i> <i>V75M</i>	<i>V106M</i> <i>E138A**</i>
3	32	Female	4.74	2021	TDF+DTG	1	<i>E157Q</i> <i>R263K</i>	None	<i>K70Q</i> <i>M184V</i>	<i>A98G</i> <i>K103N</i> <i>P225H</i>
4	39	Male	6.02	2021	TDF+DRV/r+DTG	2	<i>H51Y</i>	<i>M46I</i> <i>I50V</i> <i>T74P</i> <i>V82A</i>	<i>M41L</i> <i>M184V</i> <i>T215F</i> <i>K219R</i>	<i>Y181C</i> <i>F227L</i> <i>K238T</i>
5	33	Male	4.72	2021	ABC+DTG	0	<i>G118R</i> <i>E138T*</i>	None	<i>K65R</i> <i>Y115F</i> <i>M184V</i>	<i>K103N</i> <i>V106M</i> <i>E138A**</i>
6	35	Male	3.20	2021	TDF+DTG	1	<i>R263K</i>	None	<i>K65R</i> <i>L74I</i> <i>M184V</i>	<i>L100I</i> <i>K103N</i> <i>V106M</i> <i>E138Q**</i>
7	32	Male	5.24	2022	TDF+DTG	1	<i>R263K</i>	None	<i>K70E</i> <i>M184V</i>	None
8	36	Female	5.28	2022	AZT+DTG	0	<i>T66I</i> <i>G118R</i> <i>E138K*</i>	None	<i>D67N</i> <i>K70R</i> <i>M184V</i> <i>T215I</i> <i>K219Q</i>	<i>A98G</i> <i>K103N</i> <i>P225H</i> <i>K238T</i>
9	55	Female	4.18	2022	AZT+DTG	0.5	<i>L74M</i> <i>G118R</i> <i>E138K*</i>	None	<i>D67N</i> <i>K70R</i> <i>M184V</i> <i>K219E</i>	<i>K103N</i> <i>P225H</i>

ABC, abacavir; AZT, zidovudine; DRV/r, darunavir with boosted ritonavir; DTG, dolutegravir; ETR, etravirine; INSTI, integrase strand transfer inhibitor; NA, not available; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; TDF, tenofovir disoproxil fumarate; VL, viral load; XTC, lamivudine or emtricitabine.

Italics indicate accessory or other non-major mutations as classified by the 2022 edition of the IAS–USA drug resistance mutations list in conjunction with Stanford HIV Drug Resistance Database.

^a Year of sample collection for HIV-1 genotypic resistance testing.

^b All regimens included either lamivudine or emtricitabine.

* Classified as a minor mutation in the 2022 edition of the IAS–USA drug resistance mutations list, but as a major mutation in Stanford HIV Drug Resistance Database.

** Classified as a major mutation to rilpivirine only, in the 2022 edition IAS–USA drug resistance mutations list.

Supplementary Table S4. HIV-1 drug resistance mutations detected in genotypic resistance tests from patients in KwaZulu-Natal, South Africa.

PI (N=2735)		NRTI (N=2735)		NNRTI (N=2735)		INSTI (N=39*)	
Mutation	No. GRTs (%)	Mutation	No. GRTs (%)	Mutation	No. GRTs (%)	Mutation	No. GRTs (%)
<i>L10F</i>	552; (20.18)	A62V	62; (2.27)	<i>V90I</i>	36; (1.32)	T66A*	1; (2.56)
<i>L10I</i>	80; (2.93)	M41L	364; (13.31)	A98G	444; (16.23)	T66I	1; (2.56)
<i>L10R</i>	0; (0.00)	K65E	2; (0.07)	L100I	67; (2.45)	T66K*	0; (0.00)
<i>L10V</i>	74; (2.71)	K65N	3; (0.11)	K101E	247; (9.03)	<i>L74M</i>	1; (2.56)
<i>V11I</i>	9; (0.33)	K65R	170; (6.22)	K101H	53; (1.94)	<i>E92G</i>	0; (0.00)
<i>K20M</i>	14; (0.51)	<i>D67E</i>	3; (0.11)	K101P	51; (1.86)	<i>E92Q</i>	0; (0.00)
<i>K20R</i>	408; (14.92)	<i>D67G</i>	121; (4.42)	K103N	1302; (47.61)	<i>T97A</i>	3; (7.69)
<i>K20T</i>	170; (6.22)	<i>D67N</i>	670; (24.50)	K103S	116; (4.24)	G118R	3; (7.69)
<i>L23I</i>	106; (3.84)	<i>T69del</i>	15; (0.55)	V106A	3; (0.11)	F121Y	0; (0.00)
<i>L24I</i>	190; (6.95)	T69ins	3; (0.11)	<i>V106I</i>	66; (2.41)	E138A*	1; (2.56)
D30N	0; (0.00)	K70E	106; (3.88)	V106M	423; (15.47)	E138K*	3; (7.69)
V32I	63; (2.30)	K70R	614; (22.45)	<i>V106T</i>	0; (0.00)	E138T*	1; (2.56)
<i>L33F</i>	216; (7.90)	<i>K70Q</i>	28; (1.02)	V108I	175; (6.40)	G140A*	2; (5.13)
<i>M36I</i>	0; (0.00)	<i>L74I</i>	78; (2.85)	<i>E138A**</i>	247; (9.03)	G140C*	0; (0.00)
<i>M36L</i>	0; (0.00)	L74V	46; (1.68)	<i>E138G**</i>	49; (1.79)	G140R	0; (0.00)
<i>M36V</i>	0; (0.00)	V75A	1; (0.04)	<i>E138K**</i>	27; (0.99)	G140S*	0; (0.00)
<i>K43T</i>	77; (2.82)	V75I	58; (2.12)	<i>E138Q**</i>	52; (1.90)	Y143C	0; (0.00)
M46I	707; (25.85)	V75M	75; (2.74)	<i>E138R**</i>	2; (0.07)	Y143H	0; (0.00)
M46L	81; (2.96)	V75T	12; (0.44)	<i>V179D</i>	171; (6.25)	Y143R	2; (5.13)
I47A	51; (1.86)	V75S	0; (0.00)	<i>V179F</i>	5; (0.18)	Y143S	0; (0.00)
I47V	34; (1.24)	F77L	20; (0.73)	<i>V179L**</i>	3; (0.11)	S147G	1; (2.56)
G48M	0; (0.00)	Y115F	74; (2.71)	<i>V179T</i>	21; (0.77)	Q148H	0; (0.00)
G48V	7; (0.26)	F116Y	11; (0.40)	Y181C	218; (7.97)	Q148K	0; (0.00)
I50L	66; (2.41)	Q151M	15; (0.55)	Y181I	4; (0.15)	Q148R	2; (5.13)
I50V	41; (1.50)	M184V	2181; (79.74)	Y181V	7; (0.26)	S153F	0; (0.00)
<i>F53L</i>	50; (1.83)	M184I	13; (0.48)	Y188C	15; (0.55)	S153Y	0; (0.00)
<i>F53Y</i>	3; (0.11)	L210W	60; (2.19)	Y188H	6; (0.22)	N155H	5; (12.82)
<i>I54A</i>	12; (0.44)	T215C	2; (0.07)	Y188L	192; (7.02)	S230R	0; (0.00)
<i>I54L</i>	20; (0.73)	<i>T215D</i>	2; (0.07)	G190A	431; (15.76)	R263K	4; (10.26)
<i>I54M</i>	0; (0.00)	<i>T215E</i>	1; (0.04)	<i>G190E</i>	11; (0.40)		
<i>I54S</i>	0; (0.00)	T215F	238; (8.70)	G190S	24; (0.88)		
<i>I54T</i>	0; (0.00)	<i>T215I</i>	106; (3.88)	<i>H221Y**</i>	111; (4.06)		
<i>I54V</i>	719; (26.29)	<i>T215S</i>	6; (0.22)	P225H	517; (18.90)		
Q58E	163; (5.96)	<i>T215V</i>	17; (0.62)	F227C	0; (0.00)		
<i>I62V</i>	0; (0.00)	T215Y	140; (5.12)	<i>F227I</i>	1; (0.04)		
<i>H69K</i>	0; (0.00)	K219E	302; (11.04)	F227L	158; (5.78)		
<i>H69R</i>	0; (0.00)	K219N	14; (0.51)	F227R	0; (0.00)		
<i>A71T</i>	21; (0.77)	K219Q	340; (12.43)	F227V	0; (0.00)		
<i>A71V</i>	206; (7.53)	K219R	103; (3.77)	M230I	0; (0.00)		
G73A	1; (0.04)			M230L	36; (1.32)		
G73C	3; (0.11)			<i>L234I</i>	5; (0.18)		
G73S	34; (1.24)			<i>Y318F***</i>	0; (0.00)		
G73T	1; (0.04)						
T74P	57; (2.08)						
L76V	385; (14.08)						
V77I	0; (0.00)						
V82A	757; (27.68)						
V82C	36; (1.32)						
V82F	3; (0.11)						
V82L	13; (0.48)						
V82M	6; (0.22)						
V82S	6; (0.22)						
V82T	5; (0.18)						
N83D	10; (0.37)						
<i>I84A</i>	0; (0.00)						
<i>I84C</i>	0; (0.00)						
<i>I84V</i>	137; (5.01)						
<i>I85V</i>	4; (0.15)						
<i>N88D</i>	4; (0.15)						
N88S	29; (1.06)						
<i>L89I</i>	24; (0.88)						
<i>L89M</i>	27; (1.00)						
<i>L89V</i>	8; (0.29)						
L90M	83; (3.03)						

GRT, genotypic resistance test; INSTI, integrase strand transfer inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; No., Number; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

Italics indicate accessory or other non-major mutations as classified by the 2022 edition of the IAS–USA drug resistance mutations list in conjunction with Stanford HIV Drug Resistance Database.

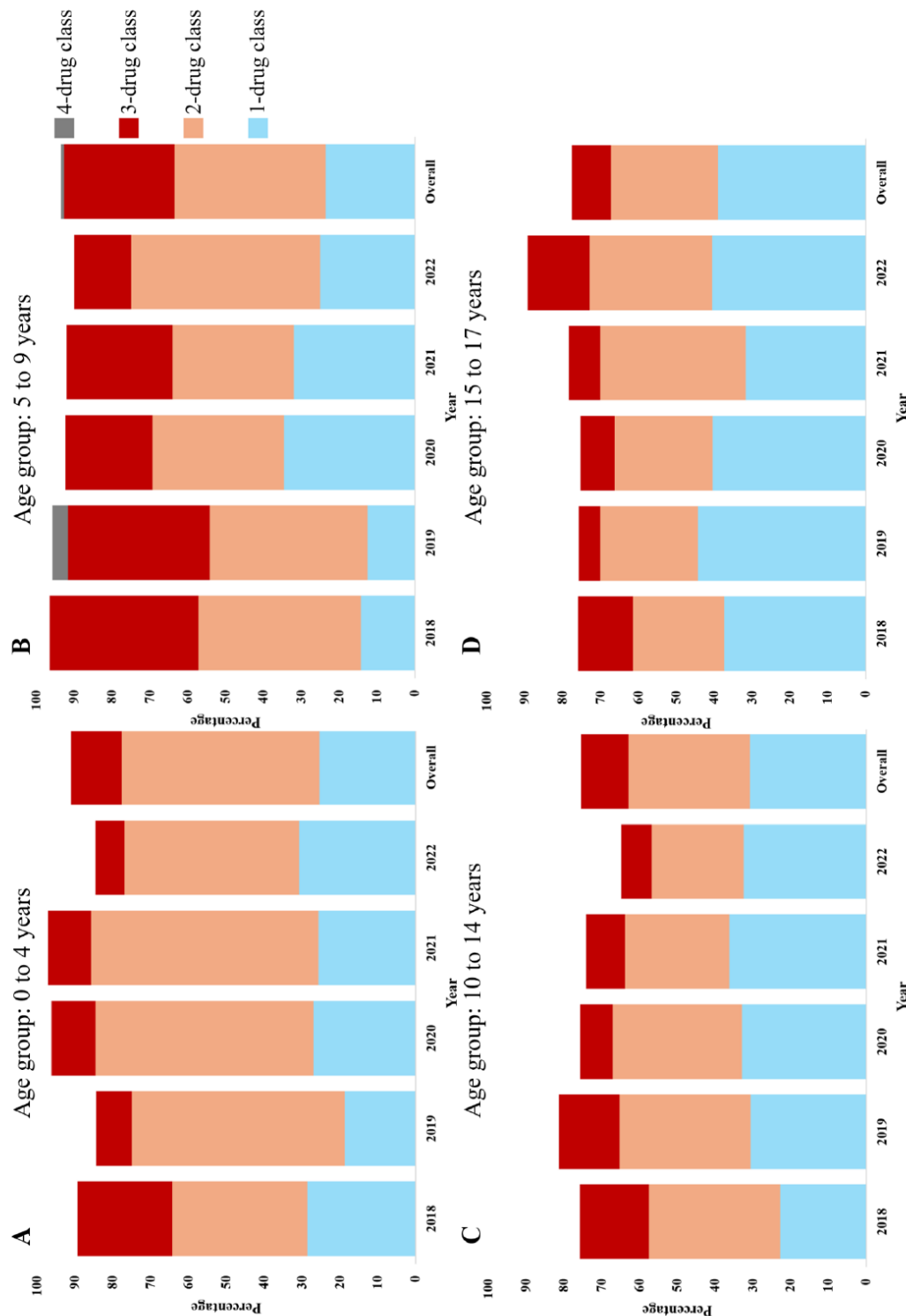
* Only 39 out of 2735 GRTs with resistance had INSTI testing done in addition to PI, NRTI and NNRTI testing.

* Classified as a minor mutation in the 2022 edition of the IAS–USA drug resistance mutations list, but as a major mutation in Stanford HIV Drug Resistance Database.

** Classified as a major mutation to rilpivirine only, in the 2022 edition IAS–USA drug resistance mutations list.

*** Classified as a major mutation to doravirine only, in the 2022 edition IAS–USA drug resistance mutations list.

Appendix 4: Supplementary material to manuscript entitled “Characterization of HIV-1 drug resistance in ART-experienced children and adolescents through geospatial analysis of routine genotypic tests in KwaZulu-Natal, South Africa”



Supplementary Figure S1. (A-D) Temporal changes in proportions of drug-class resistance observed in children and adolescents from KwaZulu-Natal, South Africa. (B) The child with four-drug class resistance was on a regimen containing zidovudine, lamivudine, ritonavir-boosted darunavir and raltegravir.

Supplementary Table S1. HIV-1 drug resistance mutations detected in genotypic resistance tests from children and adolescents in KZN, South Africa

PI (N=740)		NRTI (N=740)		NNRTI (N=740)		INSTI (N=7 ^a)	
Mutation	No. GRTs (%)	Mutation	No. GRTs (%)	Mutation	No. GRTs (%)	Mutation	No. GRTs (%)
<i>L10F</i>	86; (11.62)	M41L	64; (8.65)	<i>V90I</i>	1; (0.14)	<i>M50I</i>	1; (14.29)
<i>L10I</i>	19; (2.57)	A62V	6; (0.81)	<i>A98G</i>	41; (5.54)	<i>H51Y</i>	1; (14.29)
<i>L10R</i>	2; (0.27)	K65E	0; (0.00)	L100I	21; (2.84)	T66A*	0; (0.00)
<i>L10V</i>	5; (0.68)	K65N	0; (0.00)	K101E	31; (4.19)	T66I	1; (14.29)
<i>V11I</i>	1; (0.14)	K65R	10; (1.35)	K101H	11; (1.49)	T66K*	0; (0.00)
<i>K20M</i>	7; (0.95)	<i>D67E</i>	1; (0.14)	K101P	11; (1.49)	<i>L74M</i>	0; (0.00)
<i>K20R</i>	52; (7.03)	<i>D67G</i>	14; (1.89)	K103N	332; (44.86)	<i>E92G</i>	0; (0.00)
<i>K20T</i>	18; (2.43)	D67N	77; (10.41)	K103S	27; (3.65)	E92Q	0; (0.00)
<i>L23I</i>	7; (0.95)	<i>T69del</i>	1; (0.14)	V106A	0; (0.00)	<i>T97A</i>	1; (14.29)
<i>L24I</i>	36; (4.86)	T69ins	0; (0.00)	<i>V106I</i>	11; (1.49)	G118R	1; (14.29)
D30N	0; (0.00)	K70E	10; (1.35)	V106M	102; (13.78)	F121Y	0; (0.00)
V32I	9; (1.22)	K70R	58; (7.84)	<i>V106T</i>	1; (0.14)	E138A*	0; (0.00)
<i>L33F</i>	31; (4.19)	<i>K70Q</i>	3; (0.41)	V108I	17; (2.3)	E138K*	1; (14.29)
<i>M36I</i>	0; (0.00)	<i>L74I</i>	23; (3.11)	<i>E138A**</i>	90; (12.16)	E138T*	0; (0.00)
<i>M36L</i>	0; (0.00)	L74V	99; (13.38)	<i>E138G**</i>	21; (2.84)	G140A*	0; (0.00)
<i>M36V</i>	0; (0.00)	<i>V75A</i>	1; (0.14)	<i>E138K**</i>	7; (0.95)	G140C*	0; (0.00)
<i>K43T</i>	14; (1.89)	V75I	7; (0.95)	<i>E138Q**</i>	6; (0.81)	G140R	0; (0.00)
M46I	124; (16.76)	<i>V75M</i>	6; (0.81)	<i>E138R**</i>	0; (0.00)	G140S*	0; (0.00)
M46L	9; (1.22)	<i>V75T</i>	3; (0.41)	<i>V179D</i>	58; (7.84)	Y143C	0; (0.00)
I47A	8; (1.08)	<i>V75S</i>	0; (0.00)	<i>V179F</i>	0; (0.00)	Y143H	0; (0.00)
I47V	5; (0.68)	F77L	5; (0.68)	<i>V179L**</i>	0; (0.00)	Y143R	1; (14.29)
<i>G48M</i>	0; (0.00)	Y115F	88; (11.89)	<i>V179T</i>	3; (0.41)	<i>Y143S</i>	0; (0.00)
G48V	2; (0.27)	F116Y	0; (0.00)	Y181C	40; (5.41)	S147G	0; (0.00)
I50L	2; (0.27)	Q151M	1; (0.14)	Y181I	0; (0.00)	Q148H	0; (0.00)
I50V	9; (1.22)	M184V	491; (66.35)	Y181V	1; (0.14)	Q148K	0; (0.00)
<i>F53L</i>	5; (0.68)	M184I	1; (0.14)	Y188C	4; (0.54)	Q148R	0; (0.00)
<i>F53Y</i>	0; (0.00)	L210W	11; (1.49)	Y188H	1; (0.14)	<i>S153F</i>	0; (0.00)
<i>I54A</i>	0; (0.00)	<i>T215C</i>	3; (0.41)	Y188L	48; (6.49)	<i>S153Y</i>	0; (0.00)
I54L	2; (0.27)	<i>T215D</i>	1; (0.14)	G190A	67; (9.05)	N155H	0; (0.00)
I54M	0; (0.00)	<i>T215E</i>	0; (0.00)	<i>G190E</i>	7; (0.95)	<i>S230R</i>	0; (0.00)
<i>I54S</i>	2; (0.27)	T215F	41; (5.54)	G190S	4; (0.54)	R263K	0; (0.00)
<i>I54T</i>	0; (0.00)	<i>T215I</i>	12; (1.62)	<i>H221Y**</i>	29; (3.92)		
I54V	141; (19.05)	<i>T215S</i>	2; (0.27)	P225H	84; (11.35)		
Q58E	38; (5.14)	<i>T215V</i>	1; (0.14)	F227C	2; (0.27)		
<i>I62V</i>	0; (0.00)	T215Y	24; (3.24)	<i>F227I</i>	0; (0.00)		
<i>H69K</i>	0; (0.00)	K219E	31; (4.19)	F227L	34; (4.59)		
<i>H69R</i>	0; (0.00)	<i>K219N</i>	2; (0.27)	<i>F227R</i>	0; (0.00)		
<i>A71T</i>	1; (0.14)	K219Q	31; (4.19)	<i>F227V</i>	0; (0.00)		
<i>A71V</i>	20; (2.7)	<i>K219R</i>	8; (1.08)	M230I	0; (0.00)		
<i>G73A</i>	0; (0.00)			M230L	8; (1.08)		
<i>G73C</i>	0; (0.00)			<i>L234I</i>	1; (0.14)		
<i>G73S</i>	3; (0.41)			<i>Y318F***</i>	0; (0.00)		
<i>G73T</i>	0; (0.00)						
T74P	6; (0.81)						
L76V	73; (9.86)						
<i>V77I</i>	0; (0.00)						
V82A	137; (18.51)						
<i>V82C</i>	6; (0.81)						
V82F	2; (0.27)						
V82L	0; (0.00)						
<i>V82M</i>	1; (0.14)						
V82S	1; (0.14)						
V82T	0; (0.00)						
N83D	3; (0.41)						
<i>I84A</i>	0; (0.00)						
<i>I84C</i>	0; (0.00)						
I84V	14; (1.89)						
<i>I85V</i>	0; (0.00)						
<i>N88D</i>	1; (0.14)						
N88S	1; (0.14)						
<i>L89I</i>	3; (0.41)						
<i>L89M</i>	3; (0.41)						
<i>L89V</i>	2; (0.27)						
L90M	14; (1.89)						

GRT, genotypic resistance test; INSTI, integrase strand transfer inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; No., Number; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor

Italics indicate accessory or other non-major mutations as classified by the 2022 edition of the IAS–USA drug resistance mutations list in conjunction with Stanford HIV Drug Resistance Database.

^a Only 7 out of 740 GRTs with resistance had INSTI testing done in addition to PI, NRTI and NNRTI testing.

* Classified as a minor mutation in the 2022 edition of the IAS–USA drug resistance mutations list, but as a major mutation in Stanford HIV Drug Resistance Database

** Classified as a major mutation to rilpivirine only, in the 2022 edition IAS–USA drug resistance mutations list

*** Classified as a major mutation to doravirine only, in the 2022 edition IAS–USA drug resistance mutations list

Supplementary Table S2. Antiretroviral drug regimens with genotypic susceptibility scores for children and adolescents from public sector healthcare facilities in KwaZulu-Natal, South Africa

ART Regimen (with XTC) ^a	All GRTs	No HIVDR	HIVDR	Genotypic susceptibility score ^b		
	N=851 (100.00%)	N=165 (19.39%)	N=686 (80.61%)	<2	2	>2
ABC backbone	391 (45.95%)	73 (18.67%)	318 (81.33%)	228 (58.31%)	2 (0.51%)	161 (41.18%)
ABC+AZT+PI/r ^c	23 (2.70%)	9 (39.13%)	14 (60.87%)	3 (13.04%)	0 (0.00%)	20 (86.96%)
ABC+EFV	28 (3.29%)	5 (17.86%)	23 (82.14%)	21 (75.00%)	1 (3.57%)	6 (21.43%)
ABC+PI/r	339 (39.84%)	59 (17.40%)	280 (82.60%)	203 (59.88%)	1 (0.29%)	135 (39.82%)
ABC+PI/r+RAL	1 (0.12%)	0 (0.00%)	1 (100.00%)	1 (100.00%)	0 (0.00%)	0 (0.00%)
AZT backbone	434 (51.00%)	86 (19.82%)	348 (80.18%)	97 (22.35%)	121 (27.88%)	216 (49.77%)
AZT+DRV/r	1 (0.12%)	0 (0.00%)	1 (100.00%)	1 (100.00%)	0 (0.00%)	0 (0.00%)
AZT+DRV/r+RAL	1 (0.12%)	0 (0.00%)	1 (100.00%)	1 (100.00%)	0 (0.00%)	0 (0.00%)
AZT+DTG	4 (0.47%)	1 (25.00%)	3 (75.00%)	1 (25.00%)	1 (25.00%)	2 (50.00%)
AZT+EFV	8 (0.94%)	0 (0.00%)	8 (100.00%)	7 (87.5%)	1 (12.5%)	0 (0.00%)
AZT+PI/r	419 (49.24%)	85 (20.29%)	334 (79.71%)	87 (20.76%)	119 (28.4%)	213 (50.84%)
AZT+TDF+PI/r	1 (0.12%)	0 (0.00%)	1 (100.00%)	0 (0.00%)	0 (0.00%)	1 (100.00%)
TDF backbone	26 (3.06%)	6 (23.08%)	20 (76.92%)	10 (38.46%)	1 (3.85%)	15 (57.69%)
TDF+DRV/r+DTG	1 (0.12%)	0 (0.00%)	1 (100.00%)	0 (0.00%)	0 (0.00%)	1 (100.00%)
TDF+DTG	4 (0.47%)	2 (50.00%)	2 (50.00%)	0 (0.00%)	1 (25.00%)	3 (75.00%)
TDF+EFV	2 (0.24%)	0 (0.00%)	2 (100.00%)	2 (100.00%)	0 (0.00%)	0 (0.00%)
TDF+PI/r	19 (2.23%)	4 (21.05%)	15 (78.95%)	8 (42.11%)	0 (0.00%)	11 (57.89%)

ABC, abacavir; ART, antiretroviral therapy; AZT, zidovudine; DRV/r, ritonavir-boosted darunavir; DTG, dolutegravir; EFV, efavirenz; GRT, genotypic resistance test; HIVDR, human immunodeficiency virus drug resistance; PI/r, boosted protease inhibitor; RAL, raltegravir; TDF, tenofovir disoproxil fumarate; XTC, emtricitabine/lamivudine

^aAll regimens include XTC which is either emtricitabine or lamivudine. PI/r refers to boosted atazanavir or lopinavir and the drug with the lower penalty score in Stanford University HIV Drug Resistance Database was used. Dolutegravir is assumed to have an individual genotypic susceptibility score of 1 in three GRTs where HIV-1 *integrase* testing was not done. GRTs were done at time of antiretroviral treatment failure.

^bGenotypic susceptibility score calculated for 851 GRTs with complete ART regimen data.

^cPrescribed regimen not in keeping with the standard South African treatment guidelines.