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HIV-1 drug resistance in adults and adolescents on protease inhibitor-based antiretroviral therapy in KwaZulu-Natal Province, South Africa

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ABSTRACT

Objectives: In low–middle-income countries, increasing levels of HIV drug resistance (HIVDR) on second-line protease inhibitor (PI)-based regimens are a cause for concern given the limited drug options for third-line antiretroviral therapy (ART). We conducted a retrospective analysis of routine HIV-1 genotyping laboratory data from KwaZulu-Natal, South Africa, to describe the frequency and patterns of HIVDR mutations and their consequent impact on standardised third-line regimens.

Methods: This was a cross-sectional analysis of all HIV-1 genotypic resistance tests conducted by the National Health Laboratory Service in KwaZulu-Natal (January 2015 to December 2016) for adults and adolescents (age ≥10 years) on second-line PI-based ART with virological failure. We assigned a third-line regimen to each record based on a national treatment algorithm and calculated the genotypic susceptibility score (GSS) for that regimen.

Results: Of 348 samples analysed, 287 (82.5%) had at least one drug resistance mutation (DRM) and 114 (32.8%) had at least one major PI DRM. Major PI resistance was associated with longer duration on second-line ART (aOR per 6-months = 1.11, 95% CI 1.04–1.19) and older age (aOR = 1.03, 95% CI 1.01–1.05). Of 112 patients requiring third-line ART, 12 (10.7%) had a GSS of <2 for the algorithm-assigned third-line regimen.

Conclusion: One-third of people failing second-line ART had significant PI DRMs. A subgroup of these individuals had extensive HIVDR, where the predicted activity of third-line ART was suboptimal, highlighting the need for continuous evaluation of outcomes on third-line regimens and close monitoring for emergent HIV-1 integrase inhibitor resistance.

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1. Introduction

Human immunodeficiency virus drug resistance (HIVDR) remains a major public-health problem in the successful eradication of HIV using antiretroviral therapy (ART) [1]. The presence of HIVDR causes increases in HIV infections and deaths and has huge implications on ART programme costs [2]. Programmatic data from

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KwaZulu-Natal Province in South Africa have suggested a low compliance with viral load monitoring guidelines, with just 32% and 26% of adults having viral load tests at 12 months and 24 months, respectively [3]. This problem is perpetuated by the lack of timely action on viral load results when virological failure has been diagnosed [4]. This has resulted in several studies reporting patients remaining on failing regimens for prolonged periods of time [up to 27 months; interquartile range (IQR) 17–40 months] before treatment switch [4–7]. Delays in treatment switching further jeopardise subsequent antiretroviral drug options, as mutations accumulate on a failing ART regimen [8–10]. This poses a major challenge to achieving the Joint United Nations Programme on HIV and AIDS (UNAIDS) 90-90-90 goals, particularly in achieving 90% viral suppression in people receiving ART [2,11].

Several studies have reported high levels of acquired HIV-1 drug resistance in KwaZulu-Natal, with approximately 84–100% of all patients with unsuppressed viral loads on first-line ART having at least one known HIVDR mutation [4,12–14]. The preferred second-line ART regimen for adults in South Africa until 2019 included zidovudine (AZT) or tenofovir (TDF) depending on the previous regimen, with lamivudine or emtricitabine, and a protease inhibitor [i.e. ritonavir-boosted lopinavir (LPV/r)/ritonavir-boosted atazanavir (ATV/r)] [15]. As with first-line ART, patients with persistent viraemia on second-line ART (i.e. having at least two consecutive viral loads ≥1000 copies/mL, at least 3 months apart whilst on ART, with intensive adherence counselling) are considered to have virological failure, and only then is genotypic drug resistance testing done to guide clinical management [15].

In a pooled analysis of protease inhibitor (PI)-based failures in sub-Saharan Africa, 17% of patients had at least one major PI resistance mutation at treatment failure, with an association between time on second-line ART and development of PI resistance [16]. Similar findings were shown in a South African national survey on second-line PI-based failures between 2013 and 2014, with approximately 16% of patients having major PI resistance at virological failure [17]. As ART programmes mature and more people have longer exposure to PI-based regimens, we expect to see more PI resistance. In this analysis, we sought to describe the frequency and patterns of HIVDR mutations in adults and adolescents failing second-line PI-based ART in KwaZulu-Natal, the province with the largest ART programme in South Africa [18], and to estimate the consequent impact of the observed resistance on standardised third-line ART regimens.

2. Materials and methods

2.1. Study population

The study population comprised adult and adolescent HIV-1-positive patients with persistent viraemia (at least two consecutive viral loads ≥1000 copies/mL) on second-line PI-based ART who had samples sent for HIVDR testing between January 2015 and December 2016 to the Department of Virology, National Health Laboratory Service (NHLS) in Durban, South Africa, as per national guidelines. The NHLS is the laboratory that conducts routine HIVDR genotypic testing for people receiving care at public sector health facilities. Genotypic drug resistance test requests were done according to the standard of care in South Africa [19], and all genotypic drug resistance tests were performed at the Department of Virology, NHLS, in Durban.

2.2. Laboratory procedures

Sample extraction and Sanger sequencing were performed for samples with HIV RNA ≥1000 copies/mL. In summary, viral RNA was extracted from plasma samples using a NucliSENS easyMAG automated extraction platform (bioMérieux, Marcy-l'Étoile, France). Reverse transcription PCR was performed using a SuperScript III First Strand Synthesis System (Thermo Fisher Scientific, USA), and the *protease* and *reverse transcriptase* genes were amplified using Platinum™ *Taq* DNA Polymerase (Thermo Fisher Scientific). Integrase sequencing was not done because patient records indicated no known exposure to integrase strand transfer inhibitors (InSTIs) and we assumed there would not be transmitted integrase resistance. Details of the laboratory method have been described previously [20]. Sequencing was done on a 3730 Genetic Analyzer, and sequences were analysed for drug resistance mutations using the Stanford University HIV Drug Resistance Database (Stanford HIVdb) (http://hivdb.stanford.edu) [21].

2.3. Eligibility criteria

For this analysis, we obtained reports of HIV-1 drug resistance tests, and the inclusion criteria were all patient records from individuals aged ≥10 years with a sample successfully genotyped during the reporting period who were receiving a PI-based ART regimen at time of testing. Patient records were considered duplicates if the first name and surname were the same, they had the same date of birth, received treatment from the same clinic, and had the same national identification number. In such cases, the latest result was included in the analysis. All patient records are stored electronically in a NHLS in-house database.

2.4. Data analysis

We re-analysed each patient record based on observed mutations using the Stanford HIVdb v.8.7. We defined drug resistance as having any major PI drug resistance mutation (DRM), nucleoside reverse transcriptase inhibitor (NRTI) resistance mutation, or non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance mutation. Univariable and multivariable logistic regression were used to compare associations between patient variables and major PI resistance, and we excluded any records with missing patient variable data. A *P*-value of <0.05 was considered statistically significant. All statistical analyses were done using Stata v.14 (StataCorp LP, College Station, TX, USA). We assessed patterns of HIVDR mutations and the prevalence of major PI resistance mutations. Where drug resistance predicted a need for third-line ART, we assigned a drug regimen to each record based on the standardised national algorithm [22]. The algorithm guides selection of a regimen incorporating darunavir/ritonavir (DRV/r) + two NRTIs ± dolutegravir (DTG) ± etravirine/rilpivirine (ETR/RPV), based on observed drug resistance (Supplementary Fig. S1).

2.5. Ethics statement

Approval to conduct this study was obtained from the University of KwaZulu-Natal Biomedical Research Ethics Committee.

3. Results

We received 665 requests for genotypic resistance testing during the period January 2015 to December 2016, and excluded 59 samples that were either not approved for testing (21) or had unsuitable samples (38). We processed 606 samples for viral load testing and attempted genotyping for 551 samples with viral loads >1000 copies/mL. We obtained valid genotype results for 465 samples and included 348 genotypes in the final analysis. Fig. 1 shows details of samples processed from request to analysis.

Of the 348 samples, most (n = 325; 93.4%) were from individuals receiving a LPV/r-based second-line regimen at the time of sampling, and the median duration of second-line ART was 30

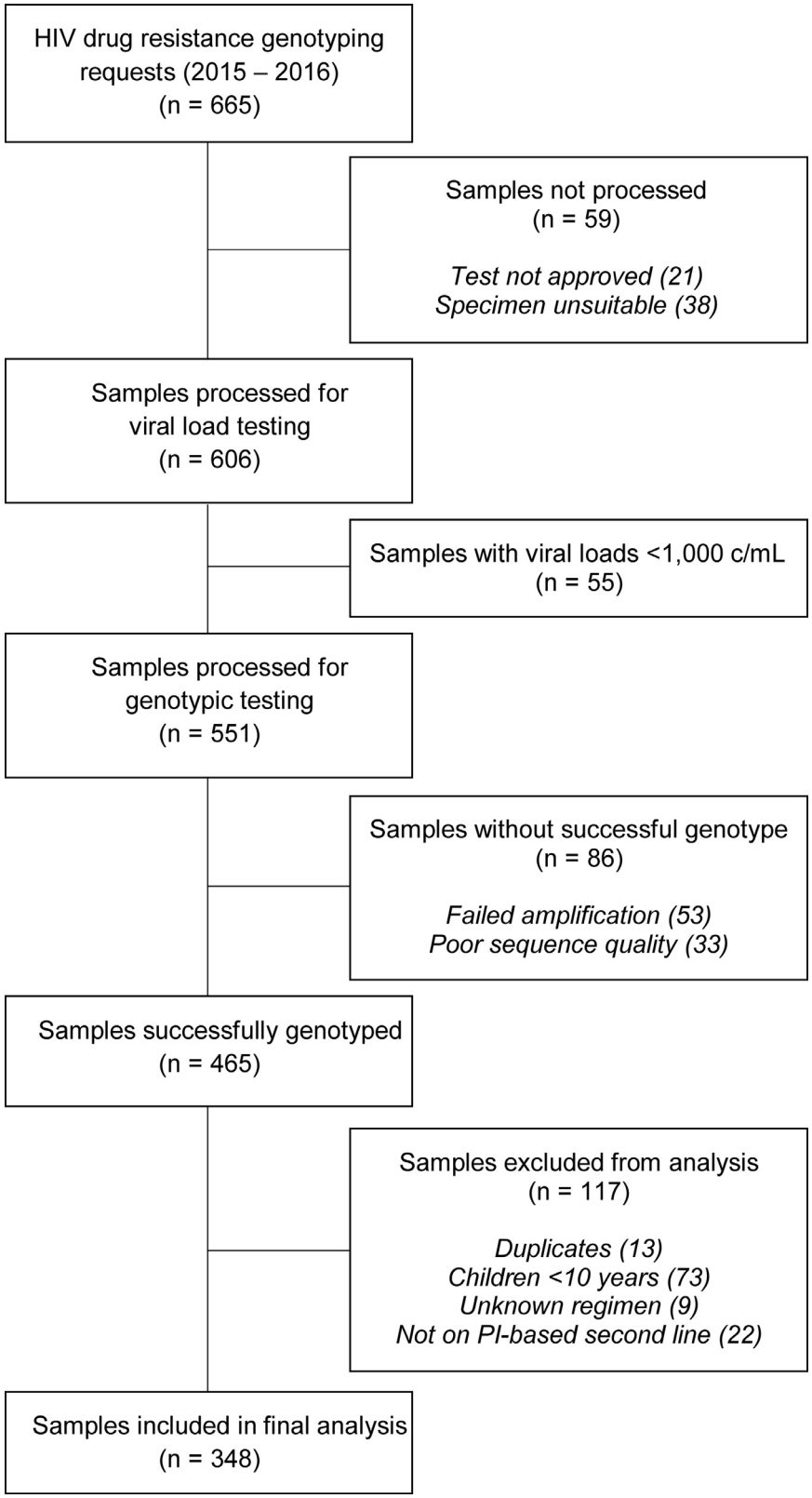

Fig. 1. Flow chart of samples from request to analysis. PI, protease inhibitor.

months (IQR 18–47 months). The clinical and demographic characteristics of the patients are summarised in Table 1.

Overall, 287 samples (82.5%) had at least one DRM and 114 (32.8%) had at least one major PI DRM. Adolescents (10–19 years) were less likely to have major PI resistance than adults aged ≥20 years (18.7% vs. 37.7%; $P = 0.001$). The patterns of drug resistance observed are shown in Supplementary Table S1. The most frequently detected major PI DRMs were V82ACS ($n = 88$), M46IL ($n = 83$), I54MTV ($n = 80$) and L76V ($n = 48$). The V82A mutation, which is known to reduce susceptibility to LPV/r and ATV/r,

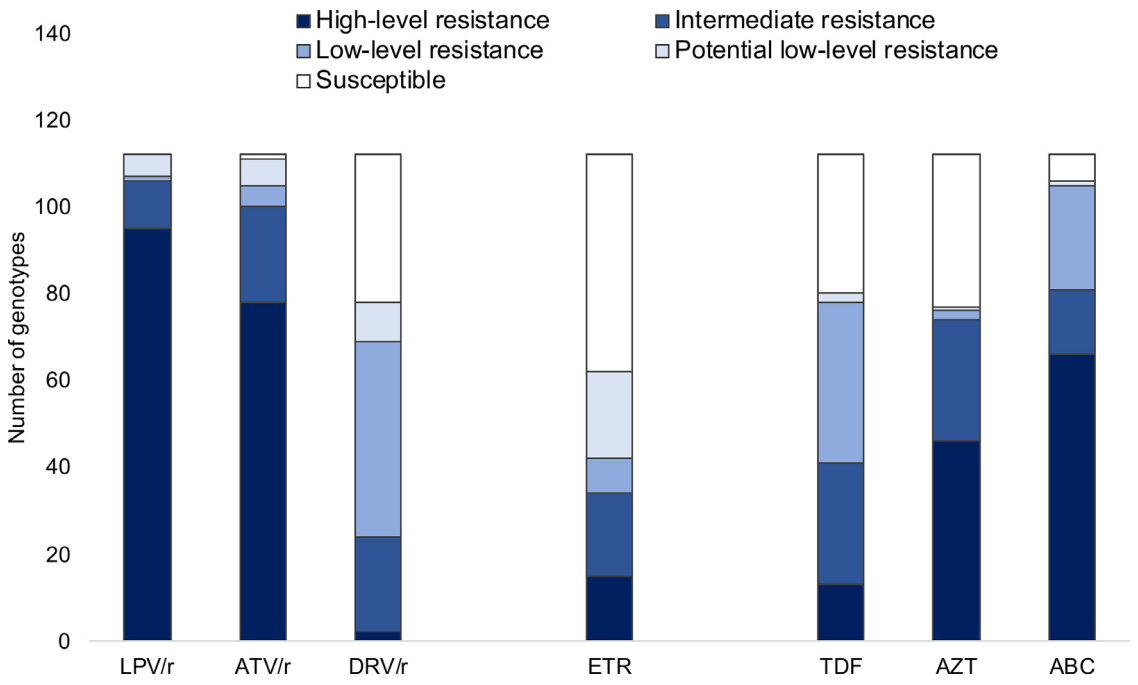

Fig. 2. Drug resistance levels among participants with major protease inhibitor drug resistance mutations. LPV/r, ritonavir-boosted lopinavir; ATV/r, ritonavir-boosted atazanavir; DRV/r, darunavir/ritonavir; ETR, etravirine; TDF, tenofovir; AZT, zidovudine; ABC, abacavir.

Table 1
Baseline characteristics of participants included in the final analysis (n = 348)

Variable	Value
Sex female [n (%)]	208 (59.8)
Age (years) [median (IQR)]	34 (19–42)
Age [n (%)]	
10–19 years	91 (26.1)
20–39 years	140 (40.2)
≥40 years	117 (33.6)
HIV-RNA, $\log_{10}$ copies/mL [median (IQR)]	4.9 (4.5–5.5)
Duration of ART (months) [median (IQR)] [a]	72 (50–96)
Duration of second-line ART (months) [median (IQR)] [b]	30 (18–47)
Second-line ART regimen (n)	
AZT+3TC+LPV/r	164
TDF+XTC+LPV/r	89
ABC+3TC+LPV/r	46
AZT+3TC+ATV/r	10
TDF+XTC+ATV/r	10
AZT+3TC+TDF+LPV/r	4
ABC+3TC+ATV/r	2
Other	23
Previous TB [n (%)]	227 (65.2)
TB treatment during second-line ART [n (%)] [c]	105 (30.2)

3TC, lamivudine; ABC, abacavir; ART, antiretroviral therapy; ATV/r, ritonavir-boosted atazanavir; AZT, zidovudine; IQR, interquartile range; LPV/r, ritonavir-boosted lopinavir; TB, tuberculosis; TDF, tenofovir; XTC, emtricitabine or lamivudine.

[a] ART start date missing for 22 participants.

[b] Second-line ART start date missing for 1 participant.

[c] TB treatment during second-line ART was assumed if the treatment start date for the latest TB episode was either after or less than 180 days prior to the start date of second-line ART; TB treatment start date missing for 4 participants.

was detected in 84 patients (Supplementary Fig. S2). Among participants with major PI DRMs, the majority had intermediate to high-levels of resistance to LPV/r and ATV/r with only a few having high-level resistance to DRV/r (Fig. 2).

Of those with major PI resistance, the median number of major PI DRMs was 3 (IQR 3–4) and the median number of accessory PI DRMs was 1 (IQR 0–2). In most cases, participants had major PI DRMs with accessory and/or other PI mutations (Supplementary Fig. S3). Major PI resistance was associated with longer duration of second-line ART [for each 6-month increase, adjusted odds ratio (aOR) = 1.11, 95% confidence interval (CI) 1.04–1.19] and with age (per year increase, aOR = 1.03, 95% CI 1.01–1.05) (Table 2).

In total, 112 patients met the criteria for requiring third-line ART (2 individuals had major PI DRMs but had a penalty score <15 for their current PI at the time of genotyping). Of these, 24 (21.4%) had intermediate or high-level resistance to DRV/r, and an additional 45 (40.2%) had low-level resistance to DRV/r. The predicted susceptibilities to selected antiretrovirals in cases requiring third-line ART are displayed in Fig. 3 and Supplementary Table S2.

Of the 112 patients requiring third-line ART, 12 (10.7%) had a genotypic susceptibility score (GSS) of <2. All of these were projected to receive a regimen of 2NRTI + DRV/r + InSTI, and all had extensive triple-class resistance including intermediate or high-level resistance to ETR. The resistance profiles for these 12 genotypes are shown in Table 3.

4. Discussion

In this study using routine laboratory data from KwaZulu-Natal, we show that major PI resistance is present in approximately one-third of people having a genotypic resistance test at the time of virological failure on second-line ART. We also show that for those with major PI resistance, the DRMs impact susceptibility to DRV/r in most cases, and for a small group of people (approximately one in ten of those requiring third-line ART) the recommended third-line ART regimen is predicted to have suboptimal activity (GSS < 2). This raises some concern about the long-term durability of current third-line ART regimens for this group and highlights the need for close monitoring of emergent InSTI resistance.

Early studies of HIV drug resistance in people with virological failure on second-line PI-based regimens from South Africa showed that major PI resistance was rare [23–25]. As exposure to PI-based regimens has increased, more recent studies have shown higher proportions with major PI resistance [17,26,27]. A meta-analysis of studies from Africa has demonstrated that, at study level, the pro-

Table 2
Logistic regression analysis of characteristics associated with the presence of at least one major protease inhibitor mutation ($n = 343$)

Characteristic		Univariable		Multivariable	
		OR	95% CI	aOR	95% CI
Duration of second-line ART	Per 6-month increase	1.12	1.05–1.19	1.11	1.04–1.19
Sex	Male	1.33	0.84–2.11	–	–
Age	Per year increase	1.03	1.01–1.05	1.03	1.01–1.05
Viral load	Per $\log_{10}$ copies/mL increase	1.19	0.89–1.60	–	–
TB during second-line ART		1.42	0.88–2.30	–	–

ART, antiretroviral therapy; CI, confidence interval; aOR, adjusted odds ratio; OR, odds ratio; TB, tuberculosis.
NOTE: This analysis included records with no missing data for all five patient variables ($n = 343$); four records were excluded for missing TB treatment date and one record for missing second-line ART start date.

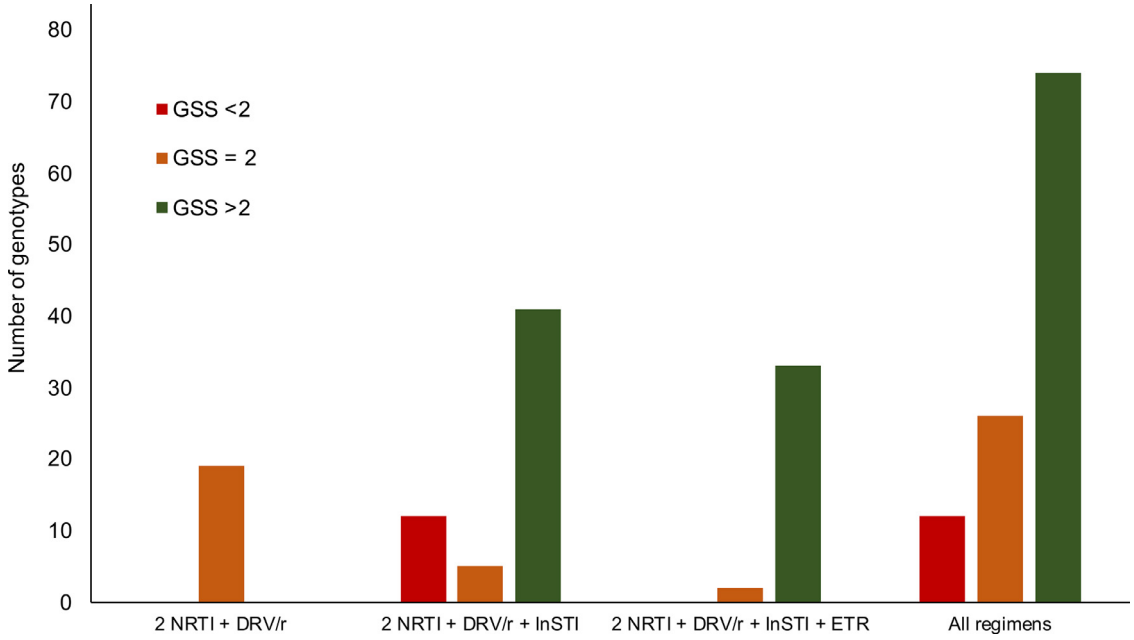

Fig. 3. Predicted genotypic susceptibility score (GSS) of third-line antiretroviral therapy regimen recommended by national algorithm. NRTI, nucleoside reverse transcriptase inhibitor; DRV/r, darunavir/ritonavir; InSTI, integrase strand transfer inhibitor; ETR, etravirine.

portion with major PI resistance is strongly associated with the average duration on second-line ART [16]. In this study, we demonstrate that at an individual level, major PI resistance is associated with longer duration on PI-based ART. This is consistent with other published studies from the region [28,29]. Moreover, a recent study using next-generation sequencing (NGS) among adults failing PI-based ART showed similar levels (34%) of major PI resistance [30], with another recent study from Namibia using dried blood spots reporting much lower levels (13%) of major PI resistance at ART failure [31]. Such findings of inadequate viral suppression with relatively low PI resistance raise questions around treatment tolerance and compliance among patients on second-line PI-based regimens.

Three out of every four cases with major PI resistance had at least three major DRMs, usually with at least one accessory mutation; very few had only a single major PI DRM. This suggests that although the initial emergence of resistance is slow, subsequent mutations may accumulate more rapidly. This was recently shown using data from the EARNEST study where, in people with viraemia on LPV/r monotherapy, the second and third PI DRM emerged more rapidly than the first [32]. A limitation was the lack of data on all viral load measurements during second-line ART, so we were unable to examine the viral load dynamics in detail or to estimate the rate of accumulation of resistance mutations. In programmatic terms, however, this may mean that there is a relatively short window in which significant PI resistance will be detected but before the accumulation of resistance that affects DRV/r susceptibility. This highlights the importance of routine viral load monitoring on second-line ART and the need for good access to genotypic resistance testing and third-line ART. If appropriate response to virological failure is slow, as has been demonstrated consistently with first-line ART regimens [7,33–35], the risk is that drug resistance will continue to accumulate and will compromise the third-line ART regimen.

For most people, the third-line ART regimen selected using the standardised algorithm was predicted to have good activity. However, there was a small group of individuals with extensive triple-class resistance [mostly involving ≥3 major PI DRMs and ≥3 thymidine analogue mutations (TAMs)] where the recommended regimen of 2NRTI + DRV/r + InSTI was predicted to have suboptimal activity (based on a GSS < 2). In these cases, ETR would not have been recommended because the genotype showed intermediate or high-level resistance to ETR. This could therefore be a population where DTG may be inadequately supported by other drugs, and where there is a risk of emergent InSTI resistance. We do not have data on third-line ART outcomes for these individuals so we do not know whether the mutation profiles accurately predicted clinical outcomes for these individuals. Whilst there is evidence that GSS can predict virological outcome, it is an imperfect predictor when used alone without viral load measurements [36]. Initial reports of outcomes on third-line ART in South Africa are encouraging, with most people achieving virological suppression in the

Table 3
Drug resistance mutations (DRMs) and genotypic susceptibility scores (GSS) for key antiretrovirals in participants with total GSS < 2 to the third-line regimen

Regimen GSS	Major PI DRMs	Accessory/other PI DRMs	NRTI DRMs	NNRTI DRMs	DRV/r GSS (PS)	NRTI GSS [a] (PS)	ETR GSS (PS)
1.25	V32I, M46I, V82A, I84V	L10V, A71IV, T74S	M41L, D67N, M184V, L210W, T215F, K219E	K103N, V108I, Y181I	0.25 (35)	0 (65)	0 (60)
1.5	M46I, I54V, L76V, V82A, I84V	L10V, Q58E	M41L, D67G, T69D, K70R, V75M, M184V, T215Y, K219Q	K103N, V108I, Y181C, G190A	0.25 (35)	0.25 (55)	0.25 (50)
1.5	M46I, I47A, I84V	–	D67N, K70R, M184V, T215F, K219Q	A98G, K101E, E138A, V179T, G190C	0.25 (30)	0.25 (35)	0.25 (45)
1.5	M46I, I54V, L76V, V82A	L10F, A71V	A62V, K65R, V75I, F77L, F116Y, Q151M, M184I, K219Q	Y181C, G190A	0.5 (25)	0 (105)	0.25 (50)
1.5	M46I, I54V, L76V, V82C	L10F, K20T, Q58E, A71V	M41L, A62V, D67G, T69D, K70R, V75I, M184V, T215F, K219Q	A98G, Y181V	0.5 (25)	0 (65)	0 (70)
1.75	M46I, I54V, V82A	L10F, K20T, L24I, L33F, A71V	M41L, D67N, K70R, L74I, M184V, T215F, K219Q	A98G, K103N, Y181C	0.75 (10)	0 (60)	0.25 (45)
1.75	M46I, I54V, L76V, V82A	L10F, A71V	M41L, D67N, T69D, V75M, M184V, L210W, T215Y	V108I, Y181V, G190A, H221Y	0.5 (25)	0.25 (55)	0 (80)
1.75	M46I, I54V, V82A, I84V	L10F	D67N, T69D, K70R, M184V, T215F, K219Q	K103N, V108I, H221Y, M230L	0.5 (20)	0.25 (35)	0.25 (40)
1.75	M46I, I54V, L76V, V82A	L10F, A71V	M41L, D67N, K70EG, L74I, V75M, M184V, T215Y	K101H, E138A, Y181C, G190A	0.5 (25)	0.25 (55)	0 (70)
1.75	M46I, I54V, L76V, V82A	L10F, K43T	M41L, D67G, V75M, M184V, L210W, T215Y	V108I, Y181C, G190A, H221Y, F227L	0.5 (25)	0.25 (55)	0 (60)
1.75	V32I, M46I, I47V, I54V, V82A	L10F, L24I, Q58E, T74S	M41L, M184V, T215Y	K101P, K103S	0.25 (35)	0.5 (15)	0 (60)
1.75	V32I, M46I, I47V, I54V, L76V, V82A	L10F, K20T	T215F	Y181C, G190S, H221Y	0 (60)	0.75 (10)	0 (60)

DRV/r, darunavir/ritonavir; ETR, etravirine; GSS, genotypic susceptibility score; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; PS, penalty score.
[a] NRTI GSS and PS refer to the highest GSS (lowest PS) for either zidovudine (AZT) or tenofovir (TDF).

first year of therapy [37,38]. However, these reports were based on very early outcomes. As more people get exposed to DTG through first-line and subsequent ART, continued monitoring of longer-term outcomes on third-line ART and close monitoring for emergent InSTI resistance will be important as third-line ART programmes expand in order to preserve long-term use of InSTIs in future ART regimens.

In addition to those already discussed, our analysis has some further limitations. First, the calculation of GSS to the third-line ART regimens is associated with a number of uncertainties. We assumed the third-line regimens that would be recommended for each individual on the basis of the genotypic resistance test only, according to the national algorithm [22]. This may not have been the actual regimen recommended by the national third-line committee. For example, some people with chronic hepatitis B virus (HBV) infection receive both TDF and AZT in the third-line regimen to ensure optimal treatment of both viruses. We did not have information about HBV infection and therefore the regimen might be incorrect and the GSS might be underestimated in these cases. Genotypic resistance testing is not routinely performed at time of switch from first-line to second-line ART in South Africa. The NNRTI DRMs detected here may therefore not reflect all mutations previously selected on first-line ART and so the predictions for ETR may overestimate susceptibility. We used only the Stanford interpretation system for the calculation of GSS. Although there are some differences between this and other established algorithms (RegaDB, ANRS), all have been shown to perform similarly in predicting clinical outcomes [39,40]. Second, these data were from routine genotypic resistance testing based on Sanger sequencing of the *pol* region, which detects drug resistance mutations that comprise more than 15–20% of the circulating viral population. We therefore do not have information about minority PI-resistant variants, which could be detected by NGS. We also do not have any information about mutations outside *pol*, for example in *gag* cleavage sites. As the clinical significance of minority PI-resistant variants and mutations outside *pol* is unclear [41,42], especially in the context of the public health approach to ART in low- and middle-income countries, this should be a focus of further investigation.

In conclusion, we show that in this analysis of routine resistance test data from KwaZulu-Natal in South Africa, one-third of adults and adolescents have major PI resistance mutations at the time of virological failure on PI-based second-line ART. This supports current guidance around genotypic drug resistance testing and suggests an ongoing need for better adherence interventions. We identify a small subgroup of those requiring third-line DTG-containing ART where the assigned regimen was predicted to not be fully active. This highlights the importance of monitoring treatment outcomes on third-line ART and strengthening systems for early detection of emergent InSTI resistance.

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Ethical approval

Approval to conduct this study was obtained from the University of KwaZulu-Natal Biomedical Research Ethics Committee [BCA143/09].

Competing interests

None declared.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2021.10.023.

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