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Emerging integrase strand transfer inhibitor drug resistance mutations among children and adults on ART in Tanzania: findings from a national representative HIV drug resistance survey

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Background: Despite the scale-up of ART and the rollout in Tanzania of dolutegravir, an integrase strand transfer inhibitor (INSTI), treatment success has not been fully realized. HIV drug resistance (HIVDR), including dolutegravir resistance, could be implicated in the notable suboptimal viral load (VL) suppression among HIV patients.

Objectives: To determine the prevalence and patterns of acquired drug resistance mutations (DRMs) among children and adults in Tanzania.

Methods: A national cross-sectional HIVDR survey was conducted among 866 children and 1173 adults. Genotyping was done on dried blood spot and/or plasma of participants with high HIV VL (≥1000 copies/mL). HIV genes (reverse transcriptase, protease and integrase) were amplified by PCR and directly sequenced. The Stanford HIVDR Database was used for HIVDR interpretation.

Results: HIVDR genotyping was performed on blood samples from 137 participants (92 children and 45 adults) with VL ≥ 1000 copies/mL. The overall prevalence of HIV DRMs was 71.5%, with DRMs present in 78.3% of children and 57.8% of adults. Importantly, 5.8% of participants had INSTI DRMs including major DRMs: Q148K, E138K, G118R, G140A, T66A and R263K. NNRTI, NRTI and PI DRMs were also detected in 62.8%, 44.5% and 8% of participants, respectively. All the participants with major INSTI DRMs harboured DRMs targeting NRTI backbone drugs.

Conclusions: More than 7 in 10 patients with high HIV viraemia in Tanzania have DRMs. The early emergence of dolutegravir resistance is of concern for the efficacy of the Tanzanian ART programme.

Introduction

Efforts to address the burden of HIV in Tanzania have attracted significant investment and involvement of the Government of Tanzania and development partners, including the US President's Emergency Plan for AIDS Relief (PEPFAR) and the Global Fund to Fight AIDS, Tuberculosis and Malaria, towards the AIDS response, especially the scale-up of ART in the country. As a result, in population-based household surveys, HIV prevalence among adults and adolescents aged 15–19 years has stabilized. The Tanzania HIV/AIDS and Malaria Indicator Survey 2011–2012 estimated a national prevalence of 5.1% (95% CIs: 4.6–5.6),[1] and a follow-up Tanzania HIV Impact Survey 2016–2017 estimated a national prevalence of 4.7% (95% CIs: 4.3–5.0).[2] The burden varies by age, sex and geographically across Tanzania, ranging from 11.4% in Njombe and 11.3% in Iringa to less than 1% in Lindi and Zanzibar.[2]

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In 2020, the Joint United Nations Programme on HIV/AIDS estimated that 82% of people living with HIV (PLHIV) were on ART in Tanzania.[3] Tanzanian HIV programme PEPFAR data further show that the country has surpassed 95% viral load (VL) suppression among the adult population. To ensure that Tanzania goes the final mile and ends the epidemic by 2030, sustaining VL suppression targets among PLHIV on ART is paramount. This can be achieved through ensuring ART adherence to treatment, management of opportunistic infections, regular scheduled VL monitoring and monitoring of emerging HIV drug resistance (HIVDR).[4,5] Delays in detection of virological failure and/or unnecessary switches to second-line treatment may reduce the effectiveness of first-line treatment, limit choices of ART regimens and subsequently facilitate the accumulation and spread of HIVDR.[6,7] Evidence indicates a high burden of HIVDR among existing ART regimens in low- and middle-income countries including Tanzania.[2,8–10]

In 2019, a potent integrase strand transfer inhibitor (INSTI), dolutegravir, was made available for public use in Tanzania. This was in addition to other ART drug classes, including NRTIs, NNRTIs and PIs.[11] Dolutegravir-based regimens have demonstrated superiority over both efavirenz and PI-based regimens, with better tolerability, fewer discontinuations and rapid suppression of VL.[12–14] Importantly, dolutegravir has a high genetic barrier to resistance, and experiences from high-income countries suggest resistance to dolutegravir rarely occurs *in vivo*.[12–14] Clinical evidence and the availability of a convenient and cost-saving fixed-dose combination (FDC) tablet, and the existence of a high burden of NNRTI pretreatment resistance have contributed to the WHO recommendation of rolling out dolutegravir-based FDC as an optimal regimen for patients on first-line ART.[2–4]

In response, the Tanzanian ART programme and other PEPFAR-funded programmes began to phase out the NNRTI-based regimen in adults and adolescents and replace it with tenofovir + lamivudine + dolutegravir as the default first-line FDC on 1 March 2019. In addition, the dolutegravir-based FDC was adopted for all eligible children weighing more than 20 kg.[15] The accelerated rollout of dolutegravir-based FDC meant that all eligible newly diagnosed patients were being initiated on the new default regimen. In addition, all adults and adolescents on the NNRTI-based regime were being switched to the dolutegravir-based FDC. In children, a PI-based regimen remains the recommended default regime while eligible children are switched to dolutegravir-based FDC.[15] PI in combination with two NRTIs remains the second-line treatment of choice for adults, adolescents and children.[15]

Despite the anticipated benefits of a dolutegravir-based first-line regimen, there is a risk that high efficacy and lack of development of dolutegravir resistance may not be realized in low-income settings. This is due to the presence of risk factors associated with selection of dolutegravir resistance, including: regimen switching without confirmation of the virological suppression;[6] high VL and low CD4 cell count in patients initiating treatment;[8–10] insufficient adherence to ART; and the predominance of HIV-1 non-B subtypes that are reported to have distinct resistance pathways.[11] Estimating the HIVDR burden amidst the rollout of an INSTI-based regimen is crucial for assessing ART programme efficiency, planning for its sustainability and informing policy.

We therefore conducted a nationally representative health facility-based survey to determine the prevalence and patterns of acquired HIV drug resistance mutations (DRMs) among children with high viraemia on ART for 12 ± 3 months and ≥ 36 months; and adults on ART for 12 ± 3 months and ≥48 months in Tanzania.

Materials and methods

Study design, participants and settings

A cross-sectional survey was conducted in 2020 for a period of 3 months. Survey participants were enrolled from 36 HIV care and treatment clinics (CTCs) distributed in 22 regions of the Tanzanian mainland. The CTC sites were randomly selected, and a probability proportional to size sampling approach was used to determine the sample size per site as per the WHO HIVDR survey guideline.[15,16]

Children on ART at early (12 ± 3 months) and late (≥36 months) timepoints; and adults on ART at early (12 ± 3 months) and late (≥48 months) timepoints as per the WHO guideline were eligible for the survey.[16] The inclusion criteria were HIV-1-positive children (<15 years) on either first- or second-line ART for 12 ± 3 months and at least 36 months; and HIV-1-positive adults (15 years and above) receiving either first- or second-line ART for 12 ± 3 months and at least 48 months.

Ethics

Ethical approval was obtained from the National Institute for Medical Research (NIMR) (NIMR/HQ/R.8a/Vol. IX/3432). The permission to conduct surveys was also obtained from the President's Office–Regional Administration and Local Government. Written informed consent was obtained from each adult survey participant (18 years and above) and in the case of children, assent (15 to 17 years) and consent (below 18 years) were obtained from their parents/caregivers.

Data collection

HIV-positive clients attending CTC were diagnosed by the national rapid HIV testing algorithm, which consists of Bioline™ HIV 1/2 3.0 (Abbott Rapid Diagnostics, Lake Forest, IL, USA) and Uni-Gold™ Recombigen® HIV-1/2 (Trinity Biotech Plc, Bray, Co. Wicklow, Ireland).[17] Eligible participants among clients attending CTC were enrolled in the survey as they attended their scheduled clinic appointment. Informed consent was obtained, and a well-structured questionnaire was used to collect participants' demographic data (age, sex, place of residence, occupation) and clinical data. In addition, participants' HIV CTC2 cards were also used to extract ART and clinical lab information [current and previous ART regimens, CD4 count and HIV viral load (HVL)]. The HIV CTC2 card is an individual-level routine data collection form that contains the client's unique CTC ID number and clinical records of all visits that a client makes to the clinic.

A total of 10 mL of blood was collected into two EDTA tubes. Samples were then processed into dried blood spot (DBS) and plasma at a designated survey hub laboratory facility. An aliquot of plasma from each participant's sample was used for HVL measurement (viral copies/mL) at a centralized testing laboratory using the Cobas® 8800 System (Roche Molecular Systems, Inc., South Branchburg, NJ, USA).[18]

Sequence analysis

HIVDR genotyping was performed at a WHO-accredited laboratory, British Columbia Centre for Excellence in HIV/AIDS (BC-CfE), in Canada. Genotyping of the HIV-1 *pol* gene, including part of reverse transcriptase (RT), protease (PR) and integrase (IN) coding regions, was performed using a conventional sequence-based standardized genotyping

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procedure established by the WHO-accredited genotyping laboratory.[19] Viral RNA was extracted from DBS and/or plasma using the standard methods.[19] A nested RT-PCR protocol was used, starting from extracted RNA, to separately amplify the protease-reverse transcriptase (PR-RT) and IN regions of the HIV *pol* gene. The RT-PCR was performed using either Applied Biosystems GeneAmp PCR System 9700 or VeritiPro Thermal Cycler. Sanger sequencing using sets of routine forward and reverse primers was performed on Applied Biosystems 3730XL DNA Analyser. All the participants' sequences were exported from the 3730XL data collection software in FASTA format and viewed in BioEdit Sequence Alignment Editor software version 7.1.[20] Using the Seqscape software version 2.7 and Gene Cutter tool, in the Los Alamos sequence database (http://www.hiv.lanl.gov/content/sequence/GENE_CUTTER/cutter.html), all the available participants' sequences were imported, and manually scrutinized to ensure sequence quality and then analysed (assembled and aligned). The Stanford HIV Drug Resistance Database (HIVdb) Program version 8.6.1 (https://hivdb.stanford.edu/hivdb) was then used for assignment of HIVDR. Prediction of phenotypic susceptibility to antiretroviral drugs was performed using HIValg program HIVDB 9.0 (https://hivdb.stanford.edu/hivalg/by-mutations). The resulting report interpreted the drug or drug classes to which there is resistance and described the five levels of resistance, which include susceptible, potential low-level resistance, low-level resistance, intermediate resistance and high-level resistance. HIV subtypes were determined using the REGA HIV-1 Subtyping Tool Version 3.0 (http://dbpartners.stanford.edu:8080/RegaSubtyping/stanford-hiv/typingtool/). Unassigned sequences to a defined subtype were regarded as inter-subtype recombinants. The identified HIV drug resistance mutations (PR-RT and IN mutations) in each participant were merged with the participants' demographic, clinical and HIV-1 subtype data in Microsoft Excel software and then subjected to further analysis.

Data analysis

Data analysis was done using the statistical software GraphPad Prism Software version 6.07 (GraphPad Software, San Diego, CA, USA). Descriptive statistical analyses involving tabular and numerical presentations were thereafter done to obtain the distribution of participant characteristics among children and adults according to their age, sex, HVL, ART therapy regimen, ART duration and HIV subtype. The associations across categorical variables were measured by chi-squared test with corresponding *P* value. The age and HVL differences among children and adults were assessed using the Mann–Whitney *U*-test. A *P* value less than 0.05 was considered statistically significant. In addition, cross-tabulations were performed to assess proportions of acquired drug resistance at early and late timepoints among children and adults by HVL and ART regimen.

Results

Study participant characteristics

This survey recruited 2039 PLHIV receiving HIV care and treatment services in Tanzania. Of these, 866 (42.5%) were children aged 1 to 14 years and 1173 (57.5%) were adults. In the survey 6.7% (137/2039) of the participants had high viraemia (HVL ≥ 1000 copies/mL) and were eligible for HIVDR genotyping. Among them, the majority were children ($n=92$; 67.2%). The mean ages and SDs of children and adults were 10 ± 3.6 and 38 ± 13.8 years, respectively. The median HVL (IQR) was 12 850 (5050–94 450) and 16 500 (5205–155 550) copies/mL in children and adults, respectively ($P>0.05$). Females comprised nearly two-thirds of the participants with high viraemia, reflecting the overall prevalence of HIV in Tanzania. The majority (82%) of eligible adult participants had been switched to or initiated on a dolutegravir-based regimen at the time of this survey. Subtype C was the predominant circulating HIV-1 subtype in this population (Table 1).

Prevalence and patterns of HIV drug resistance mutations

In this survey HIVDR genotyping was done from both DBS and plasma samples collected from the participants; DBS was the primary specimen for HIVDR genotyping, and plasma was used as a backup for samples that were not successfully sequenced from DBS. In this survey for the DBS specimens, the sequencing success rate for the IN region was 81% (111/137) and for the PR-RT region was 38.7% (53/137). In contrast, for the plasma specimen, the sequencing success rate for the IN region was 92.3% (24/26) and for the PR-RT region was 96.4% (81/84). Sequence concordance was high (median >99%) for participants where DBS and plasma sequences were available. The overall sequencing success rate for the IN region was 98.5% (135/137) and for the PR-RT region was 97.8% (134/137) (Figure 1). The overall prevalence of HIV DRM among patients with high viraemia was 71.5% (98/137) (Figure 2a). NNRTI DRMs were most prevalent, detected in 62.8% of participants, followed by 44.5% with NRTI DRMs. Dual class (NNRTI and NRTI) DRMs were detected in 39.4% of the participants (Figure 2a). Interestingly we found that 5.8% (8/157) of the participants harboured at least one INSTI DRM. DRMs were more prevalent at late timepoints (76.8%) compared with early timepoints (59.5%), consistent with accumulation of DRMs over time. We observed a higher prevalence of DRMs in children compared with adults. This observation was consistent for both timepoints: early timepoint 73.9% versus 44.4%, and late timepoint 78.3% versus 57.8%, for children and adults, respectively (Table 2). Analysis of specific DRM frequency among NNRTI DRMs revealed that the mutation K103N/S/T was the most prevalent in children (55.6%) and adults (50%) with at least one (any) DRM (Figure 2b and c). In contrast, the most frequent NRTI DRM were lamivudine-and-emtricitabine-associated mutations, at position 184, detected in 58.3% and 34.6% of children and adults with at least one (any) DRM, respectively. The patterns and frequencies of all NNRTI and NRTI DRMs detected in children and adults are summarized in Figure 2(b and c).

HIVDR among patients on dolutegravir-based ART

Overall, at least one DRM was detected in 76.7% (33/43) and 43.9% (18/41) of children and adults, respectively, with high viraemia on FDC of tenofovir + lamivudine + dolutegravir. In this survey, we detected at least one major INSTI DRM in three children and one adult. Major INSTI DRMs detected were Q148K ($n=1$), E138K ($n=2$), G118R ($n=2$), G140A ($n=1$), T66A ($n=1$) and R263K ($n=1$), all of which are known to be selected *in vivo* by dolutegravir (Table 3). It has been postulated that the substitution G118R tends to arise from a rare polymorphic codon GGA to AGA. However, codon inspection in both participants harbouring this substitution found the CGC code for arginine. Of note, the polymorphic codon GGA was present in 3.0% (4/135) of IN sequences in our survey (data not shown). Polymorphic accessory INSTI DRMs detected were T97A, Q95K and E157K. Interestingly major INSTI DRMs were detected exclusively in

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Table 1. Distribution of participants' characteristics among children and adults ($N=137$)

Participants' characteristics	Children $n=92$	Adults $n=45$	*P* value
Age (years), mean ± SD	10 ± 3.6	38 ± 13.8	<0.0001[a]
Sex (%)			
Female	54 (58.7)	28 (62.2)	0.83[b]
Male	38 (41.3)	17 (37.8)	
HIV viral load (copies/mL)			
Median	12 850	16 500	0.82[a]
IQR	5050–94 450	5205–155 550	
ART *n* (%)			
Early timepoint (12 ± 3 months)	24 (26.1)	17 (37.8)	0.92[b]
Late timepoint (≥36 and ≥48 months)	38 (41.3)	28 (62.2)	
ART regimen *n* (%)			
DTG-based	45 (48.9)	37 (82.2)	0.011[b]
PI-based	35 (38)	7 (15.6)	
NNRTI-based	1 (1.1)		
Others	3 (3.3)		
Missing	8 (8.7)	1 (2.2)	
HIV subtypes *n* (%)			
A1	28 (30.4)	15 (35.7)	0.36[b]
C	38 (41.3)	19 (45.3)	
D	6 (6.5)	0	
Recombinant between A1, C and D	20 (21.7)	8 (19.0)	

DTG, dolutegravir.
[a]Mann–Whitney *U*-test.
[b]Chi-square test.

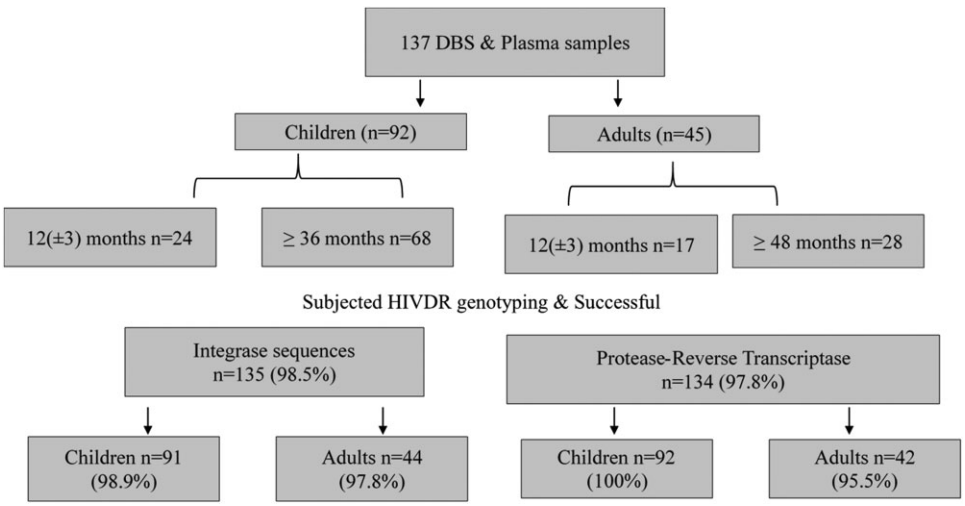


Figure 1. Distribution of participants' samples that underwent HIVDR genotyping ($n=137$).

participants who had been switched from NNRTI-based regimes. All these participants were at the late timepoint and all four participants were predicted to have high-level resistance to dolutegravir and cross-resistance to elvitegravir and raltegravir. Of four patients with major INSTI DRMs, three had their corresponding PR-RT sequences available for analysis, whereby all were demonstrated to harbour multiple NRTI and NNRTI DRMs (Table 3). In addition, the three participants were predicted to have high-level resistance to lamivudine and intermediate-level resistance to tenofovir (data not shown). These findings indicate the possible pre-existence of NRTI resistance among these participants prior to switching to a dolutegravir-based regimen.

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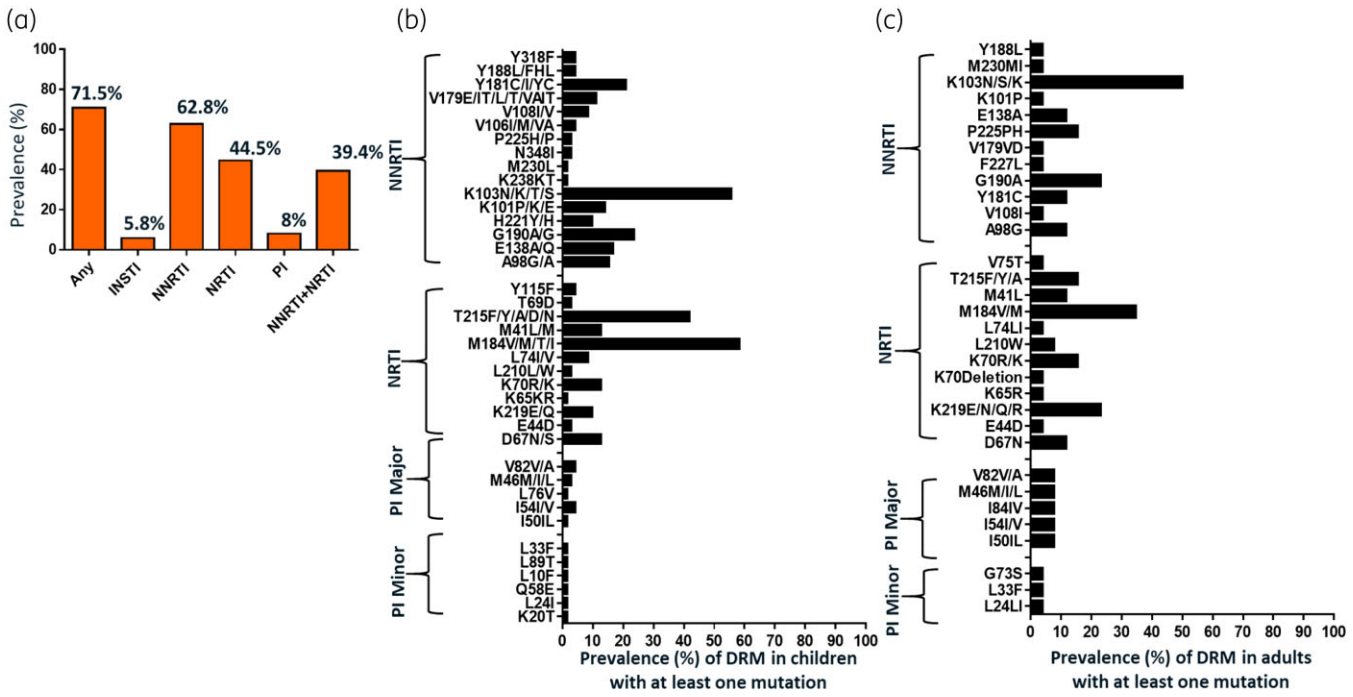


Figure 2. Prevalence and patterns of acquired HIV DRMs. (a) Prevalence estimates of acquired HIV DRMs ($n=137$). Pattern and frequency of NNRTI, NRTI, and PI major and minor DRMs in children (b) and adults (c). This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

Subsequently, HIVDR against the NRTI backbone of tenofovir + lamivudine + dolutegravir fixed combination may have led to dolutegravir functional monotherapy associated with the selection of dolutegravir resistance mutations. To investigate for potential dolutegravir functional monotherapy on the fixed combination of tenofovir + lamivudine + dolutegravir, we used the Stanford University algorithm for prediction of drug susceptibility in participants with high viraemia on a dolutegravir-based regimen. Up to 34.8% and 17.2% of children and adults, respectively, were predicted to have high-level resistance to lamivudine (Figure 3a and b). In addition, 8.7% of children and 3.4% of adults were predicted to have low- to intermediate-level resistance to tenofovir (Figure 3a and b). Nevertheless, the majority of the participants, including those with major INSTI DRM PIs, were predicted to be susceptible to PIs (Figure 3a and b).

HIVDR among patients on PI-based regimens

In participants with high viraemia on a PI-based regimen at least one DRM was detected in 81.8% (27/33) and 100% (7/7) of children and adults, respectively (Table 2). We observed that among these participants with high viraemia on a PI-based regimen only 6.1% and 28.6% of children and adults, respectively, harboured major and/or minor PI DRMs (Table 2). This suggests PI DRMs were rarely selected in the participants in this survey. Patterns and frequency of specific major and minor PI mutations are indicated in Figure 2(b and c). The majority of participants were predicted to remain susceptible to PIs, whereas 7.7% of children and 20% of adults were predicted to have intermediate- to high-level resistance to boosted atazanavir and/or lopinavir (Figure 3c and d). Despite being off NNRTIs, 61.5% of children on a PI-based regimen line were predicted to be resistant to efavirenz and nevirapine, whereas in adults resistance to either of these medications was predicted in 60% of participants who were also on PI-based ART (Figure 3c and d).

Discussion

To our knowledge, this is the first national representative large-scale survey reporting the prevalence of acquired HIVDR since the Government of Tanzania, in collaboration with PEPFAR, accelerated the rollout of tenofovir + lamivudine + dolutegravir FDC in Tanzania. The use of a dolutegravir-based regimen is expected to improve treatment efficacy and lower the burden of HIVDR in the country. In this survey, we found that the overall prevalence of acquired HIV DRMs was 71.5%, consistent with other previous findings.[8–10,21] However, the most frequently (62.8%) detected DRMs were against NNRTIs, which are currently being phased out, and only one participant was on an NNRTI-based regimen. On the other hand, NRTI DRMs were detected at a relatively lower frequency (44.5%) compared with the previous studies conducted in Tanzania among clients on ART experiencing viraemia/virological failure.[8,22] Significantly, a lower proportion (14.6%) of NRTI DRMs was detected in adults on dolutegravir-based regimens compared with previously reported proportions involving NNRTI-based regimens.[8,22] This could imply a lower accumulation of NRTI DRMs in dolutegravir-based regimens and better NRTI recyclability prospects compared with previous NNRTI-based regimens.

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Table 2. Prevalence of acquired drug resistance at early and late timepoints among children and adults by HIV viral load (HVL) suppression and ART regimen[a]

ART class	Early timepoint		Late timepoint		Total	
HIVDR among children and adults on ART with HVL ≥1000 copies/mL						
	n=42	%	*n*=95	%	*n*=137	%
Any	25	59.5	73	76.8	98	71.5
INSTI	1	2.4	7	7.4	8	5.8
NNRTI	21	50	65	68.4	86	62.8
NRTI	13	31	48	50.5	61	44.5
PI	2	4.8	9	9.5	11	8.0
NNRTI + NRTI	10	23.8	44	46.3	54	39.4
HIVDR among children on ART with HVL ≥1000 copies/mL						
	n=23	%	*n*=69	%	*n*=92	%
Any	17	73.9	55	79.7	72	78.3
INSTI	1	4.3	4	5.8	5	5.4
NNRTI	14	60.9	50	72.5	64	69.6
NRTI	12	52.2	37	53.6	49	53.3
PI	0	0	7	10.1	7	7.6
NNRTI + NRTI	9	39.1	34	49.3	43	46.7
HIVDR among adults on ART with HVL ≥ 1000 copies/mL						
	n=18	%	*n*=27	%	*n*=45	%
Any	8	44.4	18	66.7	26	57.8
INSTI	0	0	3	11.1	3	6.7
NNRTI	7	38.9	15	55.6	22	48.9
NRTI	1	5.6	11	40.7	12	26.7
PI	2	11.1	2	7.4	4	8.9
NNRTI + NRTI	1	5.6	10	37.0	11	24.4
HIVDR among children on dolutegravir-based ART						
	n=9	%	*n*=34	%	*n*=43	%
Any	5	55.6	28	82.4	33	76.7
INSTI	1	11.1	3	8.8	4	9.3
Major	0	0	3	8.8	3	7.0
Accessory	1	11.1	3	8.8	3	7.0
NNRTI	5	55.6	27	79.4	32	74.4
NRTI	2	22.2	19	55.9	21	48.8
PI	0	0.0	3	8.8	3	7.0
NNRTI + NRTI	2	22.2	19	55.9	21	48.8
HIVDR among children on PI-based ART						
	n=12	%	*n*=21	%	*n*=33	%
Any	10	83.3	17	81.0	27	81.8
INSTI	0	0.0	0	0	0	0.0
NNRTI	7	58.3	14	66.7	21	63.6
NRTI	9	75.0	10	47.6	19	57.6
PI	0	0.0	2	9.5	2	6.1
NNRTI + NRTI	6	50.0	7	33.3	13	39.4
HIVDR among adults on dolutegravir-based ART						
	n=16	%	*n*=25	%	*n*=41	%
Any	6	37.5	12	50.0	18	43.90
INSTI	0	0	2	8.0	2	4.9
Major	0	0	1	4.0	1	2.4
Accessory	0	0	1	4.0	1	2.4
NNRTI	5	31.3	11	45.8	16	39.0
NRTI	0	0	6	25.0	6	14.6
PI	1	6.3	1	4.2	2	4.9
NNRTI + NRTI	0	0	6	25.0	6	14.6
HIVDR among adults on PI-based second-line ART						
	n=1	%	*n*=6	%	*n*=7	%
Any	1	100	6	100	7	100.0
INSTI	0	100	2	33.0	2	28.6
Major	0	0	0	0	1	14.2
Accessory	0	0	1	16.7	1	14.2
NNRTI	1	100	4	67.0	5	71.4
NRTI	1	100	4	67.0	5	71.4
PI	1	100	1	17.0	2	28.6
NNRTI + NRTI	1	100	4	67.0	5	71.4

[a]Early timepoint indicates 9–15 months and late timepoint indicates ≥36 months for children and ≥48 months for adults.

However, further studies are warranted to confirm this observation.

The dolutegravir-based combination had been introduced in Tanzania within 18 months of conducting this national HIVDR survey. Dolutegravir use has a key advantage because of its superior resistance profile.[13] However, in this survey, we report for the first time in Tanzania eight patients on a dolutegravir-based regimen with no known previous exposure to INSTIs, harbouring one or more major INSTI DRMs. Interestingly, we observed distinct dolutegravir resistance pathways involving substitutions in six non-polymorphic positions: Q148K, E138K, G118R, G140A, T66A and R263K—mutations known to be selected *in vivo* by dolutegravir among other INSTIs.[23,24] The presence of a rare natural polymorphic codon, GGA, at IN position 118 in non-B HIV subtypes, particularly HIV subtype A, had been postulated to potentially increase the risk for selection of G118R (GGA→AGA) substitution. In this survey, G118R mutation was detected in two patients harbouring subtype A and recombinant A1C; however, in both sequences CGC was the responsible codon.[24] One patient harboured T66A, G118R and E138K substitutions with substantial viraemia >100 000 copies/mL. Similar sequential accumulation of the three mutations has been demonstrated in a non-B HIV subtype in an *in vitro* survey, suggesting accumulation of these mutations could be an important resistance pathway to dolutegravir in non-B subtypes.[24] Our data provide an important contribution towards understanding dolutegravir

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Table 3. Profile of participants with INSTI drug resistance mutations

S/N	Duration on ART (months)	VL (copies/mL)	HIV subtype	Previous ART regimen	Current ART regimen	INSTI DRM		NRTI DRM	NNRTI DRM	PI DRM	
						Major	Accessory			Major	Minor
Children											
1	≥36	237 000	A	ZDV/3TC/NVP	TDF/3TC/DTG	—	T97A	M41L, D67N, K70R, M184V, T215F, K219E	Y181C	—	L89T
2	≥36	1820	C	ZDV/3TC/NVP	TDF/3TC/DTG	R263K	—	M41ML, L210W, T215Y	A98AG, K103N, V108I, Y181C, H221HY	—	—
3	≥36	117 000	A1C	NA	TDF/3TC/DTG	T66A, G118R, E138K	—	M41L, M184V, T215F	K103N, V179, Y318F	—	—
4	≥36	639 000	A1D	ZDV/3TC/NVP	TDF/3TC/DTG	E138K, G140A, Q148K	Q95QK	D67N, K70R, M184V, T215F, K219Q	K101KE, E138A, G190A	—	—
5	9–15	11 200	A	ZDV/3TC/NVP	TDF/3TC/DTG	—	T97A	—	K103N	—	—
Adults											
6	≥48	8600	A	TDF/3TC/EFV	TDF/3TC/DTG	—	T97A	—	E138A	—	—
7	≥48	3020	A	TDF/3TC/EFV	TDF/3TC/DTG	G118R	—	NA			
8	≥48	61 000	C	ZDV/3TC/NVP	TDF/FTC/ATV/RTV		E157Q	NA			

ATV/RTV, atazanavir/ritonavir; DTG, dolutegravir; EFV, efavirenz; NA, not available; NVP, nevirapine; S/N, Serial number, ; TDF, tenofovir disoproxil fumarate; ZDV, zidovudine; 3TC, lamivudine.

resistance pathways in individuals infected with HIV-1 non-B subtypes.

All four participants with major INSTI DRMS in this survey had transitioned from an NNRTI-based regimen, and three whose PR-RT sequences were available harboured a significant number of NRTI and NNRTI DRMs, suggesting a potential dolutegravir functional monotherapy leading to the emergence of INSTI DRMs. In addition, a substantial fraction of children and adults with high viraemia on dolutegravir-based regimens were predicted to have resistance to the NRTI backbone drugs lamivudine and emtricitabine. This suggests dolutegravir functional monotherapy and an increased risk for selection of dolutegravir resistance in these individuals. These findings highlight the potential pitfall associated with the practice of regimen transition without confirming virological suppression during the accelerated rollout of the tenofovir + lamivudine + dolutegravir regimen in Tanzania. Therefore, virological monitoring, HIVDR testing and routine surveillance remain crucial to inform clinicians and the country's ART programme.

In this study we could not detect any DRMs in 28.5% (39/137) of participants with high viraemia. Further, about 80% or more of participants with high viraemia on dolutegravir or PI-based regimens were genotypically predicted to be susceptible to their respective regimens. This suggests that high viraemia could be because of factors other than drug resistance. This phenomenon has been reported in studies involving PI-based regimens where intensive adherence counselling has been successful to resuppress viraemia.[25] Therefore, it is logical to stipulate that high viraemia with no detectable DRMs in some patients could be a result of suboptimal adherence, which is common in Tanzania's settings.[26–28] In addition, suboptimal dosing is a known problem in paediatric patients in low-income countries and has been associated with failure to suppress viraemia.[29] Therefore, the high proportions of participants with high viraemia on a dolutegravir-based regimen without detectable DRMs reported in this survey call for further studies to elucidate the underlying factors.

Study limitations

The present study has some limitations. In this survey, we did not collect data on adherence or suboptimal dosing status. Thus, the study cannot rule out these factors as contributors to the rapid emergence of dolutegravir resistance. However, the HIV programming in the country prioritizes enhanced adherence counselling when treatment failure is suspected.[26]

Furthermore, we used the Sanger sequencing approach to target the PR-RT and IN regions. Thus, we could not rule out the presence of minority drug resistance variants or resistance mutations outside the targeted regions. However, we believe our results are representative of major drug resistance variants. Nonetheless, to mitigate the limitation, sample aliquots from each participant are archived for further analysis using the next-generation sequencing platforms, upon availability of funds.

Conclusions

This survey found that 71.5% of PLHIV with high viraemia in Tanzania have DRMs. Importantly, 5.8% of PLHIV harboured

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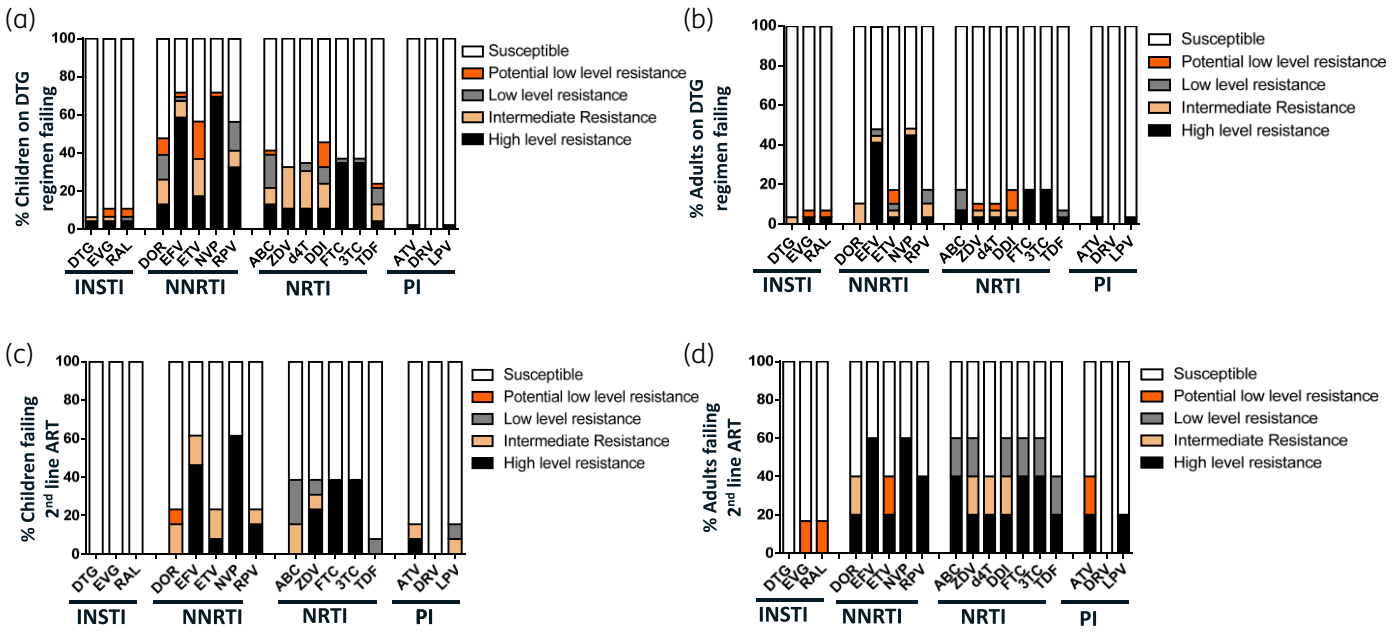


Figure 3. Genotypic prediction of INSTI, PI, NNRTI and NRTI drugs. Susceptibility predictions of INSTI, PI, NNRTI and NRTI drugs in children (a) and adults (b) with high viraemia on first-line dolutegravir-based ART. Susceptibility predictions of INSTI, PI, NNRTI and NRTI drugs in children (c) and adults (d) with high viraemia on second-line ART. ABC, abacavir; ATV, atazanavir; d4T, stavudine; ddI, didanosine; DOR, doravirine; DRV, darunavir; DTG, dolutegravir; EFV, efavirenz; ETV, etravirine; EVG, elvitegravir; FTC, emtricitabine; LPV, lopinavir; NVP, nevirapine; RAL, raltegravir; RPV, rilpivirine; TDF, tenofovir disoproxil fumarate; ZDV, zidovudine; 3TC, lamivudine. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

INSTI DRMs, including those that confer resistance to the newly introduced drug dolutegravir. In addition, we showed that NRTI drug resistance mutations are common, detected in 44.5% of patients with high viraemia. Our findings highlight the potential threat to the efficacy of the ART programme in Tanzania and similar resource-limited settings where dolutegravir rollout is being implemented without confirmation of virological suppression during switching of treatment. Therefore, the findings from this survey emphasize the need for VL testing before patients are switched to an INSTI-based regimen.

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Transparency declarations

The authors declare no conflict of interest.

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