Next-generation sequencing (NGS) reveals low-abundance HIV-1 drug resistance mutations among patients experiencing virological failure at the time of therapy switching in Uganda [version 1; peer review: awaiting peer review]

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Abstract
Background: The emergence and spread of antiretroviral drug resistant HIV-1 variants is one of the major factors associated with therapeutic failure in persons living with HIV (PLWH) as it jeopardizes the efforts to reduce the progression to AIDS. Whereas Sanger sequencing is the most appropriate conventional method for HIV drug resistance testing, it has limited capacity to detect low-abundance variants. This study assessed the suitability of next generation sequencing (NGS) to reveal low-abundance HIV-1 drug resistance mutations amongst patients experiencing virological failure at the time of therapy switching in Uganda.

Methods: Archived blood samples previously collected from 60 PLWH were used in this study. Briefly HIV viral RNA was extracted and performed targeted NGS of portions of both the HIV protease and reverse transcriptase genes on the illumina MiSeq. For performance comparison, Sanger sequencing was also performed for all the
samples targeting the highlighted genes. The sequence data generated was analyzed using HyDRA bioinformatics pipeline, accompanied by the Stanford HIV drug resistance database, to annotate and report drug resistance mutations/variants.

**Results:** Out of the 60 samples, 58 passed preliminary quality control and were considered for subsequent analysis—of which 38/58 (65.5%) registered low-abundance HIV drug resistance variants. Overall, 757 variants from the NGS data and 90 variants from the Sanger data were identified. The most prevalent minority variants included; K65R (65.5%), K14R (63.8%), K45R (63.8%), L63P (63.8%), I15V (63.8%), K70R (60.3%), V77I (60.3%), L283I (60.3%), G16E (58.6%) and L282C (58.6%).

**Conclusion:** An estimated 65.5% of the sampled population harbors low-abundance HIV-1 variants, most of which are associated with virological failure, and consequently antiviral drug resistance. NGS suitably detects drug resistance mutations even at frequencies below 20% of the viral quasi species that are occasionally missed by Sanger sequencing.

**Keywords**
HIV, Drug resistance testing, Low-abundance (minority) variants, Sanger sequencing, Next-generation sequencing, Sanger sequencing
Introduction
The widespread emergence and transmission of HIV drug resistance (HIV-DR) may jeopardize the success of the currently recommended first-line antiretroviral therapy (ART) regimens in sub-Saharan Africa (SSA) (Phillips et al., 2017). The initial ART regimen generally consists a combination of two nucleoside reverse transcriptase inhibitors (NRTIs) usually lamivudine/abacavir (3TC/ABC) or tenofovir disoproxil fumarate/emtricitabine (TDF/FTC) plus a drug from one of the three classes: a non-nucleoside reverse transcriptase inhibitor (NNRTI); usually efavirenz (EFV) or nevirapine (NVP), an integrase strand transfer inhibitor (INSTI) or a protease inhibitor (PI) (De Clercq, 2013). Additional data has shown to support the use of Dolutegravir/lamivudine (DTG/3TC) for initial treatment of some people with HIV (Cahn et al., 2020).

Recently, there has been reported existence of HIV drug resistance to both classes of first line drugs mentioned above, with reported genotypic variations. Predominantly, the prevalence of pre-treatment NNRTI resistance has been eye-catching in African populations, with notable ranges of 8% in Cameroon, and close to 15% in Uganda (de Waal et al., 2018). In 2018, the WHO recommended use of DTG in first line ART as there was increasing prevalence of pre-treatment drug resistance to most NNRTIs, especially in sub-Saharan Africa (Siedner et al., 2020). This was supported by international data documenting the presence of low-abundance variants, in addition to the known variants, among people on antiretroviral drugs. However, the policy stated that the urgency of the transition would largely depend on the country-specific prevalence of NNRTI resistance (Phillips et al., 2018a). The policy was not based on locally available data and as such calls for validation, particularly in our Uganda population—given that such in depth analyses can avail a baseline guidance on whether a patient living with HIV has a mutated form of the virus that does respond to ART or not (Clutter et al., 2016).

Several mechanisms including phenotypic and genotypic methods have been employed to offer options for HIV drug susceptibility testing. These methods include conventional HIV-1 genotyping techniques as well as Next generation sequencing approaches, that may as well be used to elaborate predominant viral quasispecies in the population (Smyth et al., 2012). The conventional HIV-1 genotyping assays, including Sanger sequencing, have to date been employed as the gold standard for HIV-DR testing although limited to only detecting HIV-1 variants present at frequencies above approximately 20% of the viral quasispecies. Such limitations lead to the collective failure of such approaches to exclusively detect low-levels of HIV-1 variants (with frequencies lower than 20% of viral quasispecies)—even when the viral species harbor HIV-DR mutations (Lee et al., 2020). Low-abundance variants, also known as minority variants, are usually present in small proportions of the virus populations, and have been reported to have a significant association with increased virological failure for first-line nucleoside reverse transcriptase inhibitors (NRTI) and NNRTI based regimens (Mbunkah et al., 2020). This type of drug resistance has been proven to be selected under the appropriate pressure of ART onto the virions during the course of treatment. And with the advent of deep (next-generation) sequencing, unlike the conventional methods, it is very possible to detect drug resistant HIV-1 variants at low frequencies, below 20% of the viral population (Simen et al., 2009). This study therefore aimed at utilizing NGS deep sequencing and correspondent bioinformatics pipelines to detect low-abundance drug resistance HIV-1 variants amongst patients on first line regimen combinations, with reported virological failure in Uganda, at the time of therapy switching.

Methods
Study design and study population
This was a retrospective quantitative cross-sectional study that analyzed 60 archived blood samples received at the National Health Laboratory and Diagnostic Services (NHLDS), a department of the Ugandan Ministry of Health (MoH), between June 2020 and October 2020. These samples had been collected from PLWH on first line ART in Uganda that were found to have virological failure defined as non-suppressed repeat (2nd) viral load of ≥1,000 copies/ml, performed consecutively within at least three months of each other, following enhanced adherence support of up to three months. The patients were on combinations; TDF+3TC+EFV, TDF+3TC+DTG, AZT+3TC+EFV, ABC+3TC+EFV AZT+3TC+NVP, ABC+3TC+DTG and ABC+3TC+LPV/r among children as reported.

Ethical consideration
Approvals to use archived samples were sought from NHLDS, Department of Immunology and Molecular Biology, NHLDS-Research and Ethics Committee (REC), Makerere University School of Biomedical Sciences Research and Ethics Committee (SBSREC, Reference SBS-2021-56), as well as the Uganda National Council of Science and Technology (UNCST, Reference HS743ES).

Study site
Samples were obtained from NHLDS, a reference laboratory for HIV viral load testing in Uganda and transported to the Genomics and Molecular Biology Laboratory at the Department of Immunology and Molecular Biology, Makerere University College of Health Sciences.
Viral RNA extraction, PCR and gel electrophoresis
Total viral RNA was extracted from 60 archived samples using the QIAamp viral RNA extraction kit according to the manufacturer’s protocol (Qiagen, USA). Total viral RNA extracted was used as template in reverse transcription PCR to obtain cDNA using SuperScript III Reverse transcriptase double stranded cDNA synthesis kit following the manufacturer’s instructions (Thermo Fisher, USA). PCR targeting a contiguous region of the HIV-1 pol gene of interest in routine HIVDR, the protease gene (codon 1-99) and amino terminus of reverse transcriptase codon (1-320) was performed. For quality control, the subsequent amplicons were analyzed by gel electrophoresis through 1.5% (w/v) agarose gels. Gels were stained with ethidium bromide and bands visualized under UV; lanes of 1 kbp DNA ladder marker (New England Biolabs, UK).

Library preparation and Next-generation sequencing (NGS)
Genomic libraries were prepared using the Illumina Nextera XT library preparation kit following manufacturer’s instruction (Illumina, San Diego, USA). Quality of the prepared libraries was assessed with the Agilent Tape Station system using the D1000 High sensitivity ScreenTape assay and reagents (Agilent Technologies, USA). Libraries were sequenced using the Illumina MiSeq V3 cartridge following the manufacturer’s protocol (Illumina, San Diego, USA) at Genomics and Molecular Biology Laboratory at Makerere University College of Health Sciences.

Sanger sequencing
To validate NGS data, sanger sequencing, a gold standard for HIV drug resistance testing was performed on the same samples. Briefly, PCR was performed targeting the reverse transcriptase and protease genes to generate Amplicons of 1200bp for each sample. The amplicons were then purified using the ExoSAP-ITTM kit and sequenced using the Big dye terminator v3.1 cycle sequencing kit according to the manufacturer’s protocol (Applied Biosystems, USA). Targeted sequencing for the HIV selected regions was run using the Applied Biosystems (ABI) SeqStudio Genetic Analyzer machine at the Genomics and Molecular Biology Laboratory, Dept. of Immunology & Molecular Biology, Makerere University College of Health Sciences.

Bioinformatics analysis
Raw sequence data in FASTQ file format were assessed for quality using FASTQC and MULTIQC software tools (Andrews, 2017/2022) that produced reports in html format about the quality of the sequences. Low-quality bases, very short reads and any adapter sequences were therefore trimmed off and removed using Trim galore tool (Babraham Bioinformatics, 2019). Using the HyDRA pipeline, an annotated reference-based bioinformatics pipeline, the generated NGS data was analyzed to determine the genotypes of HIV-1 drug resistance mutations. The annotated HXB2 sequence was used for reference mapping by Bowtie2, and stringent data quality assurance and variant calling to identify HIVDR associated mutations based on the World Health Organization (WHO) list for surveillance of transmitted HIVDR. All HIVDR mutations found in the pol gene; protease (PR), reverse transcriptase (RT), were reported according to classifications outlined in the Stanford surveillance drug resistance mutation list (WHO, 2020).

HIV variant calling and annotation
After performing all the quality checks, good quality reads were considered for the downstream analyses. Calling of the low abundance HIV-1 variants and annotation was performed using the HyDRA pipeline in the command-line to detect HIVDR mutations. The FASTQ files were analyzed to release files in aavf format, an output of HyDRA pipeline from Quasitools. The aavf report provides a compact summary of the amino acid variation obtained by conceptual translation of the NGS read pileup across the examined region of the HIV genome. It also contains information on the frequencies of matching codons (wild type or mutant), quality of the variant calling as well as the coverage of relevant loci and it is based on the variant call format (vcf) standard that has been universally adopted for recording nucleotide variants. The main output of HyDRA was a drug resistance report which used the Stanford HIVDB mutations when reporting. All HIV DR mutations found at a frequency above 1%, a default setting under minimum AA frequency were listed in the report. A summary report of HIVDR mutations identified in each sample was downloaded in the form of a CSV file and viewed in Excel.

Comparison of drug resistance mutations data generated from Sanger and NGS
Output data from Sanger sequencing in. ab1 file format was transformed using a custom script into fasta files that were also queried against the Stanford HIV drug resistance database for identification of major drug resistance mutations for each antiretroviral drug. This unveiled the drug resistant variants from the Sanger sequence data. For comparison, custom bash scripts were written to sort drug resistant mutations from both the Sanger dataset and NGS dataset. Thereafter, unique and common mutations were extracted from both datasets. The main objective of the comparison was to identify mutations of clinical significance that were missed by Sanger sequencing but captured by NGS.
Results

Patients’ demographics

A total of 58 samples passed preliminary quality control, and were successfully analyzed in this study. Among the participants, 43 (74.1%) were females and 15 (25.9%) were males with an average age of 32 years. These patients had all been on first line regimens for a period of at least 6 months where 37 (63.8%) of them were on TDF-3TC-EFV. Other ART combinations were TDF-3TC-DTG (10.3%), ABC-3TC-DTG (1.7%), AZT-3TC-NVP (12.1%), AZT-3TC-EFV (1.7%), ABC-3TC-EFV (3.4%) and ABC-3TC-LPV/r (6.9%) as summarized in Figure 1.

Summary of variants from the analyzed dataset

Sequence data was obtained from the two genes targeted in the routine monitoring of HIV drug resistance testing, namely the reverse transcriptase (RT) and protease (PR) genes. Sequence data of these genes provided information on three classes of drugs namely, NRTI, NNRTI and PI class. Overall, a total of 782 variants were identified; 757 variants from the NGS data, 90 variants from the Sanger sequencing data whereas 65/782 (8.3%) of the variants were shared between NGS and Sanger datasets. Additionally, it was observed that Sanger Sequencing was able to detect 25 variants that were not identified by NGS, whereas NGS data revealed 692 variants that were not detected by Sanger sequencing. See Table 1 below.

Distribution of minority variants amongst patients

From the NGS datasets, all variants detected at a frequencies of 20% and above within the viral quasispecies were categorized as majority, whereas all those detected at a frequency less than 20% but greater than or equal to 1% of the viral quasispecies are categorized as minority variants (Silver et al., 2018); therefore from the current study, 563/757 (74.4%) of the NGS identified variants were categorized as minority variants. The 563 minority variants were distributed variably with some mutations appearing more frequently than others. Such variants appeared in 65.5% (38/58) of the sampled population—some of which variants included HIV-DR mutations. Overall, the most prevalent minority variants present in the analyzed samples included; K65R in 38/58 (65.5%), K14R in 37/58 (63.79%), K45R in 37/58 (63.79%), L63P in 37/58 (63.79%), and I64V in 37/58 (63.79%). Additionally, I15V was identified in 60.3% (35/58), K70R in 60.3% (35/58), V77I in 60.3% (35/58), and L283I in 60.3% (35/58), G16E in 60.3% (35/58). Some of the least prevalent minority mutations found in only one (0.02%) of the analyzed samples are A71V, C67W, D25G, D30N, E21D, G16W, G27E, H69L, I15M, I50F and I64L. Others are listed in the supporting file HDR-Supp.zip.

It was also observed and important to note that a mutation categorized as a minority variant in one sample can be also identified as a more frequent variant in another sample. There was purposive selection of 15 most prevalent mutations from the analyzed samples and their positions in the corresponding genes were also highlighted in Table 2.

All variants are summarized in the supporting files HDR-Supp.zip.

Table 1. Total variants detected by Sanger Sequencing, NGS, both NGS and Sanger, NGS only but not Sanger and Sanger but not NGS.

<table>
<thead>
<tr>
<th>Sequencing Platform</th>
<th>Sanger</th>
<th>NGS</th>
<th>NGS &amp; Sanger</th>
<th>NGS but not Sanger</th>
<th>Sanger but not NGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td>90</td>
<td>757</td>
<td>65</td>
<td>692</td>
<td>25</td>
</tr>
</tbody>
</table>
Minority variants of clinical importance

This study also identified some minority variants of clinical importance that were highly prevalent in the analyzed samples but were missed by Sanger sequencing. Figure 2 shows some unusual mutations that have not been reported previously as well as the presence of drug resistance mutation. These included; K14R, K45R, L63P, I15V, L83I, V77I. Other than the drug resistant mutations, there were also several unusual mutations whose clinical significance is still unknown and they could be nominated for diagnostic targets, drug resistance targets, drug targets and so on.

Discussion

Affordable, sensitive, and scalable technologies are needed for monitoring antiretroviral treatment (ART) success with the goal of eradicating HIV-1 infection in low resource settings. For instance, in recent years, conventional HIV-1 genotyping assays, including Sanger sequencing have been the standard approaches for HIV-DR testing in sub-Saharan Africa (Manyana et al., 2021). Being the gold standard, Sanger sequencing is still able to detect HIV-DR mutations of clinical relevance as well as drug resistance, even though it is mainly limited by relatively high sequencing costs and low-throughput, henceforth evidently failing to detect the low-levels of HIV-1 variants—with reported frequencies lower than 20% of viral quasispecies—which scenario may well harbor HIV-DR mutations as reported (Lee et al., 2020). These low-abundance variants, also known as minority variants, usually present as minority members of the virus populations, and

Table 2. Most prevalent minority variants and their positions in the corresponding genes.

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Position</th>
<th>REF</th>
<th>ALT</th>
<th>ANN (variant)</th>
<th>Frequency (Out of 58) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hxb2_pol</td>
<td>65</td>
<td>K</td>
<td>R</td>
<td>K65R</td>
<td>38 (65.5)</td>
</tr>
<tr>
<td>Hxb2_pol</td>
<td>14</td>
<td>K</td>
<td>R</td>
<td>K14R</td>
<td>37 (63.8)</td>
</tr>
<tr>
<td>Hxb2_pol</td>
<td>45</td>
<td>K</td>
<td>R</td>
<td>K45R</td>
<td>37 (63.8)</td>
</tr>
<tr>
<td>Hxb2_pol</td>
<td>63</td>
<td>L</td>
<td>P</td>
<td>L63P</td>
<td>37 (63.8)</td>
</tr>
<tr>
<td>Hxb2_pol</td>
<td>15</td>
<td>I</td>
<td>V</td>
<td>I15V</td>
<td>37 (63.8)</td>
</tr>
<tr>
<td>Hxb2_pol</td>
<td>70</td>
<td>K</td>
<td>R</td>
<td>K70R</td>
<td>35 (60.3)</td>
</tr>
<tr>
<td>Hxb2_pol</td>
<td>77</td>
<td>V</td>
<td>I</td>
<td>V77I</td>
<td>35 (60.3)</td>
</tr>
<tr>
<td>Hxb2_pol</td>
<td>283</td>
<td>L</td>
<td>I</td>
<td>L283I</td>
<td>35 (60.3)</td>
</tr>
<tr>
<td>Hxb2_pol</td>
<td>16</td>
<td>G</td>
<td>E</td>
<td>G16E</td>
<td>34 (58.6)</td>
</tr>
<tr>
<td>Hxb2_pol</td>
<td>282</td>
<td>L</td>
<td>C</td>
<td>L282C</td>
<td>34 (58.6)</td>
</tr>
<tr>
<td>Hxb2_pol</td>
<td>245</td>
<td>V</td>
<td>K</td>
<td>V245K</td>
<td>33 (56.9)</td>
</tr>
<tr>
<td>Hxb2_pol</td>
<td>62</td>
<td>I</td>
<td>V</td>
<td>I62V</td>
<td>32 (55.2)</td>
</tr>
<tr>
<td>Hxb2_pol</td>
<td>49</td>
<td>K</td>
<td>R</td>
<td>K49R</td>
<td>32 (55.2)</td>
</tr>
<tr>
<td>Hxb2_pol</td>
<td>207</td>
<td>Q</td>
<td>E</td>
<td>Q207E</td>
<td>31 (53.4)</td>
</tr>
</tbody>
</table>

*Denotes the most prevalent low-abundance drug resistance variant located at position 65 of the pol gene. Other high frequency low-abundance drug resistance variants are also summarized in the Table 2 above.
thus cannot be easily detected. With such challenges of inadequate/inaccurate detection, some studies recommend the usage of high throughput sequencing technologies (Slatko et al., 2018; Manyana et al., 2021). This study therefore aimed at utilizing NGS deep sequencing approaches, and correspondent bioinformatics pipelines, to detect low-abundance drug resistance HIV-1 variants amongst patients on first line regimen combinations, with reported virological failure in Uganda, at the time of therapy switching. The study population included patients on first-line regimen with virological failure where most participants (58.6%) were on TDF, 3TC, and EFV combination; that is, two NRTIs and one NNRTI. This regimen was common for HIV treatment in many countries as reported (Gregson et al., 2016). Sequence data was obtained from the two genes targeted in the routine monitoring of HIV drug resistance testing, namely the reverse transcriptase (RT) and protease (PR) genes. These genes provide information on three classes of drugs; NRTI, NNRTI and PI class. Overall, 782 variants were identified; 757 variants from the NGS data, 90 variants from the Sanger sequencing data whereas 65/782 (8.3%) of the variants were shared between NGS and Sanger datasets. The study further observed that Sanger Sequencing was able to detect 25 variants that were not identified by NGS, whereas NGS revealed 692 variants that were not detected by Sanger sequencing. This elaborte that NGS being a deep sequencing high-throughput detects more variants compared to Sanger sequencing as observed by previous studies (Taylor et al., 2019; Schenkel et al., 2016). The current study further reports 563 (74.4%) minority variants observed within the 757 total variants identified by NGS. This elaborates that NGS is able to detect approximately 74.4% of the minority variants present above a cutoff of 1% of the viral quasispecies. The 563 minority variants were dispersed unproportionally, with some mutations appearing more frequent than others. Such variants were observed in 65.5% (38/58) of the sampled population amongst the study participants—some of which variants included HIV-DR mutations. Several reports have elucidated the presentation of similar minority variants in different populations as reported (Nicot et al., 2012; Capobianchi et al., 2013; Wensing et al., 2019). The current study also highlights the most prevalent minority variants, which included; K65R in 38/58 (65.5%), K103R in 37/58 (63.79%), K45R in 37/58 (63.79%), L63P in 37/58 (63.79%), and M184V in 37/58 (63.79%). The mutation K65R, that also presents the highest prevalence in the current study, is one of the low-abundance drug resistance mutations conferring resistance to at least one class of drugs—and within the NRTI class. However in other studies, this mutation has been categorized as a relatively rare drug resistance conferring variant that causes loss of drug susceptibility of HIV-1 to most NRTIs (tenofovir, didanosine, abacavir and stavudine), with exception to zidovudine (Brener & Coutsinos, 2009). And essentially, even though there are limited studies that have focused on understanding the incidences of minority variants more so in sub-Saharan Africa, the outstanding prevalence of K65R perfectly relates to findings in a few researches conducted in different geographical regions in HIV high burdened areas. In Israel, there was a reported high incidence of K65R is the analyzed population (Turner et al., 2009); in West Africa, particularly Togo, there was evidence of a high prevalence of K65R (Dagnan et al., 2011); whereas within the Resistance Monitoring study, a multicenter prospective observational cohort of HIV-1-infected adults who received ART in routine circumstances at 13 clinical sites in Kenya, Nigeria, South Africa, Uganda, Zambia, and Zimbabwe—also reported a significant incidence of K65R amongst samples collected from participants (Sigaloff et al., 2011). Other studies conducted in Southern Africa have elucidated significant occurrences of K65R as a key variant amongst HIV-1 positive individuals experiencing virological failure (Skhosana, 2015; Etta et al., 2017). Accordingly, in the current study, it should be noted that K65R remains the most important and significant variant identified. The results from the study findings also elaborate that there is observed co-existence of mutations amongst the analyzed samples, which findings strongly compare with other research that observed co-existence of variants, most notably K65R with S68G. The alteration S68G, has been reported to partially restore the replication defect associated with K65R as reported (Parikh et al., 2006). Additionally, the variants K14R and K70R were observed to be housed, each at a frequency of 60.3% of all the samples analyzed. The variant K14R is a single nucleotide variant that has been reported to confer no resistance to the most important ART drugs (Descamps et al., 2009), whereas K70R has been categorized as a classical Thymidine Analog mutation (TAM) known to cause intermediate-level resistance to AZT, and possibly low-level resistance to d4T and TDF (Quintana & José, 2013; Hachem et al., 2020). The current study also observed several nonpolymorphic mutations from the analyzed data as categorized by the HIV Stanford database. Such variants include; P225H, G190A, M46I, M46L, K70Q, K70N, K70S, K70T, K101E, and L100I—that are categorically believed not to occur in absence of therapy, contrary to the polymorphic mutations that occur frequently in viruses not exposed to selective drug pressure (Shafer & Schapiro, 2008; WHO, 2020). It should also be noted that the mutation P225H is an EFV-selected variant that occasionally occurs in combination with K103N. This results in synergistic reduction of susceptibility to NVP, EFV and DOR (Kouamou et al., 2021). As well, another reported mutation G190A has been associated with high-level resistance to NVP and intermediate resistance to EFV (Yang et al., 2015), whereas K101E is a primary accessory mutation that causes intermediate resistance to NVP and RVP, low-level resistance to EFV, and potentially low-level resistance to ETR as reported (Wu et al., 2015). The mutations M46I and M46L are relatively non-polymorphic Protease inhibitor-selected mutations, associated with reduced susceptibility to PIs with an exception of DRV, as reported (Watera et al., 2021). The mutations K70E and K70G are known to cause low-level resistance to TDF, ABC, 3TC and FTC, and also increase susceptibility to AZT (Siller et al., 2021). Other identified mutations included K70Q, K70N, K70S and K70T, that are rare non-polymorphic NRTI-selected mutations relating to resistance profiles of K70E and K70G described herein above. Earlier studies elaborated that the mutation L100I usually occurred in
combination with K103N even though there is evidence that such variants may cause high level resistance to NVP and EFV (Soriano & de Mendoza, 2002). Other than the drug resistant conferring variants, there were also several unusual mutations whose clinical significance is still unknown and they could be nominated and validated as potential diagnostic targets, drug resistance targets, and so on. For instance, the present study has also unveiled a number of minority variants of clinical importance that were highly prevalent in the analyzed samples. These included; K14R, K45R, L63P, I64V, I15V, L83I, V77I among others as summarized in the supporting files "HDR-Supp.zip".

**Conclusion**

Next Generation Sequencing approaches can confidently reveal low-abundance drug resistance variants (minority variants) in populations of HIV-1 resistant individuals that have been reported to have virological failure. NGS, due to its high throughput data is able to detect low abundance variants as low as 1% of the viral quasispecies. This has been a limitation of the conventional Sanger sequencing that is currently considered the gold standard for HIV-DR testing. It is therefore vital that patients experiencing virological failure are tested using NGS as Sanger misses most of the low abundance variants that are of clinical significance. The frequency of drug resistance varies among individuals suggesting that discrete viral populations have different levels of genetic diversity. An estimated 65.5% of the sampled population harbored low-abundance HIV-1 variants, most of which are associated with virological failure, and consequently antiviral drug resistance.

**Study limitations**

The current Ministry of Health consolidated guidelines for prevention and treatment of HIV in Uganda recommended transition to use of DTG-based regimens as first line ART regimen: a combination of two NRTIs plus Dolutegravir (TDF+3TC+DTG) following concern over increasing prevalence of NNRTI resistance in people on ART in low-income and middle-income countries (MoH, 2018). According to a study on cost-effectiveness of public health options in presence of pre-treatment NNRTI drug resistance in sub-Saharan Africa, the urgency of transition would depend largely on the country specific prevalence of NNRTI resistance (Phillips et al., 2018b). During sample collection for the current study in 2020, 37/58 (63.8%) of the participants were still on TDF+3TC+EFV.

**Data availability**


This project contains the following underlying data:


Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

**Author contributions**

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**Manuscript writing:** All authors have given final approval of the version to be published.
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