Next generation HIV-1 sequencing reveals complex patterns of drug resistance and transmission in a rural African setting

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INTRODUCTION

- There has been **considerable progress** made to **increase accessibility** to **antiretroviral** (ARV) therapy (ART) in Sub-Saharan Africa.
- The **HIV incidence in the uMkhanyakude district** of KZN, South Africa **remains high .**
- Acquired and transmitted **ARV resistance** is a **threat** to the **efficacy of ART.**
- The **Vukuzazi programme** is a community-based **disease phenotyping** and **health surveillance programme** aimed to measure the prevalence of HIV, tuberculosis, hypertension, and diabetes in an HIV endemic population and **directly link patients to care**

OBJECTIVES

This study aimed to:

- Assess the **prevalence of ARV resistance** in the **uMkhanyakude district** through a cross-sectional, integrated health surveillance study
- Determine **patterns of ARV resistance** using **whole-genome sequencing**
- Identify **transmission networks** of HIV between individuals and examine potential **transmitted ARV resistance**

METHODS

- A cross-sectional, integrated health surveillance study between May 25, 2018, to Nov 28, 2019. Residents aged ≥15 years were eligible for the study
- Of **34,271** eligible individuals, **17925** completed a **blood draw**, of whom **6,096 (33.8%) were HIV+.** 1,074 had a HIV-1 RNA viral load > 400 copies/ml and **1323 with >40 copies/ml.** These 1323 samples underwent **whole-genome sequencing** using the **Illumina MiSeq** platform. Poor quality sequences were excluded from analysis.
- ClusterPicker (v1.2.5) was used to identify clusters of **likely transmission** with an initial and main **support threshold of 99%**, a **genetic distance threshold of 4.5%** and allowed for large clusters to contain up to **15 tips**. Clusters were validated with a logit model. The probability of sample presence within a cluster was calculated using a backward stepwise model, considering **collection date** and **patristic distance** between **pairs of sequences** present on an **untimed maximum likelihood phylogeny**.
- Phyloscanner (1.8.1) was used to identify transmission between different individuals and within clusters.

RESULTS

	All (n=1202)
Age Group (Years)	
15-24	250 (20.8%)
25-34	422 (35.1%)
35-44	309 (25.7%)
45-54	147 (12.2%)
55-64	46 (3.8%)
65+	28 (2.3%)
ART Status At Recruitment	
Naïve	747 (62.1%)
Experienced	456 (37.9%)
Currently On ART	
Yes	603 (50.2%)*
No	34 (2.8%)
Unknown	565 (47.0%)
ART Regimen	Known regimen (n=456)
EFV, FTC, TDF	387 (85%)
3TC, TDF, LPV/r	18 (4.0%)
3TC, AZT, LPV/r	13 (2.9%)
3TC, DTG, TDF	8 (1.8%)
Other	29 (6.4%)

Table 1. Characteristics of patients with a VL>400 copies/mL

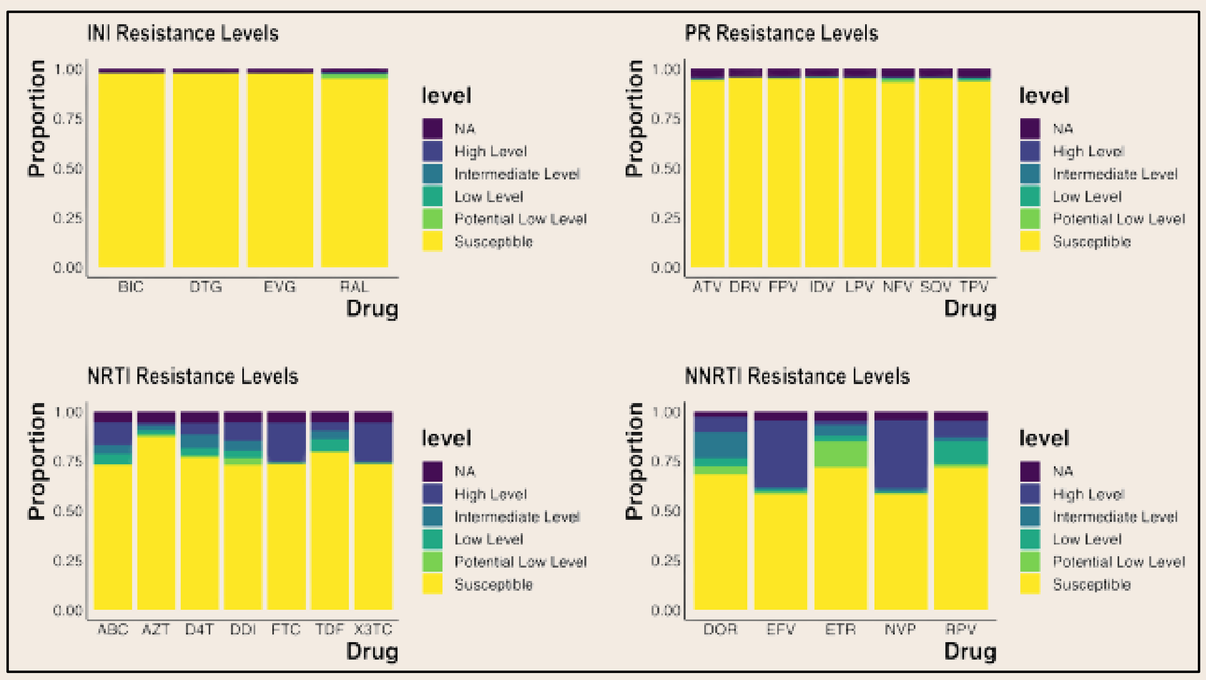

Figure 1. Drug Resistance Levels. NNRTI resistance is evident in the cohort, most of the population is susceptible to first-line and salvage therapy.

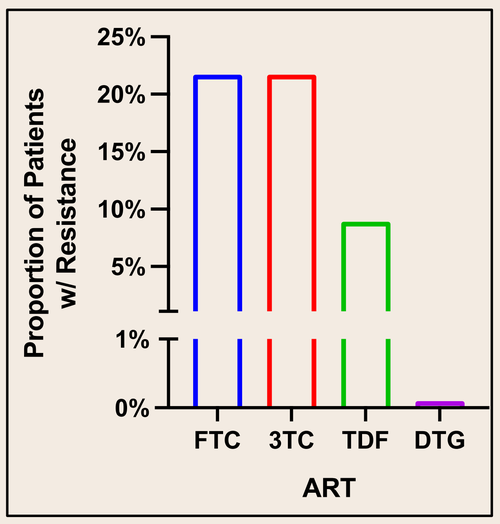

Figure 2. Proportion of Patients with resistance to FTC, 3TC, TDF and DTG (n=1050) high quality sequences.

	OR	2.5 %	97.5 %
Intercept	4.8783e+06	2.5891e+06	9.3756e+06
Patristic distance	3.9842e-33	2.4630e-34	5.8573e-32
log(p/(1-p)) = logit(p) = 15.4 + -74.60295*patristic distance			

Table 2. ClusterPicker was validated prior to further transmission analysis. The probability of a sample being present within a cluster was calculated using a backward stepwise approach. 205/1050 (19.5%) patients were linked in a cluster, 75% of which were pairs. 25% of clusters were formed of 3-6 patients.

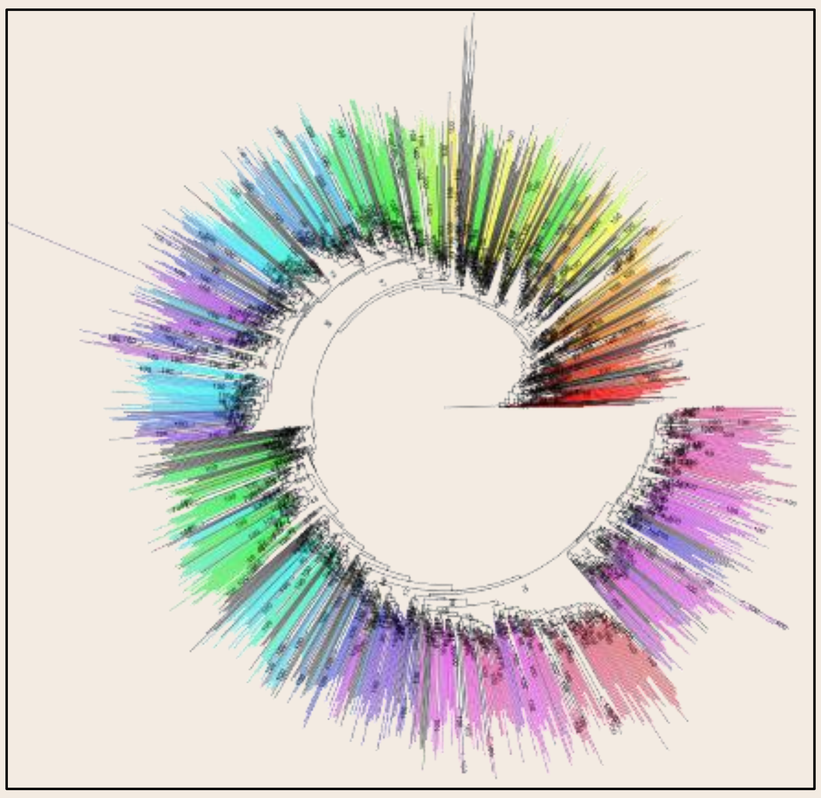

Figure 3. Maximum likelihood phylogenetic tree showing HIV whole-genome sequences. Colors indicate clusters generated through ClusterPicker. In total, **171 clusters were identified**.

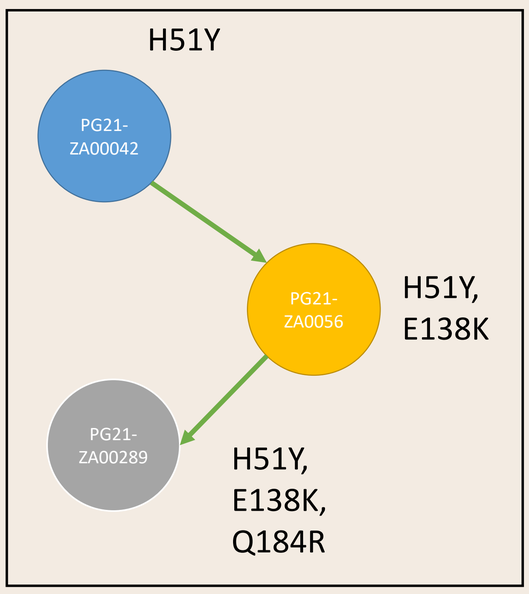

Figure 4. Onward transmission cluster of INSTI mutations between 3 patients. Accumulation of INSTI mutations was detected from one patient to another, and then passed to a third patient.

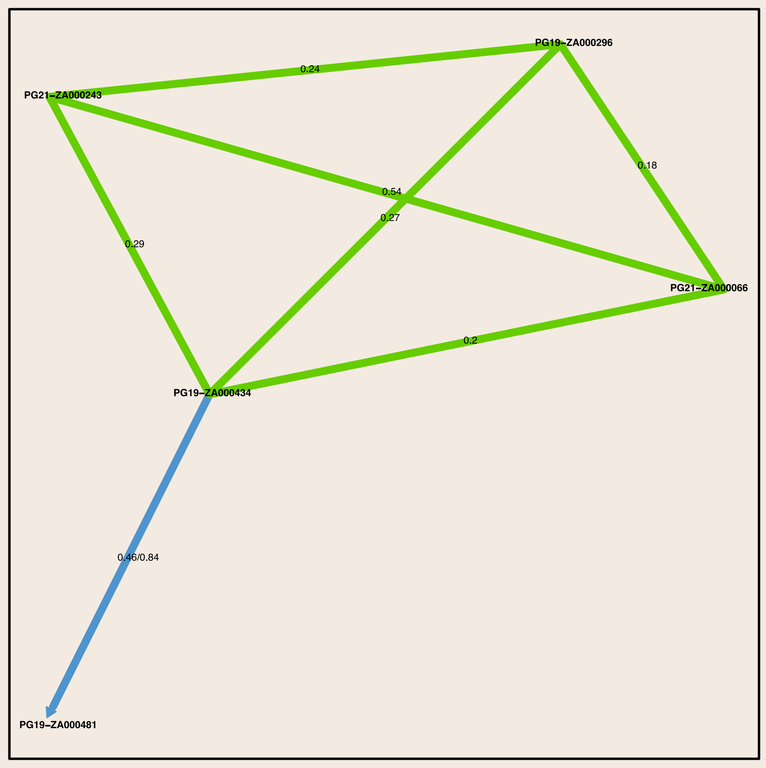

Figure 5. Transmission analysis examining a cluster of 5 patients. Linked patients are indicated with green lines. Direct transmission with directionality is highly likely between individuals linked by the blue line.

CONCLUSIONS

- As of 2019, most patients in KZN with a detectable VL i.e. >40 copies/ml, **remain susceptible to DTG**, even with **NRTI resistance**.
- **Whole-genome sequencing** and use of **phylogenetics** allows us to infer linkage and ascertain how ART resistance is transmitted between individuals in the community.
- These patients had limited instances of resistance to PIs/INSTIs - detected mutations were mostly **polymorphic** or **did not reduce susceptibility**.

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