

Short communication

Transmitted, pre-treatment and acquired antiretroviral drug resistance among men who have sex with men and transgender women living with HIV in Nigeria

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Background: Across sub-Saharan Africa, men who have sex with men (MSM) and transgender women (TGW) have disproportionately poor HIV treatment outcomes. Stigma and criminalization create barriers to health-care engagement and adherence to antiretroviral therapy (ART), potentially promoting the development of HIV drug resistance (HIVDR). We evaluated transmitted, pre-treatment and acquired HIVDR among MSM and TGW in Lagos and Abuja, Nigeria. **Methods:** Adults with HIV RNA $\geq 1,000$ copies/ml in the TRUST/RV368 cohort, including incident cases diagnosed via 3-monthly screening, underwent HIVDR testing using the Sanger sequencing method. Major mutations conferring resistance to nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs) were identified from the 2017 IAS-USA list. World Health Organization surveillance drug resistance mutations (SDRMs) were identified in ART-naive participants.

Results: From March 2013 to June 2017, 415 participants with median age 24 (interquartile range [IQR] 21–27) years, CD4⁺ T-cell count 370 (IQR 272–502) cells/mm³, and HIV RNA 4.73 (IQR 4.26–5.15) log₁₀ copies/ml underwent HIVDR testing. SDRMs were observed in 36 of 373 ART-naive participants (9.7%, 95% confidence interval [95% CI 6.8, 13.1%]), including 8 of 39 incident cases (20.5%, [95% CI 9.3, 36.5%]). Among 42 ART-experienced participants, NNRTI resistance was detected in 18 (42.9%, 95% CI 27.7, 59.0%) and NRTI resistance in 10 (23.8%, 95% CI 12.0, 39.4%). No PI resistance was detected.

Conclusions: The high prevalence of transmitted and acquired drug resistance among Nigerian MSM and TGW living with HIV suggests the need for programmatic solutions to improve uninterrupted access to ART and timely switch to second-line regimens in cases of viral failure.

Introduction

Genotypic testing for HIV drug resistance (HIVDR) is routine in resource-rich settings prior to antiretroviral therapy (ART) initiation or re-initiation in persons living with HIV (PLWH) [1] but is rarely available in

resource-limited settings such as sub-Saharan Africa [2]. Empiric ART selection based on nationally standardized regimens is common but is driven by population-level data on HIVDR prevalence that may not reflect

the epidemiology of sub-epidemics affecting marginalized populations such as men who have sex with men (MSM) and transgender women (TGW). Failing empiric therapies can promote development and transmission of resistant viruses within these populations [3]. As access to ART improves across sub-Saharan Africa, routine surveillance for HIVDR is essential to inform national HIV treatment strategies [4].

In Nigeria, sub-Saharan Africa's most populous country, HIV prevalence among reproductive-age males is about 1% but as high as 44–66% among MSM and TGW [5,6]. Criminalization and stigma associated with same-sex sexual practices impede health-care engagement and ART adherence by MSM and TGW [7,8]. Resource constraints can further complicate ART access for programmatic reasons such as drug stock-outs [9], potentially accelerating the development of HIVDR.

We report the prevalence of transmitted, pre-treatment and acquired HIVDR among MSM and TGW receiving HIV care in Lagos and Abuja, Nigeria.

Methods

The TRUST/RV368 cohort used respondent-driven sampling to recruit MSM and TGW (aged ≥ 16 years in Abuja; ≥ 18 years in Lagos) who reported anal intercourse with a male partner in the preceding year [10]. Participants were provided HIV testing, treatment and prevention services at 3-monthly visits. All participants provided written informed consent. Institutional review board approval was received from all participating institutions.

HIV was diagnosed using a parallel algorithm of rapid tests [11]. HIV RNA was measured via nucleic acid amplification using the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 (Roche Molecular Diagnostics, Pleasanton, CA, USA). Plasma samples with HIV RNA $\geq 1,000$ copies/ml at either enrolment or the first visit after HIV seroconversion underwent retrospective viral RNA extraction, cDNA generation, and near-endpoint dilution prior to PCR amplification, purification and sequencing with an ABI 3730XL capillary sequencer [12]. HIV subtype was determined by evaluating *Pol* sequences and achieving a consensus assignment [12]. *Pol* sequences were evaluated for major mutations conferring resistance to nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs) using the IAS-USA 2017 Update of the Drug Resistance Mutations in HIV [13]. Prior ART exposure was ascertained by medical record review. ART-naive participants were evaluated for World Health Organization (WHO) surveillance drug resistance mutations (SDRMs) [14].

Demographic and other characteristics were compared between ART-naive and ART-experienced participants using Wilcoxon rank-sum and Pearson's χ^2 tests, as appropriate. Prevalence of HIVDR mutations was calculated by dividing the number of participants with ≥ 1 detectable mutation by the total number of participants genotyped. 95% confidence intervals (CIs) were calculated using the Clopper-Pearson interval. Fisher's exact test was used to compare HIVDR prevalence across sites and other groups of interest. Analyses were performed using Stata 15.0 (StataCorp LP, College Station, TX, USA).

Results

From March 2013 to June 2017, 1,539 participants underwent HIV screening, 785 (51.0%) had prevalent infection at enrolment and 58 (3.8%) seroconverted during follow-up. HIV RNA $\geq 1,000$ copies/ml was observed in 553 PLWH, of whom 415 (74.7%) underwent retrospective HIV sequencing, including 376 prevalent and 39 incident cases. Participants with sequencing data had median age 24 (interquartile range [IQR] 21–27) years, CD4⁺ T-cell count 370 (IQR 272–502) cells/mm³ and HIV RNA 4.73 (IQR 4.26–5.15) log₁₀ copies/ml. HIV subtype CRF02_AG and CRF02_AG-containing recombinants were observed in 319 (76.9%) participants (Table 1).

Among the 42 ART-experienced participants, 28 (66.7%) had been treated with a combination of efavirenz, tenofovir disoproxil fumarate, and either lamivudine or emtricitabine. Twelve (28.6%) had been previously exposed to nevirapine (36.0% in Abuja and 17.6% in Lagos; $P=0.20$).

Retrospective genotyping was performed on 39 incident HIV cases at a median of 130 (IQR 84–202) days after their last negative HIV test. SDRMs were observed in 8 (21% [95% CI 9.3, 36.5%]) with no statistically significant differences between participants in Abuja and Lagos (Figure 1A).

The 39 incident cases were combined with 334 other ART-naive participants to assess pre-treatment HIVDR. SDRMs were observed in 36 (9.7% [95% CI 6.8, 13.1%]), including 28 (14.4% [9.8, 20.1%]) in Abuja and 8 (4.5% [2.0, 8.7%]) in Lagos ($P=0.001$). This included resistance to both NNRTIs and NRTIs (Figure 1B). K103N was the most common mutation overall, with higher prevalence in Abuja than Lagos (8.2% versus 1.1%; $P=0.001$). SDRM prevalence did not vary significantly by year of sample collection (14.0% [95% CI 7.4, 23.1%] in 2013 versus 5.7% [0.7, 19.2%] in 2016/2017; $P=0.38$).

Forty-two ART-experienced participants underwent genotyping at a median of 0.99 (IQR 0.36–2.19) years after ART initiation. Major resistance mutations to

Table 1. Characteristics of ART-naïve and ART-experienced Nigerian men who have sex with men and transgender women at the time of genotypic testing for drug resistance mutations

Characteristics	ART-naïve (n=373)	ART-experienced (n=42)	P-value
Age			
<25 years	205 (55.0)	23 (54.8)	0.98
≥25 years	168 (45.0)	19 (45.2)	
Gender identity			
Cisgender man	290 (77.7)	33 (78.6)	0.31
Transgender woman	50 (13.4)	3 (7.1)	
Other/unknown	33 (8.8)	6 (14.3)	
Sexual orientation			
Gay/homosexual	137 (36.7)	17 (40.5)	0.85
Bisexual	235 (63.0)	25 (59.5)	
Other/unknown	1 (0.3)	0 (0)	
Education level			
Junior secondary or less	30 (8.0)	4 (9.5)	0.93
Senior secondary	214 (57.4)	24 (57.1)	
Higher than senior secondary	124 (33.2)	13 (31.0)	
Unknown	5 (1.3)	1 (2.4)	
Occupation			
Unemployed	79 (21.2)	9 (21.4)	0.17
Student	96 (25.7)	6 (14.3)	
Professional/self-employed	77 (20.6)	10 (23.8)	
Entertainment/hospitality	42 (11.3)	2 (4.8)	
Driver/labourer	11 (2.9)	3 (7.1)	
Other/unknown	68 (18.2)	12 (28.6)	
Marital status			
Single/never married	325 (87.1)	36 (85.7)	0.43
Married/living with a woman	25 (6.7)	5 (11.9)	
Living with a man	11 (2.9)	0 (0)	
Divorced/widowed/separated/other	12 (3.2)	1 (2.4)	
Site			
Abuja	195 (52.3)	25 (59.5)	0.37
Lagos	178 (47.7)	17 (40.5)	
CD4 ⁺ T-cell count			
>500 cells/mm ³	72 (19.3)	7 (16.7)	0.65
351–500 cells/mm ³	88 (23.6)	10 (23.8)	
201–350 cells/mm ³	81 (21.7)	10 (23.8)	
≤200 cells/mm ³	37 (9.9)	7 (16.7)	
Unknown/missing	95 (25.5)	8 (19.0)	
Median HIV RNA, log ₁₀ copies/ml (IQR)	4.75 (4.30–5.17)	4.44 (3.64–4.80)	0.002
HIV subtype ^a			
CRF02_AG	222 (59.5)	21 (50.0)	0.23
CRF02_AG-containing recombinants	69 (18.5)	7 (16.7)	
G	42 (11.3)	10 (23.8)	
A1 and A1-containing recombinants	25 (6.7)	2 (4.8)	
Other	15 (4.0)	2 (4.8)	
Year of sample collection			
2013	86 (23.1)	6 (14.3)	0.17
2014	121 (32.4)	17 (40.5)	
2015	131 (35.1)	16 (38.1)	
2016	34 (9.1)	2 (4.8)	
2017	1 (0.3)	1 (2.4)	

All data are presented as n (%) unless otherwise specified. P-values were calculated using Student's *t*-test for HIV RNA as a continuous variable and Pearson's χ^2 test for all other variables. Statistically significant P-values (<0.05) are in bold. ^aHIV subtype categories are mutually-exclusive; recombinant viruses containing both CRF02_AG and A1 are included in the CRF02_AG-containing recombinants category. ART, antiretroviral therapy; EFV, efavirenz; LPV/r, ritonavir-boosted lopinavir; N/A, not applicable; NVP, nevirapine; TDF, tenofovir disoproxil fumarate; XTC, emtricitabine (FTC) or lamivudine; ZDV, zidovudine.

Table 1. Continued

Characteristics	ART-naive (n=373)	ART-experienced (n=42)	P-value
Previous ART exposure			
EFV/TDF/XTC	-	28 (66.7)	N/A
NVP/ZDV/XTC	-	10 (23.8)	
NVP/TDF/XTC	-	2 (4.8)	
LPV/r/TDF/FTC	-	1 (2.4)	
Unknown	-	1 (2.4)	

NNRTIs were detected in 18 (42.9% [95% CI 27.7, 59.0%]) and NRTIs in 10 (23.8% [12.0, 39.4%]). The most common mutations in each class were K103N and M184V, respectively (Figure 1C).

No major PI resistance mutations were detected in any participants.

Discussion

We observed a high prevalence of transmitted HIVDR among MSM and TGW with incident HIV infection in Abuja and a moderate prevalence in Lagos, driven largely by NNRTI resistance mutations such as K103N. This mutation causes high-level resistance to most available NNRTIs and is selected by both efavirenz and nevirapine, longstanding components of first-line ART and strategies to prevent mother-to-child transmission of HIV, respectively. The prevalence of transmitted HIVDR in both cities suggests the need for evaluation of early indicators at clinics, such as on-time pill pick-up and viral suppression rates, as well as assessment of HIV screening programme coverage to identify gaps that promote transmission of resistant viruses.

HIVDR mutations were less common among all ART-naive participants as compared with just the incident HIV cases. This could partially reflect overgrowth of more fit and drug-sensitive viral quasi-species [15,16]. However, much of the difference in HIVDR prevalence was driven by a lower prevalence of K103N mutations in ART-naive participants, which minimally impacts HIV replication and may be slower to revert than other HIVDR mutations, such as M184V [17]. Some data suggest that M184V reversion and wild-type overgrowth may occur more quickly when M184V is transmitted as a single substitution [16], but more slowly when linked to other mutations, including K103N [18,19]. The mechanisms underlying HIVDR reversion without selective pressure from ART in HIV subtypes CRF02_AG and G are poorly defined.

Pre-treatment HIVDR was more common in Abuja than Lagos. Developed in the 1980s for the purpose of becoming the nation's capital, Abuja is a relatively new city that has ethnically diverse and transient

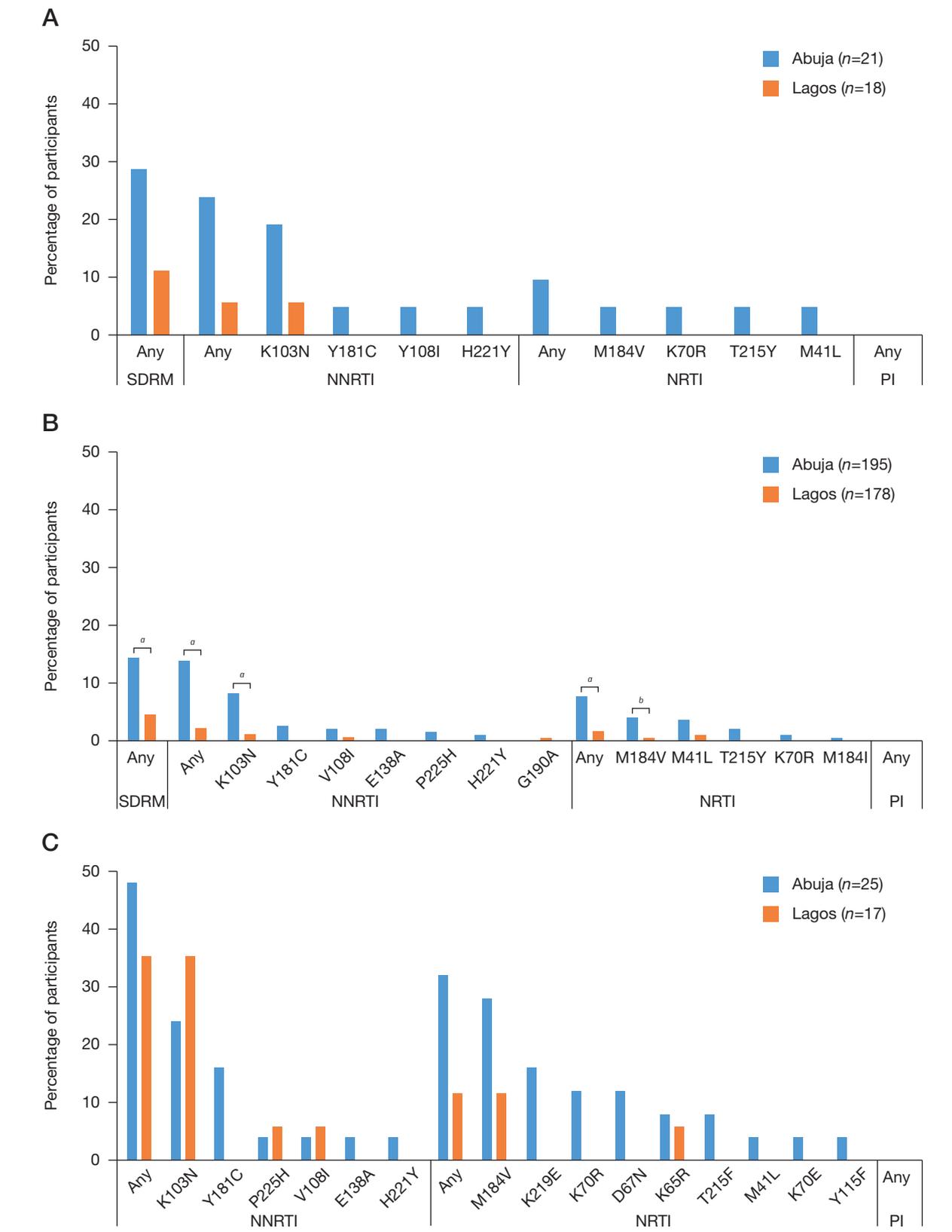
populations. It was also one of the first regions in Nigeria to undergo a massive scale-up of infrastructure to support ART delivery in 2004.

Understanding the magnitude of transmitted and pre-treatment HIVDR in MSM and TGW populations is critical for sub-Saharan Africa, where resources to prevent the development and transmission of HIVDR may inconsistently reach these marginalized populations. In similar resource-limited settings, data suggest that transmitted resistance is more common among MSM than other HIV risk groups [20]. While a recent meta-analysis described NNRTI resistance in under 3% of ART-naive patients in Western and Central Africa [21], we found a prevalence of about 10% in ART-naive participants and twice that in newly-seroconverted cases within our highly-marginalized population of MSM and TGW. WHO guidelines recommend changing empiric first-line NNRTI-based ART to an integrase inhibitor-based regimen when the prevalence of resistance exceeds 10% [22]. It is fortuitous that dolutegravir-based first-line ART is becoming the standard of care in Nigeria, one of the first countries to implement the planned continent-wide transition promoted by the U.S. President's Emergency Plan for AIDS Relief.

The high prevalence of HIVDR among ART-experienced Nigerian MSM and TGW highlights that adherence is not the only factor driving viral failure among PLWH. Guidelines that emphasize adherence counselling and delay confirmation of viral failure for months after initial detection of viraemia may delay ART regimen switches, thereby promoting the development and transmission of drug resistant viruses [23]. The resistance pattern in our study was consistent with failure of first-line therapy with NNRTI and NRTI components, including the K65R mutation that confers high-level resistance to tenofovir. Over 70% of ART-experienced participants were prescribed tenofovir-containing regimens, some of which had compromised efficacy. No major PI resistance mutations were detected, supporting continued empiric use of PI-based regimens as second-line therapy.

Our study used respondent-driven sampling to recruit a highly-marginalized population for longitudinal HIV

Figure 1. Prevalence of drug resistance mutations by site among incident cases, ART-naive and ART-experienced participants



(A) Incident cases. (B) ART-naive participants. (C) ART-experienced participants. The prevalence of World Health Organization surveillance drug resistance mutations (SDRMs) and IAS-USA drug resistance mutations to non-nucleoside reverse transcriptase inhibitors (NNRTIs), nucleoside reverse transcriptase inhibitors (NRTIs) and protease inhibitors (PIs) were tallied separately by site and compared using Fisher's exact test. Statistically significant differences between sites are indicated: ^aP<0.01 and ^bP<0.05.

screening that enabled evaluation for transmitted HIVDR within months of HIV acquisition in incident cases. However, for technical and logistical reasons, not all eligible samples underwent HIVDR testing. Under-reporting of ART use may have led to some misclassification of ART status, as is common in resource-limited settings, although extensive review of medical records likely minimized this. Our study did not include assessment of integrase inhibitor resistance, but this will be important for future research as these agents become more common in sub-Saharan Africa.

Our findings support the programmatic shift to integrase inhibitor-based first-line ART and the continued use of PI-based second-line ART. They also underscore the urgency of ensuring uninterrupted access to ART and timely switch to second-line regimens upon viral failure among marginalized communities of PLWH to prevent the development and transmission of resistance to emerging empiric therapies.

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Further acknowledgements and TRUST/RV368 Study Group membership can be found in Additional file 1.

Disclosure statement

GHK is currently employed by GSK Vaccines but his participation in this study preceded the current employment. The authors declare no relevant conflicts of interest.

Additional file

Additional file 1: Further acknowledgements and TRUST/RV368 Study Group membership can be found at https://www.intmedpress.com/uploads/documents/4576_Crowell_Addfile1.pdf

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