Open Forum Infectious Diseases

MAJOR ARTICLE

Pretreatment Human Immunodeficiency Virus (HIV) Drug Resistance Among Treatment-Naive Infants Newly Diagnosed With HIV in 2016 in Namibia: Results of a Nationally Representative Study

Michael R. Jordan,[1,2] Leonard Bikinesi,[3] Laimi Ashipala,[3] Nicholus Mutenda,[3] Mary Brantuo,[4] Gillian Hunt,[5] Andreas Shiningavamwe,[6] Gram Mutandi,[7] Anita Beukes,[7] Suzanne Beard,[7,8] Katherine Battey,[7,8] Eric J. Dziuban,[7] Elliot Raizes,[8] Paul Adjei,[1] Alice Tang,[9] Amalia Giron,[10] and Steven Y. Hong[7]

[1]Division of Geographic Medicine and Infectious Diseases, Tufts Medical Center, Boston, Massachusetts, USA, [2]Levy Center for Integrated Management of Antimicrobial Resistance, Tufts University School of Medicine, Boston, Massachusetts, USA, [3]Directorate of Special Programmes, Republic of Namibia Ministry of Health and Social Services, Windhoek, Namibia, [4]World Health Organization, Windhoek, Namibia, [5]Centre for HIV and STIs, National Institute for Communicable Diseases, National Health Laboratory Services, Johannesburg, South Africa,[6]Namibia Institute of Pathology, Windhoek, Namibia, [7]Division of Global HIV and TB, Center for Global Health, Centers for Disease Control and Prevention, Windhoek, Namibia, [8]Division of Global HIV and TB, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, Georgia, USA, [9]Department of Public Health and Community Medicine, Tufts University School of Medicine, Boston, Massachusetts, USA, and [10]Department of Global HIV, Hepatitis and STI Programmes, World Health Organization, Geneva, Switzerland

Background. The World Health Organization (WHO) recommends routine surveillance of pretreatment human immunodeficiency virus (HIV) drug resistance (HIVDR) in children <18 months of age diagnosed with HIV through early infant diagnosis (EID). In 2016, 262 children <18 months of age were diagnosed with HIV in Namibia through EID. Levels of HIVDR in this population are unknown.

Methods. In 2016, Namibia surveyed pretreatment HIVDR among children aged <18 months following WHO guidance. Reverse transcriptase, protease, and integrase regions of HIV-1 were genotyped from remnant dried blood spot specimens from all infants diagnosed with HIV in Namibia in 2016. HIVDR was predicted using the Stanford HIVdb algorithm.

Results. Of 262 specimens genotyped, 198 HIV-1 protease and reverse transcriptase sequences and 118 HIV-1 integrase sequences were successfully amplified and analyzed. The prevalence of efavirenz/nevirapine (EFV/NVP), abacavir (ABC), zidovudine, lamivudine/emtricitabine (3TC/FTC), and tenofovir (TDF) resistance was 62.6%, 17.7%, 5.6%, 15.7%, and 10.1%, respectively. No integrase inhibitor resistance was detected.

Conclusions. The high level of EFV/NVP resistance is unsurprising; however, levels of ABC and TDF resistance are among the highest observed to date in infants in sub-Saharan Africa. The absence of resistance to dolutegravir (DTG) is reassuring but underscores the need to further study the impact of ABC and 3TC/FTC resistance on pediatric protease inhibitor– and DTG-based regimens and accelerate access to other antiretroviral drugs. Results underscore the need for antiretroviral therapy optimization and prompt management of high viral loads in infants and pregnant and breastfeeding women.

Keywords. drug resistance; HIV; infants; Namibia.

Global coverage of prevention of mother-to-child transmission (PMTCT) services for human immunodeficiency virus (HIV) has increased dramatically, from 50% in 2010 to 80% by 2017 [1]. As of 2019, the number of new HIV infections among children aged 0–14 years had declined by 53% since 2010 [2]. Despite global PMTCT interventions, an estimated 150 000 (100 000–240 000) children were newly infected with HIV in 2020 [2].

PMTCT involves use of antiretroviral therapy (ART) for pregnant women living with HIV and prophylaxis of infants born to mothers living with HIV. While critical to reducing the number of new HIV infections in infants, expansion of nonnucleoside reverse transcriptase inhibitor (NNRTI)–based PMTCT has resulted in an increase in HIV drug resistance (HIVDR) among infants and children who acquire HIV infection [3]. Understanding the prevalence of HIVDR prior to ART initiation is important in children because, on average, they may have higher viral loads and more rapid disease progression compared to adults. The limited number of antiretroviral (ARV) drugs available for children necessitates use of the most potent and effective drugs possible. Moreover, HIVDR at the time of ART initiation, called pretreatment HIVDR

Received 28 November 2021; editorial decision 22 February 2022; accepted 14 March 2022; published online 24 March 2022.

Correspondence: Michael R. Jordan, MD, MPH, Division of Geographic Medicine and Infectious Disease, Tufts Medical Center and Levy Center for Integrated Management of Antimicrobial Resistance, Tufts University School of Medicine, 800 Washington St, Box 041, Boston, MA 02111, USA (michael.jordan@tufts.edu).

Open Forum Infectious Diseases®2022

https://doi.org/10.1093/ofid/ofac102

(PDR), in children is a strong predictor of treatment failure and death [4–7].

Current World Health Organization (WHO)–recommended first-line regimens for children are age- and weight-based: for neonates <1 month of age, raltegravir (RAL) + zidovudine (AZT) + lamivudine (3TC) is recommended and for children ≥4 weeks of age and weighing between 3 and 30 kg, dolutegravir (DTG) + abacavir (ABC) + lamivudine (3TC) is recommended [8]. To support country-level, evidence-based regimen selection and to accelerate the programmatic transition from NNRTI- to non-NNRTI-based first-line ART (eg, DTG or protease inhibitor [PI]–based treatment, depending on the weight of the children), WHO recommends routine (every 3 years) surveys of PDR in ART-naive infants newly diagnosed with HIV through early infant diagnosis (EID) programs [9, 10]. Results of nucleoside or nucleotide reverse transcriptase inhibitor (NRTI) resistance prevalence also inform future optimal treatment strategies.

At the time of specimen collection in Namibia in 2016, the preferred first-line ART regimen for adults including pregnant women was tenofovir (TDF) in combination with either emtricitabine (FTC) or 3TC and efavirenz (EFV) administered once daily as a fixed-dose combination. The preferred first-line regimen for infants >2 weeks of age to 2 months of age was zidovudine (AZT) in combination with 3TC and ritonavir-boosted lopinavir, with ABC being substituted for AZT from ages 3 to 35 months. Finally, at the time of specimen collection, infants received nevirapine (NVP) plus AZT for 6 weeks (high-risk mother, eg, new HIV diagnosis, women with virological failure within 3 months prior to delivery, or women on ART for <1 month) or NVP alone for 6 weeks as prophylaxis against HIV [11].

Studies of HIVDR in infants born to mothers with HIV in some sub-Saharan African countries have been published [12, 13]. This report presents the findings of the first national PDR survey conducted in ART-naive infants diagnosed with HIV in Namibia in 2016 through the national EID program. The study had the following 2 objectives: (1) to calculate the nationally representative prevalence of any HIVDR among all ART-naive children <18 months of age newly diagnosed with HIV, regardless of PMTCT exposure; and (2) to calculate the nationally representative prevalence of HIVDR to NNRTIs (NVP or EFV) among all ART-naive children <18 months of age newly diagnosed with HIV, regardless of PMTCT exposure.

MATERIALS AND METHODS

Study Design

In 2016, Namibia stored remnant diagnostic specimens from all ART-naive infants diagnosed with HIV through the country's EID program for HIV for the purpose of conducting this national survey. In total, 18 000 specimens were tested for HIV in Namibia's EID program in 2016. Of all specimens tested, 262 were confirmed to be HIV positive and were identified by the Namibia Institute of Pathology (NIP) as being from unique individual children <18 months of age. Uniqueness was assessed by NIP using name and date of birth. Remnant specimens from all 262 ART-naive children <18 months of age diagnosed with HIV in 2016 were tested for HIVDR.

Laboratory Procedures

Early Infant Diagnosis

Namibia used an EID algorithm to diagnose HIV in ART-naive children <18 months of age. The national algorithm was based on the use of a nucleic acid amplification test (NAAT) that identifies both HIV DNA and RNA in dried blood spots (DBSs). A positive initial NAAT followed by a repeat positive NAAT on the same specimen confirmed true HIV infection in a child. NAT was performed by only 1 laboratory in Namibia, NIP, using Cobas AmpliPrep/Cobas TaqMan HIV-1 qualitative test V2 (Roche Diagnostics), according to the manufacturer's instructions.

Specimen Collection, Handling, and Processing for HIVDR Testing

The study leveraged remnant DBS specimens used for NAAT confirmation of HIV infection in children tested through Namibia's EID program. DBS cards were stored at −70°C at NIP from time of confirmatory testing. DBS specimens were collected and transported per WHO guidelines for the collection of DBS specimens being collected, processed, stored, and handled for the purpose of HIVDR testing [14]. DBS specimens were shipped on dry ice from NIP to the National Health Laboratory Service, South Africa, for HIVDR testing.

HIVDR Testing

Remnant specimens were sent to the WHO-designated HIVDR genotyping laboratory at the National Institute of Communicable Diseases, Johannesburg, South Africa. HIVDR genotyping of the HIV-1 reverse transcriptase and protease regions were performed using established Sanger sequencing methods [15]. The integrase region of HIV-1 was sequenced using an internally validated in-house Sanger sequencing method.

Data Analysis

The WHO/British Columbia Centre For Excellence in HIV/AIDS HIVDR quality control tool was used for posttesting quality assurance [16]. All sequences excluded for reasons of quality were found to be duplicates (ie, likely 2 specimens from the same infant) based on distance measurements (<0.5 genetic distance).

The prevalence of HIVDR was predicted using the Stanford HIVdb algorithm (version 8.9-1) with sequences categorized as susceptible or as having potential low-level HIVDR classified as susceptible and sequences with predicted low-, intermediate-, or high-level resistance classified as drug resistant

Table 1. Prevalence of Pretreatment Human Immunodeficiency Virus (HIV) Drug Resistance by Drug Class Among Antiretroviral Therapy–Naive Children <18 Months of Age Newly Diagnosed With HIV in Namibia, 2016

Drug or Drug Class	% (95% CI)
XXX	
EFV or NVP	62.6 (55.6–69.1)
Any NNRTI	65.7 (58.7–72.0)
Any NRTI	20.7 (15.6–27.0)
NRTI or EFV/NVP	64.6 (57.7–71.0)
NRTI and EFV/NVP	18.7 (13.8–24.8)
ATV/r, DRV/r, or LPV/r	1.0 (.2–4.0)
Any ritonavir-boosted PI	1.5 (.5–4.6)
Any NRTI, EFV, NVP, ATV/r, DRV/r, or LPV/r	64.6 (57.7–71.0)
Any INI	0.0 (.0–3.2)

Human immunodeficiency virus drug resistance was assessed using the Stanford HIVdb algorithm (version 8.9-1), with virus predicted to have low-, intermediate-, or high-level resistance categorized as resistant. One hundred ninety-eight reverse transcriptase and protease sequences and 118 integrase sequences were available.

Abbreviations: ATV/r, ritonavir-boosted atazanavir; CI, confidence interval; DRV/r, ritonavir-boosted darunavir; EFV, efavirenz; INI, integrase inhibitor; LPV/r, ritonavir-boosted lopinavir; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NVP, nevirapine; PI, protease inhibitor.

[a]Study design–weighted proportion and 95% CI.

[17, 18]. HIV subtype was assigned using the Stanford HIVdb subtyping tool [19].

No de-identified demographic data, including ARV drug exposure histories, were available for analysis; thus, only national estimates of PDR among treatment-naive infants, regardless of PMTCT exposure, were estimated. Weighted statistical analysis was performed using Stata version 15.1 software (StataCorp LLC, College Station, Texas) following the WHO recommendations to adjust for genotyping failure. Study design–weighted proportion and 95% confidence interval (CI) were calculated.

RESULTS

The genotyping success rate was 92.7% (243/262) for the HIV-1 protease and reverse transcriptase regions and 53.8% (141/262) for the HIV-1 integrase region. After quality assurance, 198 protease and reverse transcriptase sequences and 118 integrase sequences were available for analysis. The prevalence of resistance to EFV/NVP was 62.6% (95% CI, 55.6%–69.1%) and the prevalence of any NNRTI resistance was 65.7% (95% CI, 58.7%–72.0%) (Table 1). Any NRTI resistance was 20.7% (95% CI, 15.6%–27.0%), and the prevalence of resistance to any NRTI in combination with EFV/NVP was 18.7% (95% CI, 13.8%–24.8%). Any ritonavir-boosted PI resistance was present at a prevalence of 1.5% (95% CI, .5%–4.6%) and the prevalence of any resistance to atazanavir, darunavir, or lopinavir was 1.0% (95% CI, .2%–4.0%). No integrase inhibitor resistance was detected. The prevalence of drug resistance by drug class is presented in Figure 1. Of the 17.7% (95% CI, 12.9%–23.7%; 35/198) with predicted ABC resistance, 34.2% (12/35) had only the M184IV mutation, predicting low-level ABC resistance, whereas 68.5% (24/35) had either M184IV in combination with K65R or a thymidine analogue mutation, or K65R with other resistance-associated mutations. The prevalence of predicted TDF resistance was 10.1% (95% CI, 6.6%–15.2%) and the TDF-associated mutation, K65NRE, was detected at a frequency of 6.6%, with the remainder for predicted TDF resistance caused by the presence of thymidine analogue mutations present in combination (eg, M41L and L210W).

The prevalence of drug resistance mutations present at ≥0.5% of all sequences analyzed is shown in Table 2. Of note, the integrase inhibitor mutations Q95K, T97A, and E157Q were detected but are insufficient to cause resistance. The most frequently observed HIV-1 subtype was subtype C (90.1%). Other HIV-1 subtypes were CRF02_AG (6.9%), subtype A (2%), subtype B (0.5%), and subtype G (0.5%).

DISCUSSION

In this study of treatment-naive infants in Namibia, detected levels of HIVDR were largely driven by NNRTI resistance, which was present in 65.7% of cases. As expected, the most frequently detected NNRTI resistance-associated mutations were at positions 103, 106, and 181. In contrast, the prevalence of NRTI resistance (20.7%) was lower and was driven by resistance to ABC, 3TC/FTC, and stavudine, with only 5.6% of cases exhibiting resistance to AZT. The prevalence of predicted tenofovir resistance (10.1%) was higher than that reported by any country providing data to WHO's 2021 global report on HIVDR [22]. In the 2021 global report, TDF resistance peaked at just over 7.7% in 1 of 10 countries reporting data [22]. Study findings highlight the increasing levels of PDR to the NRTI drug class in this population. The prevalence of ABC resistance (17.7%) is high and only 1 of 9 countries (Nigeria) reporting data to WHO in 2021 reached similar levels. However, in Namibia, 6.0% (12/199) with predicted ABC resistance had only the M184IV mutation and therefore are likely to derive clinical benefit from ABC.

Although the absence of infant ARV drug exposure and breastfeeding status is an acknowledged limitation and exposure to PMTCT may have contributed to some level of observed thymidine analogue and NNRTI resistance, observed levels of TDF and ABC resistance are high and concerning—a finding that suggests prolonged maternal virological failure in the setting of ongoing drug-selective pressure.

Current WHO-recommended first-line regimens for infants are age- and weight-based [23], and results from this study suggest the need for caution when using ABC and 3TC in combination with drugs that have a low genetic barrier for resistance (eg, NVP or RAL). The high levels of NNRTI resistance in infants are not surprising and support WHO's recommendation to accelerate access to child-friendly, non-NNRTI-based formulations to prevent poor treatment outcomes. The absence of any predicted resistance to the integrase inhibitor DTG is

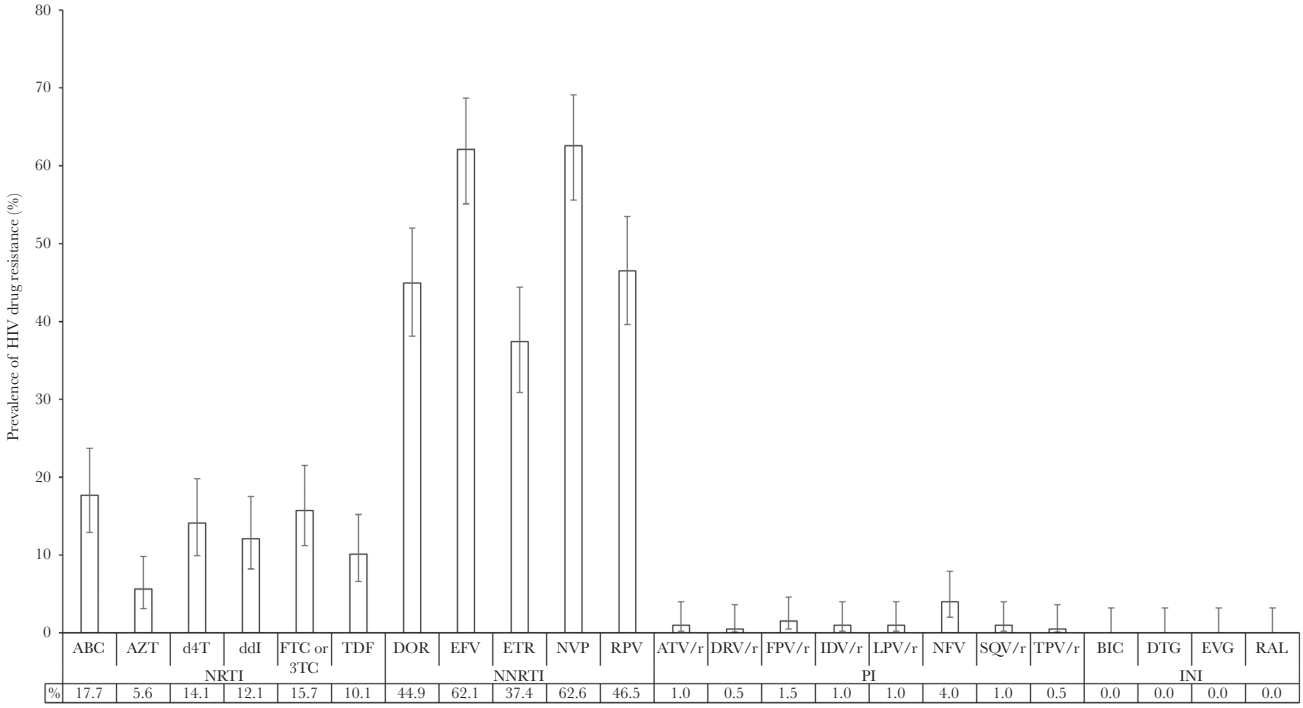

Figure 1. Prevalence of pretreatment human immunodeficiency virus (HIV) drug resistance by drug among antiretroviral therapy–naive children <18 months of age newly diagnosed with HIV in Namibia, 2016. Study design–weighted prevalence and 95% confidence interval of pretreatment HIV drug resistance among infants aged <18 months, newly diagnosed with HIV and treatment-naive, are shown. HIV drug resistance was assessed using the Stanford HIVdb algorithm (version 8.9-1), with virus predicted to have low-, intermediate-, or high-level resistance categorized as resistant. One hundred ninety-eight reverse transcriptase and protease sequences and 118 integrase sequences were available. Abbreviations: 3TC, lamivudine; ABC, abacavir; ATV/r, ritonavir-boosted atazanavir; AZT, zidovudine; BIC, bictegravir; d4T, stavudine; ddI, didanosine; DOR, doravirine; DRV/r, ritonavir-boosted darunavir; DTG, dolutegravir; EFV, efavirenz; ETR, etravirine; EVG, elvitegravir; FPV/r, ritonavir-boosted fosamprenavir; FTC, emtricitabine; HIV, human immunodeficiency virus; IDV/r, ritonavir-boosted indinavir; INI, integrase inhibitor; LPV/r, ritonavir-boosted lopinavir; NFV, nelfinavir; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NVP, nevirapine; PI, protease inhibitor; RAL, raltegravir; RVP, rilpivirine; SQV/r, ritonavir-boosted saquinavir; TDF, tenofovir; TPV/r, ritonavir-boosted tipranavir.

expected and underscores the need to understand the impact of ABC + lamivudine or emtricitabine resistance on DTG and PI regimens for children and the need to accelerate access to other NRTIs such as tenofovir alafenamide and drugs from new classes such as islatravir or lenacapavir.

Adoption of algorithms with more frequent viral load monitoring of HIV-infected infants, children, and pregnant and breastfeeding mothers compared to the general adult population as recommended by WHO is critical for early identification of virologic failure and prevention of HIVDR. Where these algorithms have already been adopted as part of national guidelines, ensuring widespread programmatic access to viral load testing should be prioritized by national programs.

CONCLUSIONS

The high levels of NNRTI drug resistance observed in this nationally representative cohort are unsurprising; however, levels of ABC and TDF resistance are among the highest observed in infants in sub-Saharan Africa. The absence of resistance to DTG is reassuring but underscores the need to further study the impact of ABC and 3TC/FTC resistance on pediatric PI- and DTG-based regimens and accelerate access to other ARV drugs. Results underscore the need for ART optimization and prompt management of high viral loads in infants and pregnant and breastfeeding women to further minimize HIV transmission including transmission of drug-resistant virus [24, 25]. Finally, results suggest that HIVDR genotyping may be considered for all children born to HIV-infected women and, in particular, those children born to mothers whose treatment has failed.

Notes

Author contributions. M. R. J. developed the original World Health Organization (WHO) concept note on which this study is based, conceived and wrote the protocol, performed data analysis and interpretation, and wrote the first draft of the manuscript. L. B. contributed to protocol development, data interpretation, and manuscript writing. L. A. supported protocol development, implementation, and manuscript writing. N. M. supported protocol development, study implementation, data interpretation, and manuscript writing. M. B. supported the finalization of the protocol, stakeholder engagement, and writing of the manuscript. G. H. performed all human immunodeficiency virus (HIV) drug resistance testing, performed sequence quality assurance, and contributed to the manuscript writing. A.

Table 2. Human Immunodeficiency Virus Drug Resistance Mutations Present at ≥0.5% of All Sequences Analyzed[a]

NRTI		NNRTI		PI		INI	
Mutation	%	Mutation	%	Mutation	%	Mutation	%
M41L	1.0	K101EHP	6.1	M46ILV	3.0	*Q95K*	*0.8*
K65ENR	6.6	K103HNST	36.9	I54VLMATS	0.5	*T97A*	*1.7*
D67EGHNSTDel	3.5	V106MA	11.1	L76V	0.5	*E157Q*	*0.8*
T69DGDelIns	1.0	V179FL	0.5	V82ATFSCML	1.0		
K70EGNQRST	3.5	Y181CFGISV	31.8	I85V	0.5		
L74VI	0.5	Y188CFHL	3.0	*L10F*	*0.5*		
V75AIMST	1.0	G190ACEQSTV	2.0	*V11IL*	*1.5*		
Y115F	0.5	P225H	4.0	*Q58E*	*0.5*		
M184VI	13.1	*A98G*	*3.5*				
L210W	1.0	*K103R*	*2.0*				
T215ACDEFILNSVY	4.0	*V108I*	*6.1*				
K219QENRW	4.5	*E138A*	*6.6*				
E44AD	*1.0*	*E138GKQR*	*1.5*				
A62V	*3.0*	*V179DE*	*3.0*				
		H221Y	*6.6*				
		F227CILV	*2.5*				

Abbreviations: INI, integrase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

[a]Unweighted proportions of sequences with surveillance drug resistance mutations (SDRMs) as defined in Bennett et al [20] and Tzou et al [21], plus other rare variants at the same positions that are not polymorphic, and other mutations (non-SDRM) that have non-zero penalty scores in the Stanford HIVdb algorithm (italicized mutations). One hundred ninety-eight reverse transcriptase and protease sequences and 118 integrase sequences were available.

S. performed diagnostic testing and supported protocol development and data interpretation. G. M. contributed to protocol development, the manuscript, and stakeholder engagement. A. B. performed coordination of sample collection, storage, and logistics including sample review, retrieval, liaison with referral laboratory, shipping of samples, and laboratory data review. S. B. performed coordination of sample logistics including sample review and retrieval and laboratory data review. K. B. contributed to the manuscript review. E. J. D. contributed to analysis, performed manuscript review, and carried out stakeholder engagement. E. R. was involved in the finalization of the protocol and in the drafting of the final version of the manuscript. P. A. wrote the original draft of the country-specific protocol. A. T. supported statistical methods. A. G. supported data cleaning and analysis and development of public health recommendations. S. Y. H. conceived the idea and planned study, carried out implementation and supervision, and supported finalization of the protocol and development of public health recommendations.

Patient consent. This study used remnant stored specimens and deidentified information collected for routine diagnostic purposes and has as its purpose to inform national antiretroviral therapy (ART) guidelines in Namibia and to provide Namibia with HIV drug resistance prevalence estimates in this population. At the time of specimen collection for HIV diagnosis, no written consent was obtained from the children's caregivers. Because this study has as its main purpose to inform optimal ART regimen selection in Namibia, a request of nonresearch determination was granted from the Research and Ethics Committee of the Namibia Ministry of Health and Social Services, the US Centers for Disease Control and Prevention (CDC), and the Health Science Independent Review Board of Tufts Medical Center.

Disclaimer. The conclusions and opinions expressed in this article are those of the authors and do not reflect those of the WHO or the US CDC.

Financial support. This work was supported by the WHO via CDC's cooperative agreement award GH002154 funded under the opportunity announcement of RFA-CDC-GH18-1850 under the US President's Emergency Plan for AIDS Relief, 2018.

Potential conflicts of interest. All authors: No reported conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

REFERENCES

1. Joint United Nations Programme on HIV/AIDS. 2018 factsheets. **2018**. http://www.unaids.org/sites/default/files/media_asset/UNAIDS_FactSheet_en.pdf. Accessed 29 January 2022.
2. Joint United Nations Programme on HIV/AIDS. 2021 factsheets. **2021**. https://www.unaids.org/en/resources/fact-sheet. Accessed 29 January 2022.
3. Kuhn L, Hunt G, Technau KG, et al. Drug resistance among newly diagnosed HIV-infected children in the era of more efficacious antiretroviral prophylaxis. AIDS **2014**; 28:1673–8.
4. Newell ML, Coovadia H, Cortina-Borja M, Rollins N, Gaillard P, Dabis F. Mortality of infected and uninfected infants born to HIV-infected mothers in Africa: a pooled analysis. Lancet **2004**; 364:1236–43.
5. Becquet R, Marston M, Dabis F, et al. Children who acquire HIV infection perinatally are at higher risk of early death than those acquiring infection through breastmilk: a meta-analysis. PLoS One **2012**; 7:e28510.
6. Richardson BA, Mbori-Ngacha D, Lavreys L, et al. Comparison of human immunodeficiency virus type 1 viral loads in Kenyan women, men, and infants during primary and early infection. J Virol **2003**; 77:7120–3.
7. Boerma RS, Boender TS, Sigaloff KC, et al. High levels of pre-treatment HIV drug resistance and treatment failure in Nigerian children. J Int AIDS Soc **2016**; 19:21140.
8. World Health Organization. Updated Recommendations on HIV Prevention, Infant Diagnosis, Antiretroviral Initiation and Monitoring. Geneva, Switzerland: WHO; **2021**.
9. World Health Organization. Surveillance of HIV Drug Resistance in Children Newly Diagnosed With HIV by Early Infant Diagnosis. Geneva, Switzerland: WHO; **2017**.
10. World Health Organization. Updated Guidance on First-Line and Second-Line Antiretroviral Regimens. Geneva, Switzerland: WHO; **2019**:14.
11. Namibia Ministry of Health and Social Services. National Guidelines for Antiretroviral Therapy. Windhoek, Namibia: Namibia Ministry of Health and Social Services; **2016**.
12. Inzaule SC, Osi SJ, Akinbiyi G, et al. High prevalence of HIV drug resistance among newly diagnosed infants aged <18 months: results from a nationwide surveillance in Nigeria. J Acquir Immune Defic Syndr **2018**; 77:e1–7.
13. Boerma RS, Sigaloff KC, Akanmu AS, et al. Alarming increase in pretreatment HIV drug resistance in children living in sub-Saharan Africa: a systematic review and meta-analysis. J Antimicrob Chemother **2017**; 72:365–71.
14. World Health Organization. WHO Manual for HIV Drug Resistance Testing Using Dried Blood Spot Specimens. Geneva, Switzerland: WHO; **2012**:26.
15. Zhou Z, Wagar N, DeVos JR, et al. Optimization of a low cost and broadly sensitive genotyping assay for HIV-1 drug resistance surveillance and monitoring in resource-limited settings. PLoS One **2011**; 6:e28184.

16. Woods CK, Harrigan PR, eds. WHO HIVDR QC tool. **2021**.https://pssm.cfenet.ubc.ca/who_qc. Accessed 5 April 2021.
17. Rhee SY, Gonzales MJ, Kantor R, Betts BJ, Ravela J, Shafer RW. Human immunodeficiency virus reverse transcriptase and protease sequence database. Nucleic Acids Res **2003**; 31:298–303.
18. Liu TF, Shafer RW. Web resources for HIV type 1 genotypic-resistance test interpretation. Clin Infect Dis **2006**; 42:1608–18.
19. Stanford University HIV Drug Resistance Database. HIVdb subtyping program. **2019**. https://hivdb.stanford.edu/page/hiv-subtyper/. Accessed 30 May 2020.
20. Bennett DE, Camacho RJ, Otelea D, et al. Drug resistance mutations for surveillance of transmitted HIV-1 drug-resistance: 2009 update. PLoS One **2009**; 4:e4724.
21. Tzou PL, Rhee SY, Descamps D, et al. Integrase strand transfer inhibitor (INSTI)–resistance mutations for the surveillance of transmitted HIV-1 drug resistance. J Antimicrob Chemother **2020**; 75:170–82.
22. World Health Organization. HIV Drug Resistance Report 2019. Geneva, Switzerland: WHO; **2019**:68.
23. World Health Organization. Consolidated Guidelines on HIV Prevention, Testing, Treatment, Service Delivery and Monitoring: Recommendations for a Public Health Approach. Geneva, Switzerland: WHO; **2021**.
24. World Health Organization. Considerations for Developing a Monitoring and Evaluation Framework for Viral Load Testing. Geneva, Switzerland: WHO; **2019**.
25. World Health Organization. HIV Molecular Diagnostics Toolkit to Improve Access to Viral Load Testing and Infant Diagnosis. Geneva, Switzerland: WHO; **2019**:48.