J Antimicrob Chemother 2018; **73**: 3476 3481
doi:10.1093/jac/dky332 Advance Access publication 27 August 2018

Journal of Antimicrobial Chemotherapy

Prevalence of pretreatment HIV drug resistance in Mwanza, Tanzania

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Received 4 May 2018; returned 4 June 2018; revised 10 July 2018; accepted 23 July 2018

Background: In a 2008–10 study, we found a pretreatment HIV drug resistance (PDR) prevalence of 18.2% in patients at Bugando Medical Centre (BMC) in Mwanza, Tanzania.

Objectives: To determine the prevalence of PDR and transmitted HIV drug resistance (TDR) in patients visiting the BMC from 2013 to 2015.

Methods: Adult outpatients were sequentially enrolled into two groups, separated by whether they were initiat ing ART. Previous exposure to antiretroviral drugs, except for prevention of mother to child transmission, was an exclusion criterion. HIV *pol* sequences were analysed according to WHO guidelines for surveillance of PDR and TDR.

Results: Two hundred and thirty five sequences were analysed (138 ART initiators, 97 non initiators). The prevalence of PDR was 4.7% (95% CI 2.6%–8.2%) overall, 3.1% (95% CI 1.1%–8.7%) for non initiators and 5.8% (95% CI 3.0%–11.0%) for ART initiators. PDR to NNRTIs and nucleoside or nucelotide reverse transcriptase inhibitors was found in 3.0% (95% CI 1.5%–6.0%) and 1.7% (95% CI 0.7%–4.3%) of patients, respectively. Resistance to PIs was not observed. The prevalence of TDR was 6.0% (95% CI 3.6%–9.8%).

Conclusions: Prevalence of PDR significantly decreased compared with 2008–10 and was below the WHO defined threshold for triggering a public health response. National and systematic surveillance is needed to inform Tanzania's public health strategy.

Introduction

The roll out of ART is one of the major success stories of global health. In Tanzania, ~690 000 patients were receiving antiretro viral drugs (ARVs) by the end of 2015,[1] compared with 19600 in 2005.[2] This trend will continue as Tanzania works towards the goal of 90% of tested HIV positive people being on ART, within the 90–90–90 strategy of the Joint United Nations Programme on HIV/AIDS. However, increased access to ART is likely to be associated with an increase in HIV drug resistance (HIVDR).[3,4] Therefore, the WHO recommends that scaling up of ART should be accompanied by surveillance of both pretreatment HIVDR (PDR) and of HIVDR acquired under ART.[4]

PDR testing is currently not recommended by the Tanzanian guidelines[5] and data about PDR in Tanzania are very sparse. Most studies report rates from 2004 to 2007, shortly after the roll out of ART in 2004, when drastically fewer people were on ART and different ARVs were used. Only three studies report more recent data. Masimba *et al.*[6] and Vairo *et al.*[7] found prevalences of PDR of 11.9% and 3.3% in 119 and 67 treatment naive patients from 2009 and 2010–11, respectively. We found a prevalence of PDR of 18.2% in treatment naive patients at Bugando Medical Centre (BMC) in 2008–10.[8] The WHO recommends that PDR surveys should be repeated every 3 years,[4] and re surveying seems espe cially appropriate if earlier studies reported high prevalence rates.

PDR can be caused by transmission of resistant HIV strains [i.e. transmitted HIVDR (TDR)] or generated intra patient by expos ure to ARVs, such as in prevention of mother to child transmission (PMTCT), pre or post exposure prophylaxis, self medication or pre vious prescribed ART.

The aim of this study was to determine the prevalence, pattern and trend of PDR and TDR in patients attending the HIV Care and Treatment Centre (CTC) at BMC in Mwanza, Tanzania, from 2013 to

2015. The analysis was conducted in accordance with WHO recommendations for the surveillance of PDR[9] and TDR.[10]

Patients and methods

Study population

This cross-sectional study was conducted at the CTC at the tertiary consultant and academic teaching hospital BMC in Mwanza, Tanzania. Participants were confirmed HIV-positive patients older than 18 years and ARV naive, except for temporary ARV use in the context of PMTCT, which was not an exclusion criterion.

Four hundred and six patients were sequentially enrolled into two groups: 196 patients initiating ART (ART-initiator group) and 210 patients attending the CTC for control examinations, not initiating ART (non-initiator group). Patients qualified for the ART-initiator group if they started ART within 90 days after blood sampling, judged by information in the medical records. We obtained sequences from 97 patients of the non-initiator group and 138 of the ART-initiator group.

Data collection and blood sampling

Demographic and clinical data were collected from questionnaires, medical records and the electronic patient database of the CTC. Twenty-one millilitres of blood per patient was collected, centrifuged and frozen in cryotubes at −20°C. The specimens were then transported by World Courier in a −20°C cold chain to Germany.

Sequencing

HIV nucleic acid was isolated from plasma using a QIAamp MinElute Virus Spin Kit and HIV *pol* sequences were amplified by RT PCR and HIV *pol*-specific PCR followed by nested PCR as previously described.[11] Positive PCRs were sequenced with the ABI Prism 310 Genetic Analyzer system.

Sequence analysis

PDR (in accordance with WHO guidelines[9]) was defined as low-, intermediate- or high-level resistance (mutation score ≥15 in Stanford's HIVDR database[12]) for one of the following drugs: any nucleoside or nucleotide reverse transcriptase inhibitor [N(t)RTI], nevirapine, efavirenz, darunavir/ritonavir, lopinavir/ritonavir or atazanavir/ritonavir.

TDR (in accordance with WHO guidelines[10]) was defined as the presence of at least one of the standard surveillance drug-resistance mutations,[13] analysed with the calibrated population resistance tool.[14]

GenBank accession numbers are MH366803 MH367013, MH444895 MH444913 and MG764290 MG764294.

Data processing and statistical analysis

Patient data were transferred to duplicate Microsoft Excel tables. Statistical analysis was performed using SPSS (version 24, IBM) and 95% CIs were calculated with Wilson's method. Patient characteristics were tested for influence on HIVDR prevalence using Fisher's exact test (categorical variables) and/or binomial logistic regression using the Wald χ^2 test (ordinal and continuous variables).

Ethics

The study was approved by the Catholic University of Health and Allied Sciences (CUHAS)/BMC Research Ethics Committee (CREC) (CREC/021/2013) and the Lake Zone Institutional Review Board of the National Institute for Medical Research, Tanzania (MR/53/100/294). All patients gave written informed consent. All clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki.

Terminology

The term 'PDR' often refers to resistance at the time of ART initiation. For simplicity, we also use it for patients not initiating ART.

Results and discussion

Patient characteristics

Demographic and clinical data are displayed in Table 1. We had already conducted a study on PDR prevalence, with similar inclusion criteria, at BMC in 2008–10.[8] Patient characteristics of the two study groups were similar.

HIV-1 subtypes

The most common subtype, A, was found in 46.0% of the samples, followed by C in 33.2%, D in 17.0% and B in 3.4% (Table 1). This is a typical subtype distribution for this area.[8,15]

Prevalence and patterns of PDR

The point prevalence of PDR in our sample was 4.7% (11/235; 95% CI 2.6%–8.2%) (Table 2). PDR prevalence was 3.1% (3/97; 95% CI 1.1%–8.7%) for the non initiators and 5.8% (8/138; 95% CI 3.0%–11.0%) for the ART initiators. The WHO outcome 1b, 'prevalence of HIVDR among ART initiators without prior exposure to ARVs',[9] was 6.3% (8/128; 95% CI 3.2%–11.8%). PDR to N(t)RTIs, NNRTIs and PIs was found in 1.7% (4/235; 95% CI 0.7%–4.3%), 3.0% (7/235; 95% CI 1.5%–6.0%) and 0.0% (0/235; 95% CI 0.0%–1.6%) of patients, respectively. Dual class resistance was not observed.

The most frequent mutation was K103N, which was found in 2.1% (5/235; 95% CI 0.9%–4.9%) of patients. Other mutations detected were M41L and A98G (both 3/235; 1.3%; 95% CI 0.4%–3.7%) and K65R and V108I (both 1/235; 0.4%; 95% CI 0.1%–2.4%). This, and the absence of PI associated mutations, is consistent with our previous observations.[8] The absence of the lamivudine associated mutation M184V is surprising given the extensive use of lamivudine in Tanzania, and not consistent with our previous results.

In the publication of our 2008–10 study,[8] we used a slightly different definition of PDR. When we re analysed the sequences according to the definition used here, the prevalence of PDR was 17.0% (15/88; 95% CI 10.6%–26.2%), and significantly higher than the prevalence found in 2013–15 (Figure 1) ($P < 0.001$, Fisher's exact test). Several factors might have contributed to this decrease in PDR prevalence. Toxic stavudine based ART regimens were phased out in 2012–15 and replaced with single pill, tolerable tenofovir disoproxil fumarate based regimens. Adherence monitoring by HIV viral load testing was introduced. More efficient supply chain management of ARVs reduced the frequency of stock running out. Increased access to free ART services led to a reduction in the use of suboptimal ARV doses as a result of sharing or purchase of insufficient doses.[16,17]

Prevalence of TDR

The prevalence of TDR was 6.0% (14/235; 95% CI 3.6%–9.8%). Most patients that had TDR also had PDR and *vice versa*. Exceptions were patients infected with viral strains carrying the mutations A98G/V108I (PDR but not TDR, two patients) and I85V/G73S/M46I

Table 1. Demographic, clinical and sequencing data

			Sequenced			Kasang *et al.*[8] 2011, sequenced (all values indicated as mean±SD or %)
		Not sequenced	all	non-initiators	ART initiators	
Total $N = 406$		$N = 171$	$N = 235$	$N = 97$	$N = 138$	$N = 88$
Demographic data						
age (years), mean±SD		36 5±10 1	36 9±10 0	36 2±9 2	37 4±10 5	35 2±11 3
gender, *n* (%)	female	141 (82 5)	178 (75 7)	79 (81 4)	99 (71 7)	78
	male	30 (17 5)	57 (24 3)	18 (18 6)	39 (28 3)	22
gravidity (only women), *n* (%)	nulliparous	19 (13 5)	29 (16 3)	18 (22 8)	11 (11 1)	
	1	26 (18 4)	36 (20 2)	13 (16 5)	23 (23 2)	
	2	30 (21 3)	36 (20 2)	14 (17 7)	22 (22 2)	
	3	35 (24 8)	37 (20 8)	18 (22 8)	19 (19 2)	
	4	17 (12 1)	13 (7 3)	7 (8 9)	6 (6 1)	
	>4	14 (9 9)	27 (15 2)	9 (11 4)	18 (18 2)	
Clinical data						
time since HIV diagnosis (years), median (IQR)		1 30 (0 28–3 53)	0 71 (0 12–2 52)	1 79 (0 41–3 41)	0 30 (0 08–1 69)	1 30±1 75
WHO stage, *n* (%)	1	85 (49 7)	89 (37 9)	56 (57 7)	33 (23 9)	
	2	55 (32 2)	67 (28 5)	39 (40 2)	28 (20 3)	
	3	23 (13 5)	59 (25 1)	2 (2 1)	57 (41 3)	
	4	8 (4 7)	20 (8 5)	0 (0 0)	20 (14 5)	
current CD4 cell count (cells/mm^3), median (IQR)		491 (345–669) (8 values missing)	382 (190–529) (19 values missing)	513 (445–600) (7 values missing)	210 (114–317) (12 values missing)	490±222
nadir CD4 cell count (cells/mm^3), median (IQR)		451 (281–605) (4 values missing)	334 (187–483) (8 values missing)	462 (391–567) (1 value missing)	209 (114–315) (7 values missing)	
sexual partner on ART, *n* (%)	not indicated	0 (0 0)	1 (0 4)	0 (0 0)	1 (0 7)	
	no	111 (64 9)	161 (68 5)	70 (72 2)	91 (65 9)	
	yes	60 (35 1)	73 (31 1)	27 (27 8)	46 (33 3)	
history of PMTCT, *n* (%)	no	160 (93 6)	223 (94 9)	95 (97 9)	128 (92 8)	
	yes	11 (6 4)	12 (5 1)	2 (2 1)	10 (7 2)	
time between study visit and ART initiation (days), median (IQR; minimum, maximum)					0 (0–0; −1, 83) (2 values missing)	
Sequencing data						
subtype, *n* (%)	A		108 (46 0)	44 (45 4)	64 (46 4)	34 (+7 A1D)
	B		8 (3 4)	2 (2 1)	6 (4 3)	1
	C		78 (33 2)	35 (36 1)	43 (31 2)	26
	CRF10_CD		1 (0 4)	0 (0 0)	1 (0 7)	4
	D		40 (17 0)	16 (16 5)	24 (17 4)	28

Table 2. Patients with PDR and/or TDR according to WHO surveillance criteria

Sample no.	ART initiation	Age (years)	Gender	Subtype	Time since HIV diagnosis (years)	WHO stage	Current CD4 cell count (cells/mm^3)	History of PMTCT monotherapy	PI	N(t)RTI	NNRTI	Resistance to ARVs included in WHO surveillance criteria for PDR			Resistance to other ARVs	Definition of mutation (PDR, TDR)
												low	intermediate	high		
G2151	no	58	female	A	0.28	1	592	no	none	none	A98G, K103N			EFV, NVP	RPV	PDR, TDR
G2156	no	46	female	D	18.36	2	535	no	none	M41L	none	ZDV, d4T				PDR, TDR
G3042	yes	27	female	A	1.41	1	256	no	none	none	K103N			EFV, NVP		PDR, TDR
G3056	yes	40	female	A	0.15	3	197	no	L10LFI[a]	M41L	none	ZDV, d4T			FPV/r, NFV	PDR, TDR
G3061	yes	32	female	C	0.36	3	51	no	none	none	K103N, E138A			EFV, NVP	RPV	PDR, TDR
G3084	yes	38	female	CRF10_CD	0.88	3	220	no	F53L[a]	M41L	none	ZDV, d4T			SQV/r	PDR, TDR
G3122	yes	29	female	A	1.16	2	69	no	none	K65R	none		ABC, FTC, 3TC	d4T, ddI, TDF		PDR, TDR
G3146	yes	36	male	C	0.36	1		no	none	none	K103N, E138EA			EFV, NVP	RPV	PDR, TDR
G3171	yes	33	female	A	0.31	1	173	no	none	none	K103N, E138A			EFV, NVP	RPV	PDR, TDR
G1019	no	25	female	D	0.08	1	1144	no	none	none	A98G	EFV	NVP		RPV	PDR
G3138	yes	38	female	A	0.13	3		no	none	none	A98G, V108VI	EFV	NVP		RPV	PDR
G1006	yes	22	female	A	0.72	1	478	no	I85V	none	none					TDR
G1018	no	20	female	C	0.12	1	449	no	I85V	none	none					TDR
G3080	yes	24	male	C	5.29	2	226	no	G73S	none	E138A				RPV, IDV/r, NFV, SQV/r	TDR
G3109	yes	34	female	C	5.81	1	188	no	I85V	none	none					TDR
G3118	yes	27	female	A	5.73	2	323	no	M46MI	none	none				NFV	TDR

3TC, lamivudine; ABC, abacavir; d4T, stavudine; ddI, didanosine; EFV, efavirenz; FPV/r, fosamprenavir/ritonavir; FTC, emtricitabine; IDV/r, indinavir/ritonavir; NFV, nelfinavir; NVP, nevirapine; RPV, rilpivirine; SQV/r, saquinavir/ritonavir; TDF, tenofovir disoproxil fumarate; ZDV, zidovudine

[a]Mutation conveys resistance only to PIs not included in the WHO surveillance criteria for PDR

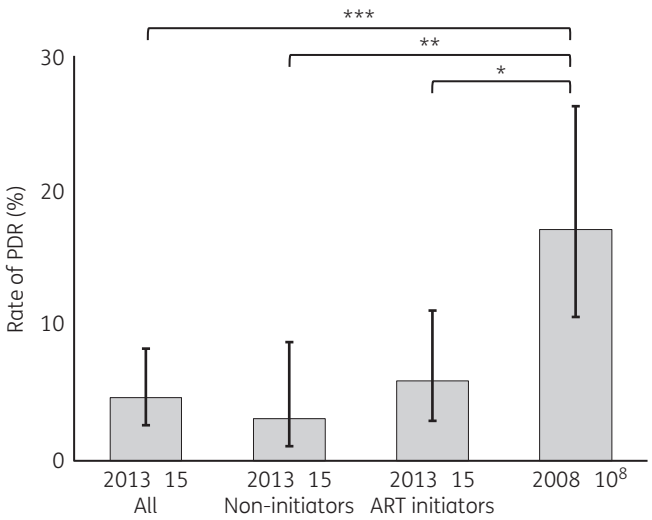

Figure 1. Decrease in prevalence of PDR in patients visiting BMC from 2008 to 2015. The sequences from 2008 to 2010 were re-analysed according to the process used for PDR in this study. Fisher's exact test. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

(TDR but not PDR, five patients). A98G and V108I mediate resistance to efavirenz and nevirapine,[18] but occur at polymorphic positions and are therefore not included in the WHO surveillance list for TDR.[13] I85V and G73S are non polymorphic and selected by PIs,[13] but confer only very weak resistance to the PIs relevant for WHO defined PDR.[18]

Prevalence of HIVDR to other ARVs

An additional 6.0% (14/235; 95% CI 3.6%–9.8%) of patients did not fall under the criteria for PDR or TDR, but carried viral strains resistant to other ARVs (mutation score ≥15 in Stanford's HIVDR database;[12] data not shown). The mutations were E138A (13 patients) and E138G (1 patient). E138A is present in ∼5% of treatment naive patients and most common in subtype C.[19] It conveys resistance to rilpivirine and etravirine,[19] with implications for future use in resource limited settings. Etravirine is proposed to be included in third line ART regimens in Tanzania.

Risk factor analysis

Prevalence of PDR or TDR did not differ significantly between different groups with regard to gender, age, time since first HIV diagnosis, WHO stage, current CD4 cell count, ART status of sexual partner, history of PMTCT or HIV subtype ($P > 0.05$; data not shown).

Study limitations

See the Supplementary data available at *JAC* Online.

Conclusions

The WHO defined the threshold for triggering a public health response (the implementation of an NNRTI free first line regimen or PDR testing) as a 10% rate of PDR to NNRTIs.[20] Resistance rates to NNRTIs found in this and the other three recent studies from Tanzania are <10%.[6 8] However, this is not a definite all clear signal. None of the studies was generally representative of ART initiators in Tanzania and a recent systematic review found a universal increase in PDR rates over the last 20 years across 63 low and middle income countries.[3] This highlights the need for country wide, systematic PDR surveillance in Tanzania.

Acknowledgements

We would like to acknowledge the technical support provided by the members of laboratories of the BMC and the Institute of Virology and Immunobiology of the University of Wuerzburg. We are indebted to our study nurse, Lydia Makenge, our documentation assistant, Theresia Gabriel Juma, our laboratory technician, John Myombe, and laboratory manager, Medard Beyanga. The National Institute of Medical Research in Mwanza, headed by John Changalucha, kindly provided sample storage facilities. We thank Friederike Grosse-Holz for feedback on the manuscript and Judith K. Paulus and Philippe V. Jutras for scientific discussion (prior to submission).

Funding

This work was supported by the Georg Ludwig Rexroth Foundation (http://www.rexroth-stiftung.de/), Deutscher Akademischer Austauschdienst (DAAD) (https://www.daad.de/de/index.html) and German Gilead Foerderprogramm Infektiologie (http://www.gilead-foerderprogramm-infektiologie.de/home.html). The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

Transparency declarations

None to declare.

Author contributions

C. K. and C. Sc. conceived the study design and C. K. supervised study conduction. L. R., S. E. K., C. Sc. and C. K. designed the experiments. L. R., J. E., C. Se., C. Sc., C. K. and K. P. conducted the experiments. J. M. B. and L. R. analysed the data and wrote the manuscript. C. K., B. R. K. and S. E. K. edited and reviewed the manuscript critically. All authors read and approved the manuscript.

Supplementary data

Supplementary data are available at *JAC* Online.

References

1 UNAIDS. *Prevention Gap Report*. 2016. http://www.unaids.org/sites/default/files/media_asset/2016-prevention-gap-report_en.pdf.

2 Nyombi BM, Holm-Hansen C, Kristiansen KI *et al*. Prevalence of reverse transcriptase and protease mutations associated with antiretroviral drug resistance among drug-naive HIV-1 infected pregnant women in Kagera and Kilimanjaro regions, Tanzania. *AIDS Res Ther* 2008; **5**: 13.

3 WHO. *HIV Drug Resistance Report 2017*. 2017. http://apps.who.int/iris/bitstream/10665/255896/1/9789241512831-eng.pdf.

4 WHO. *HIV Drug Resistance Surveillance Guidance 2015 Update*. 2015. http://www.who.int/hiv/pub/drugresistance/hiv-drug-resistance-2015-update/en/.

5 National AIDS Control Programme. *National Guidelines for the Management of HIV and AIDS, Fifth Edition*. 2015. https://aidsfree.usaid.gov/sites/default/files/04_11_2016.tanzania_national_guideline_for_management_hiv_and_aids_may_2015._tagged.pdf.

6 Masimba P, Kituma E, Klimkait T *et al*. Prevalence of drug resistance mutations and HIV type 1 subtypes in an HIV type 1-infected cohort in rural Tanzania. *AIDS Res Hum Retroviruses* 2013; **29**: 1229 36.

7 Vairo F, Nicastri E, Liuzzi G *et al*. HIV-1 drug resistance in recently HIV-infected pregnant mother's naïve to antiretroviral therapy in Dodoma urban, Tanzania. *BMC Infect Dis* 2013; **13**: 439.

8 Kasang C, Kalluvya S, Majinge C *et al*. HIV drug resistance (HIVDR) in antiretroviral therapy-naïve patients in Tanzania not eligible for WHO threshold HIVDR survey is dramatically high. *PLoS One* 2011; **6**: e23091.

9 WHO. *Surveillance of HIV Drug Resistance in Adults Initiating Antiretroviral Therapy (Pre-Treatment HIV Drug Resistance) Concept Note*. 2014. http://www.who.int/hiv/pub/drugresistance/pretreatment_drugresistance/en/.

10 Bennett DE, Myatt M, Bertagnolio S *et al*. Recommendations for surveillance of transmitted HIV drug resistance in countries scaling up antiretroviral treatment. *Antivir Ther* 2008; **13** Suppl 2: 25 36.

11 Jacobs GB, Laten A, van Rensburg EJ *et al*. Phylogenetic diversity and low level antiretroviral resistance mutations in HIV type 1 treatment-naive patients from Cape Town, South Africa. *AIDS Res Hum Retroviruses* 2008; **24**: 1009 12.

12 Liu TF, Shafer RW. Web resources for HIV type 1 genotypic-resistance test interpretation. *Clin Infect Dis* 2006; **42**: 1608 18.

13 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.

14 Gifford RJ, Liu TF, Rhee S-Y *et al*. The calibrated population resistance tool: standardized genotypic estimation of transmitted HIV-1 drug resistance. *Bioinformatics* 2009; **25**: 1197 8.

15 Shao ER, Kifaro EG, Kimaro J *et al*. HIV-1 diversity in Tanzania and its implication toward development of effective vaccines: a review article. *J Vaccines Vaccin* 2014; **5**: 249.

16 Geng EH, Hunt PW, Diero LO *et al*. Trends in the clinical characteristics of HIV-infected patients initiating antiretroviral therapy in Kenya, Uganda and Tanzania between 2002 and 2009. *J Int AIDS Soc* 2011; **14**: 46.

17 Ramadhani HO, Thielman NM, Landman KZ *et al*. Predictors of incomplete adherence, virologic failure, and antiviral drug resistance among HIV-infected adults receiving antiretroviral therapy in Tanzania. *Clin Infect Dis* 2007; **45**: 1492 8.

18 Rhee S-Y, Gonzales MJ, Kantor R *et al*. Human immunodeficiency virus reverse transcriptase and protease sequence database. *Nucleic Acids Res* 2003; **31**: 298 303.

19 Sluis-Cremer N, Jordan MR, Huber K *et al*. E138A in HIV-1 reverse transcriptase is more common in subtype C than B: implications for rilpivirine use in resource-limited settings. *Antiviral Res* 2014; **107**: 31 4.

20 WHO. *Guidelines on the Public Health Response to Pretreatment HIV Drug Resistance: July 2017*. 2017. http://www.who.int/hiv/pub/guidelines/hivdr-guidelines-2017/en/.