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Association between HIV-1 subtype and drug resistance in Nigerian infants

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Background: Many lines of evidence point to HIV-1 subtype-specific differences in the development of drug resistance mutations. While variation between subtype C and others has been extensively explored, there has been less emphasis on subtypes common to West Africa. We examined a previously described national survey of pretreatment drug resistance in HIV-1-infected Nigerian children aged <18 months, to explore the association between subtypes and patterns of resistance.

Methods: Five hundred and forty-nine dried blood spots, from 15 early infant diagnostic facilities in Nigeria, were amplified and HIV-1 polymerase was sequenced. Four hundred and twenty-four were analysed for surveillance drug resistance mutations (SDRMs). Associations between subtype and SDRMs were evaluated by Fisher's exact test and logistic regression analysis, controlling for geographical region and exposure.

Results: Using the sub-subtypes of HIV-1 G defined by Delatorre *et al.* (*PLoS One* 2014; **9**: e98908) the most common subtypes were CRF02_AG (174, 41.0%), G_{WA-I} (128, 30.2%), G_{WA-II} (24, 5.7%), G_{CA} (11, 2.6%), A (21, 5.0%) and CRF06_cpx (18, 4.2%). One hundred and ninety infants (44.8%) had ≥1 NNRTI mutation, 92 infants (21.7%) had ≥1 NRTI mutation and 6 infants (1.4%) had ≥1 PI mutation. By logistic regression, 67N was more common in G_{WA-II}/G_{CA} than CRF02_AG (OR 12.0, $P = 0.006$), as was 70R (OR 23.1, $P = 0.007$), 184I/V (OR 2.92, $P = 0.020$), the presence of ≥1 thymidine analogue mutation (TAM) (OR 3.87, $P = 0.014$), ≥1 type 2 TAM (OR 7.61, $P = 0.001$) and ≥1 NRTI mutation (OR 3.26, $P = 0.005$).

Conclusions: This dataset reveals differences among SDRMs by subtype; in particular, between the G_{WA-II} and G_{CA} subclades, compared with CRF02_AG and G_{WA-I}.

Introduction

Over 88% of the 37 million people living with HIV-1 worldwide reside in Africa, Asia or Eastern Europe.[1] Among the various types of HIV-1 infections, HIV subtype C dominates globally, accounting for about half of the world's HIV-1 infections. This is followed by HIV-1 subtypes A (12%), B (11%), CRF02_AG (8%), CRF01_AE (5%) and G (5%).[2] The greatest diversity of HIV-1 subtypes is found in Africa.[3] In West Africa, CRF02_AG and subtype G are the most common HIV-1 infections, although subtype A, CRF06_cpx and other circulating recombinant forms are often described.[4]

While it is often difficult to compare subtypes, because their prevalence correlates with geographical region, ethnic group and standard of care, many lines of evidence point to subtype-specific differences in the development of drug resistance mutations (DRMs). Early studies showed a difference in the mutational pathways that develop in response to a failing regimen of stavudine in subtype B versus C[5] and these same differences were seen in a

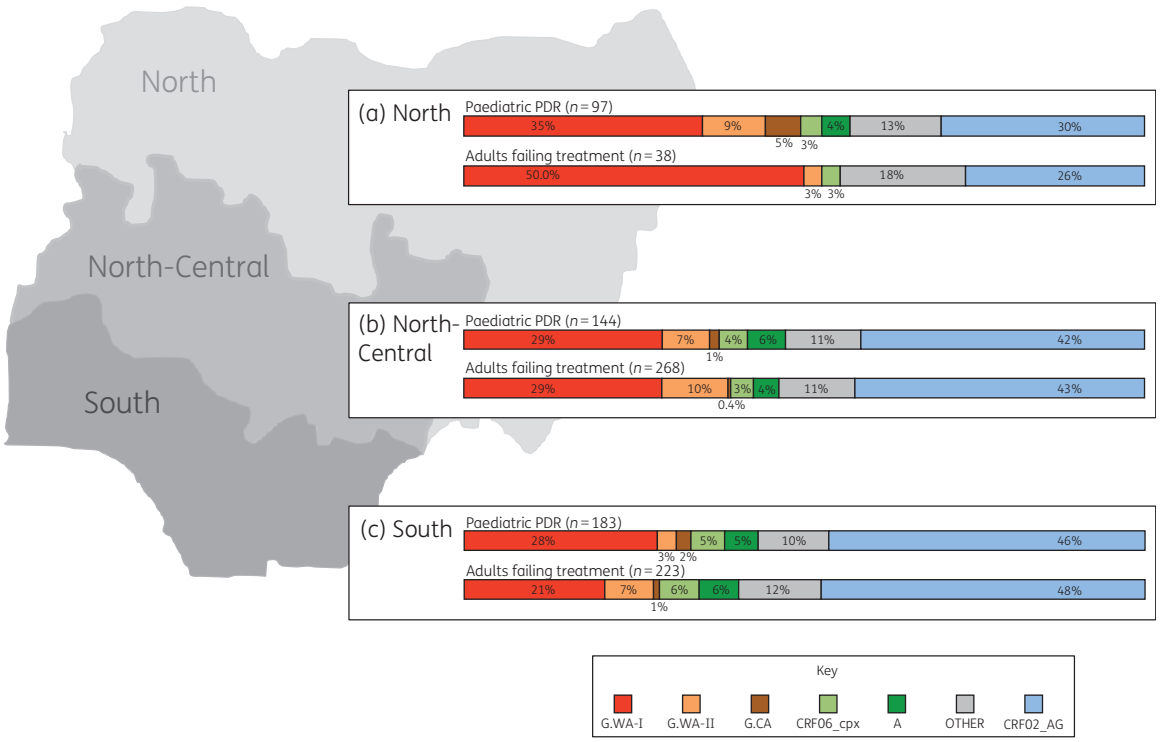

Figure 1. Distribution of HIV-1 subtype, based on *pol* sequence, in (a) North Nigeria (NE and NW zones), (b) North-Central Nigeria (NC zone) and (c) South Nigeria (SS, SW and SE zones). Paediatric pretreatment drug resistance samples were drawn from EID facilities distributed around the country, as described by Inzaule *et al.*,[12] and comprise the dataset of 424 sequences in this article. Samples from adults failing treatment were taken from Borno State in North Nigeria, Plateau State in North-Central Nigeria and Lagos and Oyo States in South Nigeria, between 2004 and 2011, as previously published.[13,24] PDR, primary drug resistance. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

setting with primarily G and CRF02_AG.[6] A meta-analysis of 35 publications describing 1825 individuals showed different DRMs in C and CRF01_AE.[7]

A study by Koning *et al.*[8] investigated a UK database with large numbers of subtype B and C patients and found that different accessory mutations were associated with the development of NNRTI DRMs as well as thymidine analogue mutations (TAMs), between the two subtypes. Armstrong *et al.*[9] showed that individual TAMs had widely varying fitness costs between viral backbones that were subtype B or C. This could help explain the observation that while resistance to zidovudine in subtype B infection is more likely to be mediated by type 1 TAMs (TAM-1; 41L, 215Y, 210W), infection with many non-B subtypes results in type 2 TAMs (TAM-2; 67N, 70R, 215F, 219E/Q).[8]

While many of these studies have identified distinct characteristics of subtype C as compared with other clades, there has been less focus on subtype G due to its smaller numbers worldwide and its occurrence primarily in resource-limited settings. In addition, previous reports from West Africa have not consistently distinguished between the different clades of G, so that any distinct patterns of mutations may have been lost as all G sequences were often grouped together.

In the context of non-B subtype infection, recent surveys of transmitted drug resistance have revealed an increasing prevalence of NNRTI DRMs in both adults and infants in sub-Saharan Africa.[10,11] Nigeria, with >3 million people living with HIV-1,[1] has an estimated mother-to-child transmission rate of 23%.[12] A recent survey in Nigeria examining pretreatment paediatric drug resistance found an alarming overall prevalence of 48%[12] in children before reaching 18 months of age. Because we have previously observed a geographical association between subtypes in Nigerian adults,[13] and because this survey of paediatric patients was representative of the country, examining this dataset would allow us to address this question.

As described by Inzaule *et al.*,[12] a national Nigerian survey of children aged <18 months, from 15 early infant diagnostic (EID) DNA-PCR laboratories distributed throughout the country, revealed that 205 of 430 had DRMs conferring resistance to NNRTIs (45%), NRTIs (22%), multiclass NNRTIs/NRTIs (20%) or PIs (2%). We examined this dataset to explore further the association between HIV-1 subtypes and the emergence of specific patterns of resistance.

Methods

The collection methods and results for this study population have been previously published.[12] Briefly, 549 dried blood spot samples were obtained from 15 EID facilities, with the sample contribution based on a probability-proportional-to-size approach that incorporated the contribution of each laboratory to the HIV drug resistance survey and the number of HIV-1-positive diagnoses. Sequences from the 5′ region of the HIV-1 *pol* gene (nucleotide positions 224–1200) were generated using a two-step RT and nested PCR and analysed on an ABI Prism 3130 Genetic Analyser (Applied Biosystems); 430 were genotyped at a WHO-accredited KRMI/CDC HIV drug resistance regional reference laboratory in Kisumu, Kenya. As described by

Table 1. Demographic and clinical characteristics of included patients [*n* (%)]

	CRF02_AG	G_{WA-I}	G_{WA-II}	G_{CA}	CRF06_cpx	A	Other	Total	*P*
Number of observations	174	128	24	11	18	21	48	424	
Neonatal intervention									NS
sd-NVP	21 (12.1)	23 (18.0)	1 (4.2)	2 (18.2)	3 (16.7)	2 (9.5)	7 (14.6)	59	
extended prophylaxis	32 (18.4)	25 (19.5)	3 (12.5)	2 (18.2)	4 (22.2)	5 (23.8)	8 (16.7)	79	
unknown	2 (1.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2	
not administered	50 (28.7)	28 (21.9)	7 (29.2)	3 (27.3)	3 (16.7)	5 (23.8)	17 (35.4)	113	
information missing	69 (39.7)	52 (40.6)	13 (54.2)	4 (36.4)	8 (44.4)	9 (42.9)	16 (33.3)	171	
Maternal ART									NS
monotherapy or dual therapy	7 (4.0)	10 (7.8)	1 (4.2)	0 (0)	0 (0)	1 (4.8)	3 (6.3)	22	
triple regimen	48 (27.6)	36 (28.1)	3 (12.5)	7 (63.6)	6 (33.3)	5 (23.8)	11 (22.9)	116	
unknown regimen	9 (5.2)	10 (7.8)	2 (8.3)	0 (0)	0 (0)	3 (14.3)	2 (4.2)	26	
not administered	67 (38.5)	39 (30.5)	9 (37.5)	1 (9.1)	5 (27.8)	7 (33.3)	21 (43.8)	149	
information missing	43 (24.7)	33 (25.8)	9 (37.5)	3 (27.3)	7 (38.9)	5 (23.8)	11 (22.9)	111	
Exposure									NS
not administered	59 (33.9)	34 (26.6)	9 (37.5)	1 (9.1)	5 (27.8)	5 (23.8)	18 (37.5)	131	
neonatal or maternal	80 (46.0)	66 (51.6)	7 (29.2)	7 (63.6)	8 (44.4)	11 (52.4)	22 (45.8)	201	
information missing	35 (20.1)	28 (21.9)	8 (33.3)	3 (27.3)	5 (27.8)	5 (23.8)	8 (16.7)	92	
Geographical region									NS[a]
North Nigeria	29 (16.7)	34 (26.6)	9 (37.5)	5 (45.5)	3 (16.7)	4 (19.1)	13 (27.1)	97	
North-Central Nigeria	60 (34.5)	42 (32.8)	10 (41.7)	2 (18.2)	6 (33.3)	8 (38.1)	16 (33.3)	144	
South Nigeria	85 (48.9)	52 (40.6)	5 (20.8)	4 (36.4)	9 (50.0)	9 (42.9)	19 (39.6)	183	
DRMs									
≥1 NNRTI mutation	73 (42.0)	60 (46.9)	13 (54.2)	6 (54.6)	7 (38.9)	9 (42.9)	22 (45.8)	190	NS
≥1 NRTI mutation	29 (16.7)	27 (21.1)	10 (41.7)	4 (36.4)	3 (16.7)	4 (19.1)	15 (31.3)	92	0.050
≥1 TAM	11 (6.3)	10 (7.8)	4 (16.7)	3 (27.3)	3 (16.7)	2 (9.5)	6 (12.5)	39	NS
≥1 TAM-1	7 (4.0)	8 (6.3)	0 (0)	1 (9.1)	3 (16.7)	1 (4.8)	2 (4.2)	22	NS
≥1 TAM-2	6 (3.5)	5 (3.9)	4 (16.7)	3 (27.3)	0 (0)	2 (9.5)	4 (8.3)	24	0.003
M184I/V	21 (12.1)	19 (14.8)	6 (25.0)	4 (36.4)	1 (5.6)	3 (14.3)	12 (25.0)	66	NS
≥1 PI mutation	2 (1.2)	2 (1.6)	0 (0)	0 (0)	0 (0)	0 (0)	2 (4.2)	6	NS
Resistance to									
zidovudine	11 (6.3)	12 (9.4)	4 (16.7)	2 (18.2)	2 (11.1)	3 (14.3)	6 (12.5)	40	NS
tenofovir	4 (2.3)	12 (9.4)	3 (12.5)	2 (18.2)	1 (5.6)	1 (4.8)	5 (10.4)	28	NS
emtricitabine	21 (12.1)	19 (14.8)	6 (25.0)	4 (36.4)	1 (5.6)	3 (14.3)	12 (25.0)	66	NS
lamivudine	21 (12.1)	19 (14.8)	6 (25.0)	4 (36.4)	1 (5.6)	3 (14.3)	12 (25.0)	66	NS
efavirenz	73 (42.0)	61 (47.7)	13 (54.2)	6 (54.6)	7 (38.9)	9 (42.9)	22 (45.8)	191	NS
nevirapine	73 (42.0)	61 (47.7)	13 (54.2)	6 (54.6)	7 (38.9)	9 (42.9)	22 (45.8)	191	NS
lopinavir	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0	NA

sd-NVP, single dose nevirapine; NA, not applicable; NS, not significant. Geographical region: North Nigeria includes Gombe and Taraba States (NE zone) and Kaduna, Kano and Sokoto States (NW zone); North-Central Nigeria includes Abuja, Benue and Plateau states (NC zone); and South Nigeria includes Anambra State (SE zone), Akwa Ibom and Edo States (SS zone) and Lagos and Osun States (SW zone).
Patients with a score of low-level resistance, intermediate resistance or high-level resistance, by the five-level scale from the Stanford University HIV Drug Resistance Database hivDB program, were classified as resistant to that particular antiretroviral medication. Those with a score of susceptible or potential low-level resistance were not classified as resistant to that drug.
[a]There is no overall association between subtype and geographical region; however, G (of any subcluster) is more common than other subtypes in the North versus the South and CRF02_AG is more common than other subtypes in the South versus the North.

Inzaule *et al.*,[12] sequence data were assembled with RECall,[14] and assessed for quality as per WHO recommendations, using RECall, MEGA v7.0[15] and Stanford HIVdb tools.[16] Resistance was assessed using the Stanford HIVdb algorithm (v7.0). Six sequences were later removed and 424 retained for this current analysis. Surveillance DRMs recommended by WHO for surveillance of pretreatment drug resistance were used in all analyses.[17]

Subtypes were originally assessed with the Rega HIV subtyping tool v2.0,[18] as reported by Inzaule *et al.*[12] In this analysis, subtype classifications were refined using alignments in ClustalX[19] and neighbour-joining phylogenetic trees in njplot.[20] References added to the alignments to determine subtype included those downloaded from the curated sequences available at the Los Alamos HIV Database Tools,[21] in-house references and additional controls that further distinguished monophyletic sub-subtypes of G, contributed by Delatorre *et al.*[4] Sequences were further examined for recombination by the Los Alamos Recombinant Identification Program.[22]

Table 2. Logistic regression, comparison with G_{WA-II}/G_{CA}

	G_{WA-I}		CRF02_AG	
	OR	*P*	OR	*P*
67N	0.219 (0.050–0.962)	0.044	0.083 (0.014–0.496)	0.006
70R	0.110 (0.005–0.417)	0.015	0.043 (0.005–0.417)	0.007
≥1 TAM	0.305 (0.102–0.907)	0.033	0.258 (0.088–0.760)	0.014
≥1 TAM-2	0.139 (0.039–0.490)	0.002	0.131 (0.039–0.444)	0.001
184I/V	0.395 (0.158–0.986)	0.047	0.342 (0.138–0.845)	0.020
>1 NRTI mutation	0.369 (0.160–0.852)	0.019	0.306 (0.160–0.852)	0.005

Associations between subtype and individual mutations or classes of mutations, which showed a significant difference by Fisher's exact test, were subjected to logistic regression, controlling for exposure and region. Selective results are shown.

The Government of Nigeria has established six geopolitical zones and the dataset of patients was originally categorized into these zones based on the origin of the sample. Because we have seen previous evidence of an association between HIV-1 subtype and geography in the country,[13] along a North–South gradient, these zones were grouped into three bands. The South-West (SW), South-South (SS) and South-East (SE) geopolitical zones were grouped as South Nigeria, the North-Central (NC) zone corresponded to North-Central Nigeria and the North-West (NW) and North-East (NE) zones were grouped as North Nigeria, as shown in Figure 1. Infants with a record of exposure to neonatal (prevention of mother to child transmission) prophylaxis or maternal ART or prophylaxis were classified as 'exposed', those with no record of exposure as 'unexposed' and those with no recorded information about either category as 'unknown'.

Associations between subtype and individual or classes of mutations were initially assessed by Fisher's exact test. Those that showed a significant difference were subjected to logistic regression, controlling for exposure and region, for categorical variables such as the presence or absence of an individual mutation or a class of mutations.

Results

Subtype G is an important subtype in West Africa, but it has a highly varied distribution. In previous work, we have noticed distinct clades of subtype G in Nigeria, with evidence of subtype-specific differences in the emergence of DRMs.[13] The clade that we have referred to as G-prime has been further defined by the work of Delatorre *et al.*,[4] named G_{WA-I}, described as a lineage that was introduced into Nigeria in the mid-1970s and that currently predominates in that country.[4] A separate clade, G_{WA-II}, was hypothesized to have been introduced into the Togo/Ghana region in the late 1970s and subsequently spread to Nigeria. G_{CA} represents a diverse set of G sequences that emerged in Central Africa in the 1960s and are present in small numbers. The reference sequences used to assess subtype included these sub-subtypes of G. The most common subtype was CRF02_AG (174, 41.0%), followed by G_{WA-I} (128, 30.2%), G_{WA-II} (24, 5.7%), G_{CA} (11, 2.6%), A (21, 5.0%) and CRF06_cpx (18, 4.2%). For this analysis, 48 sequences (11.3%) were classified as 'other'; these included 2 B, 2 C, 2 D, 1 CRF09_cpx, 1 CRF11_cpx, 1 CRF37_cpx and 39 unidentified recombinant forms.

Of the 424 sequences assayed: 97 (22.9%) were from North Nigeria, which included Gombe, Taraba, Kaduna, Kano and Sokoto States; 144 (34.0%) were from North-Central Nigeria, which included Abuja (the Federal Capital Territory), Benue and Plateau States; and 183 (43.2%) were from South Nigeria, which included Anambra, Akwa Ibom, Edo, Lagos and Osun States. Information on exposure is detailed in Table 1. Employing a variable that combined maternal and infant exposure, 201 infants were exposed to either maternal ART or neonatal prophylaxis, 131 were unexposed and, for 92, the information was unavailable.

One hundred and ninety infants (44.8%) had ≥1 NNRTI mutation, 92 infants (21.7%) had ≥1 NRTI mutation and 6 infants (1.4%) had ≥1 PI mutation. The presence of ≥1 NRTI mutation was marginally different between subtypes ($P = 0.050$) and this appeared to be driven by the presence of ≥1 TAM-2 ($P = 0.003$).

Surveillance DRMs that appeared in one subtype category significantly more or less often than others (75M, 41L, ≥1 TAM-1, 67N, 70R, 219Q, 219E, ≥1 TAM-2, ≥1 TAM, 184V, 184I/V and ≥1 NRTI mutation) were explored by logistic regression. Based on the preliminary associations, subtype categories were collapsed into G_{WA-I}, G_{WA-II}/G_{CA}, CRF02_AG and others. Those with significant differences between subtypes are shown in Table 2. Although no subtype-specific variation was found among the NNRTI or PI mutations, the TAM-2 mutations, among the NRTI mutations, exhibited variability in bivariate analysis that held up under logistic regression. When compared with CRF02_AG, 67N, 70R, ≥1 TAM, ≥1 TAM-2 or ≥1 NRTI mutation, as well as M184I/V, were significantly more frequent in G_{WA-II}/G_{CA}. These mutations were also less common in G_{WA-I}, compared with G_{WA-II}/G_{CA}.

Discussion

In this study of pretreatment drug resistance in paediatric patients, both 184I/V and TAM-2 mutations 67N and 70R emerge more frequently in G sub-subtypes WA-II and CA, as compared with G_{WA-I} or CRF02_AG. In addition, the total number of TAMs and the total number of NRTIs are higher in these subsets.

We cannot discount the possibility that the subtype-based differences in TAMs could be linked to a confounding factor, such as site-specific differences in care, including the administration of maternal prophylaxis containing zidovudine. However, these differences are in accordance with other studies that have shown that the viral backbone can influence the residual fitness of individual DRMs, including TAMs.[9]

In looking back at other repositories of resistance testing in Nigeria, we have noticed evidence of similar differences in the prevalence of TAM-2 mutations, when it is possible to confine the

dataset to those relatively early in the development of resistance (data not shown).

Despite the high level of resistance in this paediatric population, the majority of DRMs were to NNRTIs. The ability to elucidate subtype-specific differences in NRTIs would undoubtedly increase with a larger dataset. Further, the clinical impact of the differences that were found remains to be fully studied. However, a meta-analysis of treatment-naive HIV-1-infected individuals has shown differences in progression, with subtypes C and D leading more rapidly to death, progression to AIDS or CD4 or viral load changes, followed by G, CRF01_AE, CRF02_AG and A.[23] This and other studies have shown subtype-specific differences in the response to specific antiretroviral medications. As larger datasets are gathered globally, based on sequencing of both pretreatment drug resistance and treatment failure, careful analysis of subtypes may reveal more information on the influence of viral subtypes towards the varying pathways of resistance.

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Transparency declarations

None to declare.

References

1 UNAIDS. *AIDSInfo: Number of People Living with HIV*. http://aidsinfo.unaids.org.

2 Hemelaar J, Gouws E, Ghys PD *et al*. Global trends in molecular epidemiology of HIV-1 during 2000-2007. *AIDS* 2011; **25**: 679–89.

3 Tatem AJ, Hemelaar J, Gray RR *et al*. Spatial accessibility and the spread of HIV-1 subtypes and recombinants. *AIDS* 2012; **26**: 2351–60.

4 Delatorre E, Mir D, Bello G. Spatiotemporal dynamics of the HIV-1 subtype G epidemic in West and Central Africa. *PLoS One* 2014; **9**: e98908.

5 Hosseinipour MC, van Oosterhout JJG, Weigel R *et al*. The public health approach to identify antiretroviral therapy failure: high-level nucleoside reverse transcriptase inhibitor resistance among Malawians failing first-line antiretroviral therapy. *AIDS* 2009; **23**: 1127–36.

6 Hawkins CA, Chaplin B, Idoko J *et al*. Clinical and genotypic findings in HIV-infected patients with the K65R mutation failing first-line antiretroviral therapy in Nigeria. *J Acquir Immune Defic Syndr* 2009; **52**: 228–34.

7 Tang MW, Rhee SY, Bertagnolio S *et al*. Nucleoside reverse transcriptase inhibitor resistance mutations associated with first-line stavudine-containing antiretroviral therapy: programmatic implications for countries phasing out stavudine. *J Infect Dis* 2013; **207**: 70–7.

8 Koning FA, Castro H, Dunn D *et al*. Subtype-specific differences in the development of accessory mutations associated with high-level resistance to HIV-1 nucleoside reverse transcriptase inhibitors. *J Antimicrob Chemother* 2013; **68**: 1220–36.

9 Armstrong KL, Lee TH, Essex M. Replicative capacity differences of thymidine analog resistance mutations in subtype B and C human immunodeficiency virus type 1. *J Virol* 2009; **83**: 4051.

10 Boerma RS, Sigaloff KCE, 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.

11 WHO. *HIV Drug Resistance Report* 2017. http://apps.who.int/iris/bitstream/handle/10665/255896/9789241512831-eng.pdf; jsessionid=443E1847827CCE3AB8E798A56C3DFF50?sequence=1.

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 Chaplin B, Eisen G, Idoko J *et al*. Impact of HIV type 1 subtype on drug resistance mutations in Nigerian patients failing first-line therapy. *AIDS Res Hum Retroviruses* 2011; **27**: 71–80.

14 Woods CK, Brumme CJ, Liu TF. Automating HIV drug resistance genotyping with RECall, a freely accessible sequence analysis tool. *J Clin Microbiol* 2012; **50**: 1936–42.

15 Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 2016; **33**: 1870–4.

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

17 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.

18 Peña ACP, Faria NR, Imbrechts S *et al*. Automated subtyping of HIV-1 genetic sequences for clinical and surveillance purposes: performance evaluation of the new REGA version 3 and seven other tools. *Infect Genet Evol* 2013; **19**: 337–48.

19 Larkin MA, Blackshields G, Brown NP *et al*. Clustal W and Clustal X version 2.0. *Bioinformatics* 2007; **23**: 2947–8.

20 Perrière G, Gouy M. WWW-query: an on-line retrieval system for biological sequence banks. *Biochimie* 1996; **78**: 364–9.

21 Los Alamos National Laboratory. *HIV Databases*. http://www.hiv.lanl.gov/.

22 Siepel AC, Halpern AL, Macken C *et al*. A computer program designed to screen rapidly for HIV type 1 intersubtype recombinant sequences. *AIDS Res Hum Retroviruses* 1995; **11**: 1413–6.

23 Pant Pai N, Shivkumar S, Cajas JM. Does genetic diversity of HIV-1 non-B subtypes differentially impact disease progression in treatment-naive HIV-1-infected individuals? A systematic review of evidence: 1996-2010. *J Acquir Immune Defic Syndr* 2012; **59**: 382–8.

24 Meloni ST, Onwuamah CK, Agbaji O *et al*. Implication of first-line antiretroviral therapy choice on second-line options. *Open Forum Infect Dis* 2017; **4**: ofx233.