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ORIGINAL RESEARCH

HIV drug resistance levels in adults failing first-line antiretroviral therapy in an urban and a rural setting in South Africa

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Objectives

Urban and rural HIV treatment programmes face different challenges in the long-term management of patients. There are few studies comparing drug resistance profiles in patients accessing treatment through these programmes. The aim of this study was to perform such a comparison.

Methods

HIV drug resistance data and associated treatment and monitoring information for adult patients failing first-line therapy in an urban and a rural programme were collected. Data were curated and managed in SATuRN RegaDB before statistical analysis using Microsoft Excel 2013 and STATA Ver14, in which clinical parameters, resistance profiles and predicted treatment responses were compared.

Results

Data for 595 patients were analysed: 492 patients from a rural setting and 103 patients from an urban setting. The urban group had lower CD4 counts at treatment initiation than the rural group (98 *vs.* 126 cells/μL, respectively; $P = 0.05$), had more viral load measurements performed per year (median 3 *vs.* 1.4, respectively; $P < 0.01$) and were more likely to have no drug resistance mutations detected (35.9% *vs.* 11.2%, respectively; $P < 0.01$). Patients in the rural group were more likely to have been on first-line treatment for a longer period, to have failed for longer, and to have thymidine analogue mutations. Notwithstanding these differences, the two groups had comparable predicted responses to the standard second-line regimen, based on the genotypic susceptibility score. Mutations accumulated in a sigmoidal fashion over failure duration.

Conclusions

The frequency and patterns of drug resistance, as well the intensity of virological monitoring, in adults with first-line therapy failure differed between the urban and rural sites. Despite these differences, based on the genotypic susceptibility scores, the majority of patients across the two sites would be expected to respond well to the standard second-line regimen.

Keywords: drug resistance, HIV-1, rural, urban.

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Introduction

Highly active antiretroviral therapy (HAART) significantly decreases morbidity and mortality associated with HIV-1 infection, transforming it from an inevitably fatal illness into a manageable, chronic condition [1–4]. Significant

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increases in patient lifespan and quality of life are a result of reduced HIV-1 viraemia with a corresponding partial reconstitution of the immune system. The development of HIV-associated drug resistance (HIVDR) in the presence of suboptimal drug levels, however, poses a threat to the long-term success of the HIV treatment programme as it limits treatment options, increases costs and leads to a reservoir of resistant virus that can be transmitted to other individuals [5].

Similar treatment responses to HAART have been reported across HIV-1 subtypes [6]. Most data on drug resistance, however, concern subtype B, the predominant subtype found in the developed world. In contrast, there is still relatively little information on subtype C, which accounts for the majority of infections world-wide [7] and is predominantly found in sub-Saharan Africa. More research is needed, especially as it has been reported that subtype C might have a higher propensity for the development of mutations conferring resistance to common first-line drugs [8–10]. For instance, is has been shown that subtype C contains a valine codon 106 polymorphism (GTG) that facilitates a V106M mutation (GTG < –ATG) after selection with efavirenz (EFV), which confers high-level cross-resistance to the class of nonnucleoside reverse transcriptase inhibitors (NNRTIs) [11]. In addition, subtype C has a different template nucleotide sequence around codon 65 that causes transcription errors in this region, with the subsequent rapid development of resistance to tenofovir (TDF) [12,13].

Even a relatively small increase in HIVDR in subtype C might be significant, considering the magnitude of the treatment programme in countries such as South Africa, where approximately 2.5 million people were accessing HAART by the end of 2013 [14]. Up to 85% of patients failing first-line line HAART have evidence of at least one drug resistance-associated mutation [15]. Overall, based on data from 12 studies in eight countries, between 3.7% and 49% of individuals on first-line HAART in sub-Saharan Africa fail virologically because of drug resistance [16].

Although South Africa has a few, large, highly populated urban centres, much of the population (38%) is rural [17]. Nevertheless, most published studies on ART outcomes and HIVDR in South Africa have come from urban treatment cohorts or from provinces with primarily urban populations [10,15,18–26]. Rural HIV treatment programmes face different, and possibly greater, challenges such as: (1) longer travel distances to access health care facilities coupled with inadequate access to transportation; (2) limited access to virological monitoring; (3) increased stigma; and (4) significant human resource challenges [16,27–30]. Human resource constraints may impact on the ability to provide sufficient adherence counselling, and the latter has repeatedly been shown to account for the majority of treatment failures [3,29].

Very few studies have investigated whether treatment outcomes, and specifically HIVDR, are different in rural compared with urban areas. The aim of this study was therefore to describe and compare select clinical and HIVDR profiles of patients failing first-line HAART across two sites in South Africa – one urban and one rural. The urban site was a district hospital in central Pretoria (Tshwane District Hospital) and the rural site was located in northern KwaZulu-Natal (Hlabisa subdistrict). We also examined the accumulation of resistance mutations as a function of the duration of treatment failure.

Methods

The terms “urban” and “rural” were defined according to Urban Influence codes as described by Larson & Fleishman (2003) [31]. The urban group is from a large metropolitan statistical area of more than 1 million people, Pretoria in the Gauteng province. The rural group is from the Hlabisa subdistrict (1430 km^2) of the KwaZulu-Natal province, a geographical setting that is very heterogeneous in terms of population density, with 2–3000 people/km^2. The subdistrict is served by one district hospital and 17 primary health care clinics [13].

All patients were ≥ 18 years old, had started HAART after 2004 as part of the South African national HIV treatment plan and were treated according to the National Department of Health HIV guidelines (2004 and 2010) operative during this time. All patients had failed a first-line NNRTI-based HAART regimen as defined by at least one HIV-1 RNA [viral load (VL)] measurement of > 1000 HIV-1 RNA copies/mL plasma after at least 6 months of HAART. The duration of virological failure was estimated as the time from the date of the first VL > 1000 copies/mL to the date of genotyping. If there was a VL < 50 copies/mL between the above dates, the duration was then measured from the next VL > 1000 copies/mL. If all VL values were > 1000 copies/mL then the duration was taken as the time from baseline to the date of genotyping. Immunological failure was defined according to World Health Organization (WHO) criteria as: (1) a decline of CD4 T-cell count to lower than or equal to the baseline value; (2) a persistent CD4 T-cell count of < 100 cell/μL, or (3) a decline of ≥ 50% from the on-treatment peak value.

Genotypic drug resistance testing (DRT) became available to the urban group in 2008 as part of a drug resistance surveillance project. Samples were collected between the beginning of 2008 and the end of 2012. This

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was a doctor-based programme and patients were referred for DRT after two measurements of VL > 1000 copies/mL when interventions to improve adherence had failed to result in virological suppression and if the clinician was satisfied that all other obvious causes of treatment failure had been excluded. Clinicians sent three ethylenediaminetetraacetic acid (EDTA) tubes of blood to the Department of Immunology at the University of Pretoria together with the patient's history consisting of all available CD4 T-cell counts, VLs and HAART history.

DRT started in the rural group in December 2010 as part of the implementation of genotypic resistance testing in 17 primary health care clinics in the area [13,32] and samples were collected between the end of 2010 and the beginning of 2013. Details of the rural programme have been published previously [33]. In summary, routine HIV treatment, care and monitoring were delivered largely by nurses and HIV counsellors at each primary health care clinic. People with complications of treatment or evidence of treatment failure were referred to the medical officer who made weekly visits to each clinic. Patients were referred for DRT after one VL > 1000 copies/mL. Clinicians or nurses collected one EDTA tube of blood, which was sent to the Africa Centre virology laboratory, together with a detailed clinical history.

For HIV drug resistance genotyping, the rural group used the open access SATuRN Life Technology HIV drug resistance testing [34]. Briefly, the method involved generating cDNA using the Superscript III kit (Invitrogen Corporation, Carlsbad, CA, USA) and a gene-specific primer. This was followed by a nested polymerase chain reaction (PCR) using Platinum taq polymerase (Invitrogen Corporation) to amplify a 1315-bp polymerase (pol) fragment. The purified amplicons were sequenced using four bidirection primers covering the full protease gene and the first 300 codons of the reverse transcriptase (RT) gene. Sequencing electrophoresis was performed on a 3130 xL genetic analyzer (Applied Biosystems Inc., Foster City, Carlsbad, CA, USA). This method was developed in collaboration with Life Technologies (Applied Biosystems Inc.) as an affordable method to be implemented in resource-limited settings [35]. It has been validated against the commercial and Food and Drug Administration (FDA)-approved Viroseq genotyping method (Celera Diagnostics, Alameda, CA, USA) [36]. The SatuRN Life Technologies method has been validated with a panel of proficiency testing samples obtained from the French National Agencies for Research on AIDS and Viral Hepatitis (ANRS). The SATuRN Life Technologies and ViroSeq methods were 100% concordant in identifying all clinically important drug resistance-associated mutations [34]. In addition, the laboratory participates in an HIV-1 drug resistance genotyping proficiency testing programme from Quality Control for Molecular Diagnostics (QCMD, Glasgow, Scotland). Genotying for the urban group was performed using the commercial and FDA-approved Trugene HIV-1 genotyping kit (Siemens Healthcare Diagnostics, Tarrytown, NY, USA) as per the manufacturer's protocol. Sequences were assembled and manually edited using the DNA Workbench 5.7.1 software (CLC bio, Aarhus, Denmark).

The two groups followed the same protocol for sequence quality assessment and analysis, which has also been previously described [34]. Briefly, the quality of the sequences was assessed using the quality analysis tool and the HIVDB programme on the Stanford HIV database [37]. HIV subtyping was performed using the Rega HIV-1 subtyping tool version 3.0 [38]. In addition, phylogetic analysis was used to assess possible contamination. All sequence data were anonymously managed in a relational database, the SATuRN RegaDB [39].

Genotypic susceptibility scores (GSSs) were calculated for each antiretroviral agent using the Stanford HIVSeq algorithm version 6.0.5 [40] and a total score was then calculated for the standard second-line regimens. This was done to assess the impact of observed drug resistance mutations on the predicted effectiveness of standard second-line regimens. Total GSS for the standard second-line regimen was calculated depending on the patient's treatment history: for participants on stavudine (d4T) or zidovudine (ZDV) at the time of genotyping, GSS was calculated for a regimen of TDF, lamivudine (3TC) and lopinavir/ritonavir (LPV/r), while for those on TDF at the time of genotyping, GSS was calculated for a regimen of ZDV, 3TC and LPV/r. These standard second-line regimens were consistent with the recommendations in the 2013 South African national HIV treatment guidelines [41]. For the purposes of this analysis, a compromised second-line regimen was defined as GSS < 2. Data from the two groups were combined to assess the accumulation of resistance mutations for the various drug classes as a function of treatment failure.

The urban and rural group data were exported from SATuRN RegaDB into Excel 2013 files. Pivot tables with IF/AND and VLOOKUP functions were used to calculate variable categories while the SOLVER function, using the minimization of the square of the residuals, was used to fit specified nonlinear functions to the accumulated mutations data. Excel data were also exported to stata version 14 (Statacorp, College Station, TX, USA), using which descriptive statistics were calculated, and to statistix version 9, using which *P*-values were calculated for differences between variables. For count data, two-proportion tests were used and for continuous data Wilcoxon

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rank sum tests were used. Alpha (α) was set on the 95th percentile and a *P*-value ≤ 0.05 was considered significant.

The study was approved by the research ethics committees of the Faculty of Health Sciences at the University of Pretoria (46/2011) and the University of KwaZulu Natal (BF052/10). No personal participant information was entered in the database and all participants were allocated a unique identifying code. Results of the DRT were made available to the treating doctors and nurses in real time and support was given regarding selection of next regimens and access to newer medication, as needed.

Results

Demographic and clinical characteristics of the two patient groups are presented in Table 1. There was a large difference in the size of the groups: 492 patients in the rural group *vs*. 103 in the urban group. Patient proportions were not significantly different in terms of sex and age. In the urban group, the median CD4 T-cell count prior to ART initiation was significantly lower than that of the rural group (98 *vs*. 126 cells/μL, respectively; *P* = 0.05); however, the range was large in both groups.

At the time of genotyping, the differences in CD4 count and the proportion of patients with immunological failure in the two groups were not significant, but the median CD4 T-cell count was still very low (in the 130–140 cells/μL range) and over 41% of patients had immunological failure in both groups. Both groups reported VL values > 4.0 $\log_{10}$ copies/mL; however, the urban group had a higher median value than the rural group (4.18 *vs*. 4.08 $\log_{10}$ copies/mL, respectively; *P* = 0.03). The urban group had more intensive monitoring, as evidenced by the significantly larger median number of VL tests per year (three for the urban group *vs*. 1.4 for the rural group; *P* < 0.01). Consequently, the majority of patients in the urban group had < 6 months of virological treatment failure, while the majority of patients from the rural group had been failing for more than 24 months.

Table 2 shows the comparison of the HAART regimens, the number of patients with resistance mutations and GSSs in the two groups. Consistent with the duration of virological failure, the duration of treatment in the two institutions was significantly different. Initial ART regimens were essentially comparable, although a larger proportion of rural patients had been started on regimens consisting of d4T/3TC/EFV and TDF/3TC/EFV, while a larger proportion of urban patients had been started on ZDV-based regimens. Similarly, at genotyping, a significantly larger proportion of rural patients were still on d4T-containing regimens, while more urban patients were on ZDV-based regimens. Urban patients were more likely to have changed regimen, either in terms of a nucleos(t)

Table 1 Patient demographic and clinical characteristics compared

Characteristic	Africa centre (*n* = 492)	Pretoria (*n* = 103)	*P*-value
Sex			
Male	136 (27.7)	26 (27.4)	0.95
Female	355 (72.3)	69 (72.6)	
Age (years) [median (IQR)]	36 (31–42)	36 (31–40)	0.27
< 20 years	19 (3.9)	1 (1.1)	
20–29 years	90 (18.3)	19 (20.0)	
30–39 years	212 (43.2)	53 (55.8)	
40–49 years	105 (21.4)	19 (20.0)	
≥ 50 years	19 (3.9)	3 (3.2)	
CD4 count at entry (cells/mL) [median (IQR)]	126 (60–191)	98 (40–185)	0.05
< 50 cells/mL	98 (19.9)	30 (29.7)	
50–99 cells/mL	97 (19.7)	21 (20.8)	
100–149 cells/mL	94 (19.1)	12 (11.9)	
150–199 cells/mL	95 (19.3)	16 (15.8)	
200–249 cells/mL	36 (7.3)	12 (11.9)	
≥ 250 cells/mL	72 (14.6)	10 (9.9)	
CD4 count at genotyping (cells/mL) [median (IQR)]	138 (110–173)	131 (103–162)	0.12
< 50 cells/mL	51 (10.4)	17 (16.5)	
50–99 cells/mL	4 (0.8)	0 (0)	
100–149 cells/mL	237 (48.2)	53 (51.5)	
150–199 cells/mL	128 (26.0)	17 (16.5)	
200–249 cells/mL	31 (6.3)	9 (8.7)	
≥ 250 cells/mL	41 (8.3)	7 (6.8)	
Immunological failure at time of genotyping*	203 (41.3)	43 (41.7)	0.56
VL at time of genotyping (log copies/mL) [median (IQR)]	4.08 (3.08–4.48)	4.18 (3.19–5.11)	0.03
VLs per patient per year [median (IQR)]	1.4 (1.1–1.8)	3.0 (1.9–5.4)	< 0.01
Viral suppression			
Ever < 1000 copies/mL	344 (70.1)	78 (75.8)	0.37
Ever < 50 copies/mL	267 (54.4)	62 (60.2)	0.4
Duration of virological failure (months) [median (IQR)]**	20.0 (10.8–33.2)	8.2 (3.1–17.7)	< 0.01
< 6 months	48 (9.8)	38 (36.9)	< 0.01
6–12 months	84 (17.1)	26 (25.2)	0.07
13–24 months	168 (34.2)	26 (25.2)	0.1
> 24 months	192 (39.0)	13 (12.6)	< 0.01

Values are *n* (%), unless otherwise stated.
IQR, interquartile range; VL, HIV viral load.
*Immunological failure was defined according to World Health Organization (WHO) criteria: (i) a decline of CD4 count to lower than or equal to the baseline value; (ii) a persistent CD4 count of < 100 cells/μL; (iii) a decline of ≥ 50% from the on-treatment peak value.
**Virological failure duration was estimated as follows: (i) the duration of virological failure was taken as the time from the first VL > 1000 copies/mL to the date of genotyping; (ii) if there was a VL < 50 copies/mL between the above dates, the duration was then measured from the next VL > 1000 copies/mL; (iii) if all VL values were > 1000 copies/mL, then the duration was taken from the baseline to the date of genotyping.
P-values were obtained using either the Wilcoxon rank sum test or the two-proportion test.
HIV viral load tests were repeated at the time of genotyping in the urban group, while the rural group used the latest routine programme viral load, which was a median of 3 months prior to genotyping [32].

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Table 2 Comparison of antiretroviral treatment regimens, genotype susceptibility scores and number of resistance mutations

Characteristic	Africa centre	Pretoria	*P*-value
Duration of ART (months) [median (IQR)]	49.2 (36.7–61.0)	25.9 (14.2–41.5)	< 0.01
< 24 months	54 (11.0)	40 (43.5)	< 0.01
24–48 months	179 (36.4)	39 (42.4)	0.47
> 48 months	259 (52.6)	13 (14.1)	< 0.01
Initial ART regimen			
d4T/3TC/EFV	297 (60.4)	53 (52.0)	0.05
d4T/3TC/NVP	131 (26.6)	24 (23.5)	0.73
TDF/3TC/EFV	48 (9.8)	3 (3.1)	0.05
TDF/3TC/NVP	8 (1.6)	1 (1)	0.97
ZDV/3TC/EFV	5 (1.0)	11 (10.8)	< 0.01
ZDV/3TC/NVP	3 (0.6)	8 (7.8)	< 0.01
ddI/3TC/EFV		1 (1)	
Total	492 (100)	102 (100)	
ART regimen at time of genotyping			
d4T/3TC/EFV	218 (44.3)	19 (18.6)	< 0.01
d4T/3TC/NVP	109 (22.2)	10 (9.8)	< 0.01
TDF/3TC/EFV	99 (20.1)	12 (11.8)	0.07
TDF/3TC/NVP	22 (4.5)	7 (6.9)	0.44
ZDV/3TC/EFV	32 (6.5)	23 (22.6)	< 0.01
ZDV/3TC/NVP	12 (2.4)	16 (15.7)	< 0.01
ddI/3TC/EFV		12 (7.8)	
Total	492 (100)	102 (100)	
Patients with any substitutions*	362/492 (74.0)	87/102 (85.3)	< 0.01
Patients with NRTI substitutions	105 (21.3)	57 (56.4)	< 0.01
Patients with NNRTI substitutions	65 (13.2)	18 (17.8)	0.31
Patients with both NRTI and NNRTI substitutions	19 (4.0)	13 (11.3)	< 0.01
Time from ART start to first substitution (months) [median (IQR)]	20.0 (10.8–33.2)	13.0 (6.0–13.0)	< 0.01
Number of regimen changes [median (min–max)]	2 (1–4)	3 (1–6)	< 0.01
GSS for current regimen at genotyping [median (IQR)]	1.0 (0.5–1.0)	1.0 (1.0–2.0)	
< 2	409 (83.3)	53 (63.9)	< 0.01
≥ 2	82 (16.7)	30 (36.1)	< 0.01
Predicted second-line GSS [median (IQR)]	2.0 (2.0–2.0)	2.0 (2.0–3.0)	
< 2	58 (12.0)	14 (16.9)	0.27
≥ 2	433 (88.0)	69 (83.1)	0.27
No mutations (wild type)	55 (11.2)	37 (35.9)	< 0.01
NNRTI mutations	418 (84.4)	57 (55.3)	< 0.01
NRTI mutations	414 (83.6)	57 (55.3)	< 0.01
Any RT mutations			
< 2	82 (16.7)	39 (47.6)	< 0.01
2–3	255 (51.9)	28 (34.1)	0.01
4–6	62 (12.7)	3 (3.7)	0.03
> 6	92 (18.7)	12 (14.6)	0.54
TAMs	152 (30.9)	15 (14.6)	< 0.01
0	343 (69.7)	87 (84.5)	< 0.01
1–2	90 (18.3)	11 (10.7)	0.08
≥ 3	62 (12.6)	4 (3.9)	0.02
PI mutations	4 (0.8)	3 (2.9)	0.45

Values are *n* (%) unless otherwise stated.
ART, antiretroviral therapy; IQR, interquartile range; d4T, stavudine; 3TC, lamivudine; EFV, efavirenz; NVP, nevirapine; TDF, tenofovir; ZDV, zidovudine; ddI, didanosine; GSS, genotypic susceptibility score; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleos(t)ide reverse transcriptase inhibitor; RT, reverse transcriptase; TAM, thymidine analogue mutation; PI, protease inhibitor.
*All substitutions were for reasons of toxicity/intolerability and all occurred before drug resistance testing.
P-values were either the Wilcoxon rank sum test or two-proportion tests.

ide reverse transcriptase inhibitor (NRTI) substitution or both NRTI and NNRTI substitutions ($P < 0.01$). Urban patients also had a significantly shorter time before the first treatment change and had more regimen changes during the course of treatment ($P < 0.01$).

At genotyping, all patients, except one in the urban group who was infected with subtype B, were found to have HIV-1 subtype C infection. A significantly larger proportion of urban patients than rural patients had no HIVDR mutations detected, indicating wild-type virus (35.9% *vs.* 11.2%, respectively; $P < 0.01$). In addition, a larger proportion of urban patients had fewer than two RT mutations detected, while rural patients were more likely to have between two and six mutations. The proportion with more than six mutations was, however, comparable in the two groups (14.6% in the urban group *vs.* 18.7% in the rural group; $P = 0.54$). Rural patients were more likely than urban patients to have thymidine analogue mutations (TAMs) (30.9% *vs.* 14.6%, respectively; $P < 0.01$) as well as multiple (three or more) TAMs (12.6% *vs.* 3.9%, respectively; $P = 0.02$). The exact resistance mutations are depicted in Figure 1. The most common mutations in both groups were M184V, K103NS and V106AM. Interestingly, the GSSs for second-line regimens were essentially identical, with 88% of the rural group and 83.1% of the urban group having scores ≥ 2.

By combining the results of the two groups, we also assessed whether the duration of treatment failure was associated with the number of drug resistance mutations. Table 3 demonstrates a pattern of an increased number of NRTI mutations in patients failing for between 6 and 12 months compared with those failing for < 6 months (mean 1.4 *vs.* 1.1, respectively), and a further increase in patients failing for between 13 and 24 months (mean 1.8), after which time there was a slight decline (mean 1.6 in patients with failure for > 24 months). NNRTI mutations accumulated slightly more slowly than NRTI mutations in patients failing for < 6 months or between

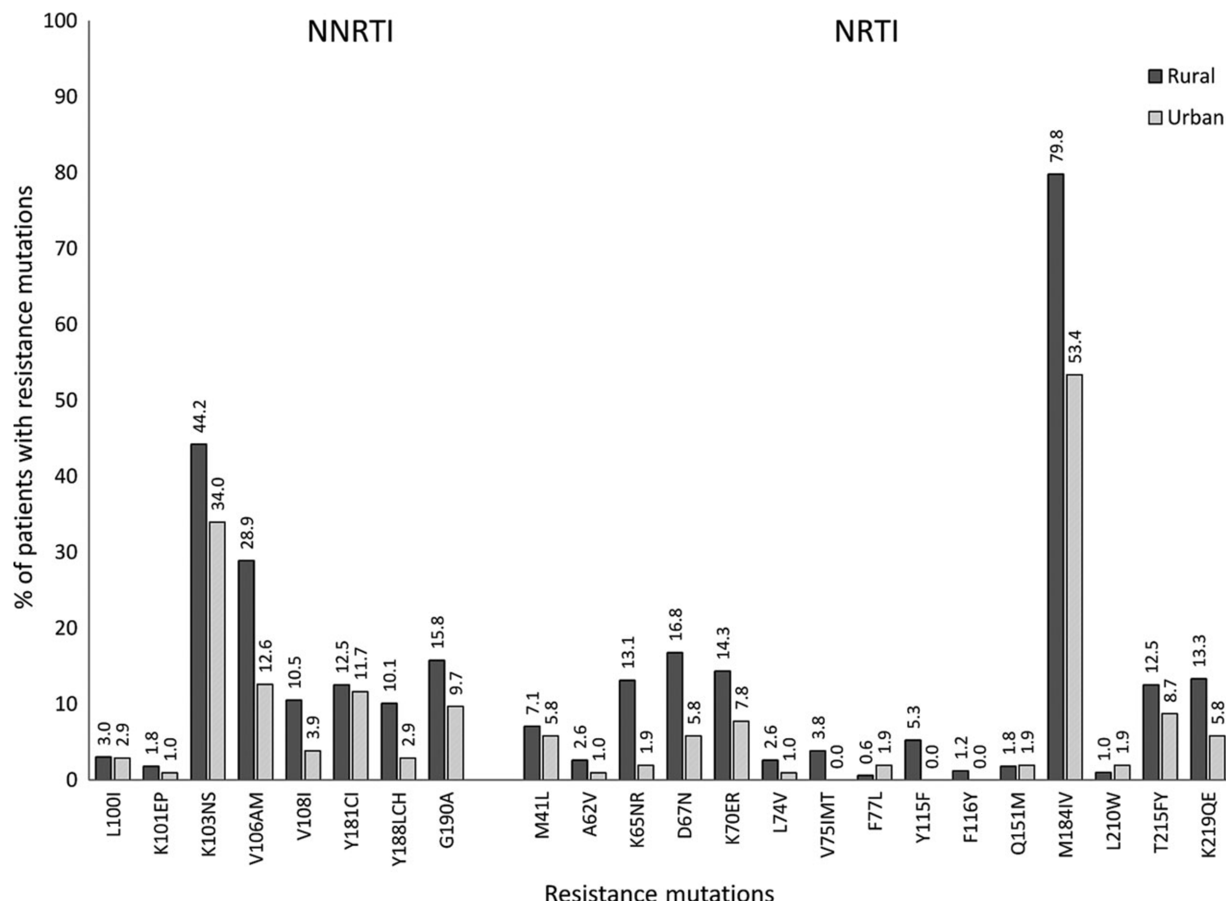

Fig. 1 The patterns of drug resistance mutation formation in the two cohorts. NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleos(t)ide reverse transcriptase inhibitor.

6 and 12 months (mean 1 *vs.* 1.1, respectively), while those failing for > 1 and > 2 years had means of 1.3 and 1.2, respectively. TAMs accumulated mostly after 1 year of failure (0.38 for failure < 6 months; 0.45 for failure 6–12 months; 0.85 for failure 13–24 months; and 0.81 for failure > 24 months). Figure 2 demonstrates a sigmoidal accumulation of mutations over failure duration. Using the minimization of the square of residuals, the following function provided a near ideal fit to the data for total mutations:

$$Y(\% \text{ of mutations}) = \frac{100}{1 + A.e^{-Bt}}$$

where $A = 12$, $B = 0.125$ and $t =$ the failure duration in months.

Given that the sigmoidal is also the integral of a logistic function, mutation accumulation would appear to follow a binary additive pattern over time. Analysis of the data did not reveal any relationship between the VL at the time of genotyping and the number of mutations (data not shown).

Discussion

To our knowledge, this is the first study to compare HIVDR profiles in patients failing first-line NNRTI-based HAART in an urban and a rural setting in South Africa. Urban patients were more aggressively managed than their rural counterparts: they had more therapy switches within a shorter duration of therapy, had more VL tests per annum and had been failing for a shorter time before being referred for DRT. The HIVDR results reflect these different approaches to monitoring and treatment, although causation cannot be claimed; there were significantly more urban patients with no resistance mutations and rural patients had higher percentages of NNRTI and NRTI mutations. The proportion of patients with no mutations detected on genotyping despite repeat VL testing and an adherence intervention in the urban group was higher than previously reported in the literature (36% *vs.* 19–30%, respectively) [19,20,23] and may be attributable to more intensive monitoring, coupled with the absence

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Table 3 All patient data grouped by categorical failure duration

Failure category (months)	GSS category < 2 *vs.* ≥ 2 [n (%)]	Predicted GSS to standard 2nd line regimen < 2 *vs.* ≥ 2 [n (%)]	NRTI substitution No *vs.* yes [n (%)]	NNRTI substitution No *vs.* yes [n (%)]	Both substitutions No *vs.* yes [n (%)]	BL VL (mean ± SD)	Genotype VL (mean ± SD)	Mutations: NNRTI [mean ± SD sum (%)]	Mutations: NRTI [mean ± SD sum (%)]	Mutations: PI [mean ± SD sum (%)]	Mutations: TAM [mean ± SD sum (%)]	Mutations: All [mean ± SD sum (%)]	*n*
< 6	58 (10.1)	9 (1.6)	54 (9.1)	71 (12.0)	12 (9.9)	3.891 ± 1.122	3.708 ± 1.232	1 ± 0.78	1.1 ± 1.1	0	0.38 ± 0.94	2.5 ± 2.3	86
	vs. 19 (3.3)	*vs.* 68 (11.9)	*vs.* 31 (5.2)	*vs.* 14 (2.4)	*vs.* 8 (6.6)			90 (41.5)	94 (43.3)	0	33 (15.2)	217 (10.5)	
6–12	80 (14.0)	6 (1.1)	83 (14.0)	96 (16.2)	21 (17.4)	4.165 ± 1.108	3.797 ± 1.33	1.1 ± 0.85	1.4 ± 1.3	0.02 ± 0.19	0.45 ± 0.91	3 ± 2.5	110
	vs. 25 (4.4)	*vs.* 99 (17.3)	*vs.* 26 (4.4)	*vs.* 13 (2.2)	*vs.* 8 (6.6)			126 (37.8)	156 (46.9)	2 (0.6)	49 (14.7)	333 (16.1)	
13–24	163 (28.4)	23 (4.0)	140 (23.6)	170 (28.7)	24 (19.8)	4.086 ± 1.068	3.818 ± 1.139	1.3 ± 0.73	1.8 ± 1.4	0.04 ± 0.31	0.85 ± 1.4	3.9 ± 2.8	194
	vs. 25 (4.4)	*vs.* 165 (28.8)	*vs.* 54 (9.1)	*vs.* 24 (4.1)	*vs.* 10 (8.3)			245 (32.1)	347 (45.5)	7 (0.9)	164 (21.5)	763 (37.0)	
> 24	161 (28.1)	34 (5.9)	154 (26.0)	173 (29.2)	32 (26.5)	4.437 ± 0.953	4.05 ± 1.114	1.2 ± 0.77	1.6 ± 1.4	0.029 ± 0.3	0.81 ±	3.7 ± 3	205
	vs. 42 (7.3)	*vs.* 169 (29.5)	*vs.* 5 (0.8)	*vs.* 32 (5.4)	*vs.* 6 (5.0)			247 (32.9)	331 (44.1)	6 (0.8)	1.3 166 (22.1)	750 (36.4)	
Total	462 (80.6)	72 (12.6)	431 (72.7)	510 (86.0)	89 (73.6)	4.202 ± 1.058	3.878 ± 1.186	1.2 ± 0.78	1.6 ± 1.4	0.03 ± 0.26	0.69 ± 1.2	3.5 ± 2.8	595
	vs. 111 (19.4)	*vs.* 501 (87.4)	*vs.* 162 (27.3)	*vs.* 83 (14.0)	*vs.* 32 (26.5)			708 (34.3)	928 (45.0)	15 (0.7)	412 (20.0)	2063 (100)	

Mutation percentages total towards the right.
GSS, genotypic susceptibility score; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; BL, baseline; VL, HIV viral load; PI, protease inhibitor; TAM, thymidine analogue mutation; SD, standard deviation; *vs.*, versus.

of skilled adherence counsellors and objective adherence measurement tools.

Although the individual percentages were different in the urban and rural environments, the overall patterns of resistance formation were similar. Taken together with the findings shown in Figure 1, the underlying trend is the accumulation of similar mutations with increasing duration of failure in both environments, with the rural group having accumulated more mutations as a result of the longer time spent with actively replicating virus while on HAART. This is consistent with other studies that observed that prolonged HAART failure leads to the accumulation of drug resistance [42,43].

The more aggressive management of urban patients might be attributable to the different management approaches, easier access to information, increased access to treatments, and better human resource allocation. Differential management might also be attributable to the ease of referring patients to tertiary services and requesting blood investigations, as a consequence of the proximity of the referral hospital and laboratory in the urban setting. A Ugandan study assessing paediatric responses to ART similarly found that urban children were more likely to be switched to second-line regimens than their rural counterparts [44].

It is interesting to note that, despite more significant HIVDR in the rural group, the two groups had comparable predicted GSSs for second-line therapy. This is in keeping with the effectiveness of second-line protease inhibitor-based therapy in suppressing VL, at least in the short term, even in the absence of a fully active NRTI backbone [24,45,46]. When the two groups were combined and mutations assessed *vs.* the duration of failure, it was apparent that a sigmoidal accumulation of mutations occurred in the population. This observation has value in that a per mutation “cost value”, be it health or financial, might be associated with this accumulation, giving the public health practitioner an ability to plan for the future of the particular patient population. Further, a higher proportion of patients in the urban setting received ZDV *vs.* TDF at the time of genotyping, with the converse in the rural setting. TAMs are expected to accumulate more on ZDV and d4T (potentially causing cross-resistance to TDF) and less so on TDF [47]. This may in part explain why GSSs were similar in the urban and rural groups despite longer average failure duration in the latter.

This study has limitations. It did not assess the larger groups receiving ART at the different sites, or all the patients failing ART in the clinics, but rather focused on the subgroups that had been referred for DRT; hence, there are no clinic denominators or mortality statistics.

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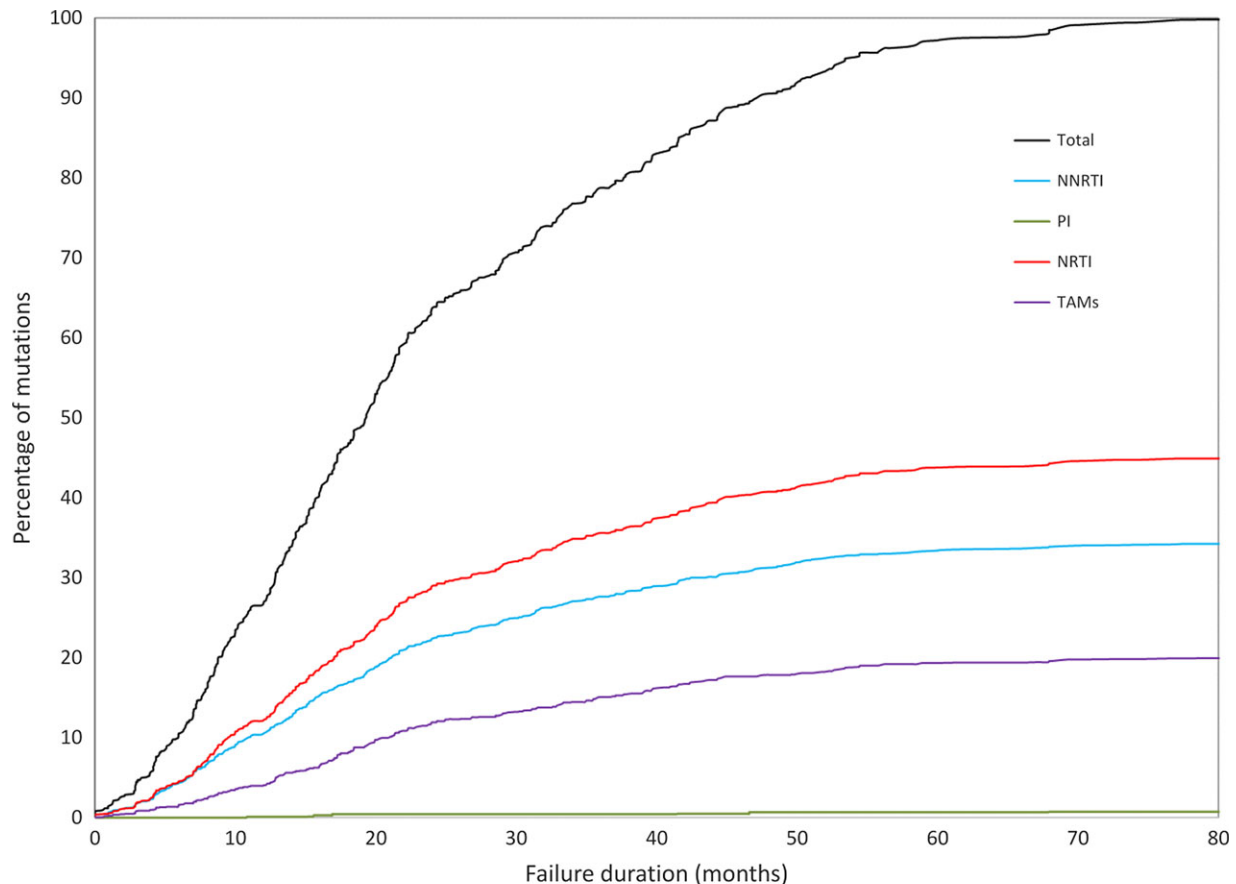

Fig. 2 Accumulation of drug resistance mutations in all patients in different antiretroviral drug classes over time. The pattern of mutation accumulation followed a near ideal fit to an asymptotic sigmoidal. NNRTI, nonnucleos(t)ide reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; TAM, thymidine analogue mutation.

Variables that might have impacted on the development of HIVDR, such as adherence and use of traditional medication or alcohol, were not routinely collected and could thus not be compared between the groups. There was also no active follow-up of patients in the research studies after change to second-line regimens and so we do not have data on actual treatment responses on second-line ART. There were some methodological differences in terms of referral pathways and eligibility criteria at the two sites, but these have been taken into account. In addition, DRT methodology differed between the urban and rural centres, but this is not expected to make a difference to the DRT results [48]. Despite these limitations, this study presents one of the largest data sets of HIVDR results in sub-Saharan Africa and addresses an important, yet understudied, area of HIV research.

In conclusion, this study showed that urban and rural patients received significantly different HIV care, with urban patients being more aggressively managed. This translated into fewer patients with evidence of HIVDR and, specifically, severe HIVDR at the time of genotyping and supports the notion that increased duration on failing HAART increases drug resistance. Despite these differences, the expected response to second-line ART remained comparable between the groups. These findings have important implications for management of patients in large treatment programmes where financial constraints may limit the feasibility of intensive patient monitoring. It seems that a balance can be obtained between limited virological monitoring on the one hand and prevention of severe, treatment-limiting HIVDR on the other. Future studies might investigate the association between the rate and type of mutations accumulated and the cumulative (integrated) VL, that is, the “viral fitness”, during the treatment failure duration.

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References

1 Gulick RM, Mellors JW, Havlir D *et al.* Simultaneous vs sequential initiation of therapy with Indinavir, Zidovudine, and Lamivudine for HIV-1 infection. 100-week follow-up. *JAMA* 1998; **280**: 35–41.

2 Chen TK, Aldrovandi GM. Review of antiretroviral drug resistance. *Pediatr Infect Dis J* 2008; **27**: 749–752.

3 Zdanowicz MM. The pharmacology of HIV drug resistance. *Am J Pharm Educ* 2006; **70**: 100.

4 Miller CD, Crain J, Tran B, Patel N. Rilpivirine: a new addition to the anti-HIV-1 armamentarium. *Drugs Today (Barc)* 2011; **47**: 5–15.

5 Hamers RL, Kityo C, Lange JMA, Rinke de Wit TF, Mugyenyi P. Global threat from drug resistant HIV in sub-Saharan Africa. *BMJ* 2012; **344**: e4159.

6 Nkengasong JN, Adje-Toure C, Weidle PJ. HIV antiretroviral drug resistance in Africa. *AIDS Rev* 2004; **6**: 4–12.

7 Abraha A, Nankya IL, Gibson R *et al.* CCR5- and CXCR4-tropic subtype C human immunodeficiency virus type 1 isolates have a lower level of pathogenic fitness than other dominant group M subtypes: implications for the epidemic. *J Virol* 2009; **83**: 5592–5605.

8 Tang M, Rhee S-Y, 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**: S70–S76.

9 Brenner BG, Oliveira M, Doualla-Bell F *et al.* HIV-1 subtype C viruses rapidly develop K65R resistance to tenofovir in cell culture. *AIDS* 2006; **20**: F9–F13.

10 Sunpath H, Wu B, Gordon M *et al.* High rate of K65R for antiretroviral therapy-naive patients with subtype C HIV infection failing a tenofovir-containing first-line regimen. *AIDS* 2012; **26**: 1679–1684.

11 Brenner B, Turner D, Oliviera M *et al.* A V106M mutation in HIV-1 clade C viruses exposed to efavirenz confers cross-resistance to non-nucleoside reverse transcriptase inhibitors. *AIDS* 2003; **17**: F1–F5.

12 Li JF, Lipscomb JT, Wei X *et al.* Detection of low-level K65R variants in nucleoside reverse transcriptase inhibitor–naive chronic and acute HIV-1 subtype C infections. *J Infect Dis* 2011; **203**: 798–802.

13 Lessells RJ, Stott KE, Manasa J *et al.* Implementing antiretroviral resistance testing in a primary health care HIV treatment programme in rural KwaZulu-Natal, South Africa: early experiences, achievements and challenges. *BMC Health Serv Res* 2014; **14**: 116.

14 WHO. Global update on HIV treatment 2013: results, impact and opportunities. Available at: www.who.int/hiv/data/global_treatment_report_presentation-2013.pdf (accessed 15 November 2015).

15 El-Khatib Z, Delong AK, Katzenstein D *et al.* Drug resistance patterns and virus re-suppression among HIV-1 subtype C infected patients receiving non-nucleoside reverse transcriptase inhibitors in South Africa. *J AIDS Clin Res* 2011; **2**: 117.

16 Hamers RL, Derdelinckx I, van Vugt M *et al.* The status of HIV-1 resistance to antiretroviral drugs in sub-Saharan Africa. *Antivir Ther* 2008; **13**: 625–639.

17 Mahajan S. *Economics of South African Townships: Special Focus on Diepsloot. A World Bank study.* Washington, DC, World Bank Group, 2014. http://documents.worldbank.org/curated/en/2014/08/19925268/economics-south-african-townships-special-focus-diepsloot.

18 Mutevedzi PC, Lessles RJ, Heller T, Bärnighausen T, Cooke GS, Newell ML. Scale-up of a decentralized HIV treatment programme in rural KwaZulu-Natal, South Africa: does rapid expansion affect patient outcomes? *Bull World Health Organ* 2010; **88**: 593–600.

19 Orrell C, Walensky RP, Losina E, Pitt J, Freedberg KA, Wood R. HIV type-1 clade C resistance genotypes in treatment-naive patients and after first virological failure in a large community antiretroviral therapy programme. *Antivir Ther* 2009; **14**: 523–531.

20 Wallis CL, Papathanasopolous MA, Fox M *et al.* Low rates of nucleoside reverse transcriptase inhibitor resistance in a well-monitored cohort in South Africa on antiretroviral therapy. *Antivir Ther* 2012; **17**: 313–320.

21 Marconi VC, Sunpath H, Lu Z *et al.* Prevalence of HIV-1 drug resistance after failure of a first highly active antiretroviral therapy regimen in KwaZulu Natal, South Africa. *Clin Infect Dis* 2008; **46**: 1589–1597.

22 Hoffmann CJ, Charalambous S, Sim J *et al.* Viremia, resuppression, and time to resistance in human immunodeficiency virus (HIV) subtype C during first-line

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antiretroviral therapy in South Africa. *Clin Infect Dis* 2009; **49**: 1928–1935.

23 Hamers RL, Sigaloff KC, Wensing AM *et al.* Patterns of HIV-1 drug resistance after first-line antiretroviral therapy (ART) failure in 6 sub-Saharan African countries: implications for second-line ART strategies. *Clin Infect Dis* 2012; **54**: 1660–1669.

24 Sigaloff KC, Ramatsebe T, Viana R, de Wit TF, Wallis CL, Stevens WS. Accumulation of HIV drug resistance mutations in patients failing first-line antiretroviral treatment in South Africa. *AIDS Res Hum Retroviruses* 2012; **28**: 171–175.

25 El-Khatib Z, Ekstrom AM, Ledwaba J *et al.* Viremia and drug resistance among HIV-1 patients on antiretroviral treatment: a cross-sectional study in Soweto, South Africa. *AIDS* 2010; **24**: 1679–1687.

26 van Zyl GU, van der Merwe L, Claassen M, Zeier M, Preiser W. Antiretroviral resistance patterns and factors associated with resistance in adult patients failing NNRTI-based regimens in the Western Cape, South Africa. *J Med Virol* 2011; **83**: 1764–1769.

27 Heckman TG, Somlai AM, Peters J *et al.* Barriers to care among persons living with HIV/AIDS in urban and rural areas. *AIDS Care* 1998; **10**: 365–375.

28 Wilson DP, Kahn J, Blower SM. Predicting the epidemiological impact of antiretroviral allocation strategies, in KwaZulu-Natal: the effect of the urban-rural divide. *Proc Natl Acad Sci USA* 2006; **103**: 14228–14233.

29 Bangsberg DR. Preventing HIV antiretroviral resistance through better monitoring of treatment adherence. *J Infect Dis* 2008; **197**: S272–S278.

30 Cooke R, Couper I, Versteeg M. Human Resources for Rural Health. In: Padarath A, English R, eds. *South African Health Review 2011*. Durban, Health Systems Trust, 2011: 101. http://www.hst.org.za/publications/south-african-health-review-2011.

31 Larson SL, Fleishman JA. Rural-urban differences in usual source of care and ambulatory service use: analyses of national data using Urban Influence Codes. *Med Care* 2003; **41**: III65–III74.

32 Manasa J, Lessles RJ, Skingsley A *et al.* High levels of acquired drug resistance in adult patients failing first-line antiretroviral therapy in a rural HIV treatment programme in Kwa-Zulu Natal, South Africa. *PLoS One* 2013; **8**: e72152.

33 Houlihan C, Bland R, Mutevedzi P *et al.* Cohort profile: Hlabisa HIV treatment and care programme. *Int J Epidemiol* 2011; **40**: 318–326.

34 Manasa J, Danaviah S, Pillay S *et al.* An affordable HIV-1 drug resistance monitoring method for resource limited settings. *J Vis Exp* 2014; **85**: 51242.

35 Life_Technologies. Life technologies and SATuRN collaborate to increase access to hiv testing in Africa. Available at: http://www.prnewswire.com/news-releases/life-technologies-and-saturn-collaborate-to-increase-access-to-hiv-testing-in-africa-163526506.html (2012) (accessed 29 March 2016).

36 Eshleman SH, Crutcher G, Petrauskene O *et al.* Sensitivity and specificity of the ViroSeq human immunodeficiency virus type 1 (HIV-1):genotyping system for detection of HIV-1 drug resistance mutations by use of an ABI PRISM 3100 genetic analyzer. *J Clin Microbiol* 2005; **43**: 813–817.

37 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.

38 Pineda-Peña AC, 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**: 337–348.

39 Manasa J, Lessells R, Rossouw T *et al.* Southern African Treatment Resistance Network (SATuRN) RegaDB HIV drug resistance and clinical management database: supporting patient management, surveillance and research in southern Africa. *Database (Oxford)* 2014; **2014**: bat082.

40 Liu TF, Shafer RW. Web resources for HIV type 1 genotypic-resistance test interpretation. *Clin Infect Dis* 2006; **42**: 1608–1618.

41 National Department of Health. The South African antiretroviral treatment guidelines. 2013. Available at: http://www.sahivsoc.org/upload/documents/2013%20ART%20Treatment%20Guidelines%20Final%2025%20March%202013%20corrected.pdf (accessed 10 October 2015).

42 Hosseinipour MC, van Oosterhout JJ, 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**: 1127–1134.

43 Sigaloff KC, Hamers RL, Wallis CL *et al.* Second-line antiretroviral treatment successfully resuppresses drug-resistant HIV-1 after first-line failure: prospective cohort in Sub-Saharan Africa. *J Infect Dis* 2012; **205**: 1739–1744.

44 Musiime V, Kayiwa J, Kiconco M *et al.* Response to antiretroviral therapy of HIV type 1-infectedchildren in urban and rural settings of Uganda. *AIDS Res Hum Retroviruses* 2012; **28**: 1647–1657.

45 Hosseinipour MC, Kumwenda JJ, Weigel R *et al.* Second-line treatment in the Malawi antiretroviral programme: high early mortality, but good outcomes in survivors, despite extensive drug resistance at baseline. *HIV Med* 2010; **11**: 510–518.

46 Paton NI, Kityo C, Hoppe A *et al.* Assessment of second-line antiretroviral regimens for HIV therapy in Africa. *N Engl J Med* 2014; **371**: 234–247.

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47 Miller MD, Margot N, Lu B *et al*. Genotypic and phenotypic predictors of the magnitude of response to tenofovir disoproxil fumarate treatment in antiretroviral-experienced patients. *J Infect Dis* 2004; **2004** : 837–846.

48 Li J, Jiang Y, Lü C, Wang J, Yao J. Evaluation of the consistency of three methods for testing HIV-1 genotype drug resistance. *Zhonghua Yu Fang Yi Xue Za Zhi* 2013; **47**: 1050–1055.

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