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Original article

Provincial and national prevalence estimates of transmitted HIV-1 drug resistance in South Africa measured using two WHO-recommended methods

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ABSTRACT

Introduction: Sentinel surveillance of transmitted HIV drug resistance (TDR) among recently infected populations within a country was recommended by the World Health Organization from 2004-2015.

Methods: Serum specimens collected as part of the 2010, 2011 and 2012 National Antenatal Sentinel HIV Prevalence Surveys were used to estimate provincial and national TDR prevalence in South Africa.

Results: Moderate (5-15%) levels of transmitted non-nucleoside reverse transcriptase inhibitors (NNRTI) drug class resistance were detected in 3 of 5 provinces surveyed in 2010 and 2011 (Eastern Cape, Free State and KwaZulu-Natal). Inclusion of all 9 of South Africa's provinces in the 2012 survey enabled calculation of a national TDR point prevalence estimate: TDR to the NNRTI drug class was 5.4% (95% CI 3.7 – 7.8%), with K103N and V106M being the most frequently detected mutations. TDR estimates for the nucleoside reverse transcriptase inhibitor (NRTI) drug class were 1.1% (95% CI 0.5 – 2.4%) and 0.6% (95% CI 0.1 – 1.6%) for protease inhibitors (PI).

Conclusions: These data provide national TDR estimates for South Africa in 2012 and indicate that levels of TDR were low to moderate for the NNRTI drug class and low for NRTIs and PIs in the population surveyed.

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INTRODUCTION

South Africa has adopted a public-health approach to antiretroviral therapy (ART) provision, using standardized regimens recommended by the World Health Organization (WHO). In 2012, 6.4 million (12.4%) South Africans were living with HIV. By 2017, the number living with HIV had increased to 7.1 million (12.6%) [1]. Begun in 2004, the national HIV treatment program had enrolled 1.7 million people by 2012 [2], and by 2016 approximately 3.4 million people were receiving ART [3], making South Africa's ART program the largest in the world. In 2010, national treatment guidelines introduced tenofovir plus lamivudine or emtracitabine in combination with efavirenz or nevirapine as the recommended first-line combination antiretroviral therapy for all patients with CD4 cell counts <200 cells/μl [4]. Studies from South Africa published during initial years of ART expansion showed levels of HIV drug resistance among patients failing first-line regimens to be >80% with mutations reflecting the drug regimens used [5–11].

Despite high prevalence estimates of drug resistant HIV documented among patients failing first-line therapies in various resource-limited countries in the early to mid-2000s [12,13] levels of HIV drug resistance amongst recently infected and antiretroviral (ARV) drug naive individuals, so called transmitted drug resistance (TDR), remained low prior to 2010 [13,14]. A global analysis performed by the World Health Organization (WHO) in 2012 signaled a slow but steady annual increase in TDR prevalence to the NNRTI drug class, particularly in sub-Saharan Africa [13]. In countries where routine HIV drug resistance testing is not available, WHO recommended from 2004-2015 that levels of TDR be monitored using remnant specimens, from people likely to have been recently infected such as those attending antenatal clinics or Voluntary Counselling and Testing centers [15] [16]. Initially, a minimum-resource method using truncated sequential sampling was proposed [17,18]. The method yielded prevalence classifications of TDR using ≤47 drug resistance genotypes from individuals consecutively enrolled and identified as HIV infected. TDR was classified as low (<5%), moderate (5-15%) or high (>15%) depending on the number of sampled individuals with detected drug resistant genotypes. This minimum resource method is referred to as the "threshold survey". The recommended survey population and required epidemiological criteria used to maximize the likelihood of inclusion of recently infected individuals were age <25 years (or <21 years, where feasible) and no previous pregnancies if female. If available, a first HIV-risk defining event within the past three years, self-reported ARV drug naiveté, and CD4 >500 cells/μl were also used to maximize inclusion of individuals likely to be recently infected. Threshold surveys were most frequently embedded into pre-existing sentinel surveys designed to estimate the prevalence of HIV in populations such as pregnant women. Modifications to WHO recommendations, made in 2012, did not alter TDR survey inclusion criteria, but deemphasized reporting of prevalence classifications generated by the threshold survey method in favor of calculating point prevalence estimates of TDR. In addition, rather than using genotypes from ≤47 consecutively enrolled survey participants to classify TDR prevalence, WHO encouraged genotyping and analysis of all available specimens from individuals meeting epidemiological inclusion criteria [16].

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The National Antenatal Sentinel HIV Prevalence Survey (ANSUR) was conducted annually in South Africa, across all 9 provinces and 52 health districts, using a cross-sectional unlinked and anonymous design to estimate the HIV sero-prevalence among first-time attendee pregnant women aged 15 - 49 years in public health facilities [19–21]. Between 2010 and 2012, the surveys recruited and tested approximately 33,000 women annually, with HIV prevalence estimates plateauing around 30%. Results from TDR threshold surveys conducted in Gauteng Province between 2002 - 2004 and 2005 - 2009 showed low levels of TDR to all drug classes [22]; however, levels of TDR to NNRTIs in KwaZulu-Natal province increased to moderate (5-15%) in 2009 [23]. Additional studies by others from KwaZulu-Natal province have shown similar increases in the prevalence of HIV drug resistance amongst ARV drug naïve individuals [24] [25].

In this study, we performed a retrospective analysis of HIV drug resistance using ANSUR specimens collected in 2010, 2011, and 2012, a period during which the number of South Africans receiving ART increased from about 730,000 to 1.8 million [26]. Our analysis provides the first national prevalence estimates of TDR for all three drug classes in South Africa and demonstrates that levels of NNRTI increased, while those to nucleoside reverse transcriptase inhibitors (NRTI) and protease inhibitors (PI) remained low.

METHODS

Specimen collection and HIV testing

All participants were from the national ANSUR surveys performed in 2010, 2011 and 2012. Anonymised demographic data were recorded on standardized collection forms. All individuals selected for this TDR sub-analysis met the WHO-required inclusion criteria of primigravid females aged <21 years. Serum specimens were collected during routine antenatal care and tested anonymously for HIV infection by ELISA (Abbot AxSYM System for HIV-1 and HIV-2, Abbott Laboratories, USA). Ethical approval for drug resistance testing was obtained from the University of the Witwatersrand Human Research Ethics Committee.

The 2010 analysis focused on surveys from Gauteng and KwaZulu-Natal, as both provinces had higher HIV prevalence and population densities. These provinces had also been surveyed in previous years [23]. In 2011, TDR estimates were performed using specimens from Gauteng and KwaZulu-Natal provinces and expanded to include data from three additional provinces: Eastern Cape, Free State and Western Cape. Eastern Cape and Free State provinces both have high HIV burdens, whilst the Western Cape was the first province to provide ART through state programs. Following WHO's updated TDR survey recommendations [16], the 2012 survey was expanded to included analysis of specimens from North West, Northern Cape, Mpumalanga and Limpopo, bringing the total number of provinces surveyed

to nine. Genotypic results from all nine provinces were aggregated and a weighted national point prevalence estimate of TDR was calculated.

Genotyping

HIV drug resistance genotyping was performed on remnant HIV-infected serum specimens, which had been stored at -70°C following serological testing. Sequencing of the HIV-1 *pol* gene was done using a validated in-house assay [22], and sequence quality was assured following WHO guidance [27]. The presence of TDR in a specimen was defined by the presence of one or more HIV surveillance drug resistance mutations (SDRM) per WHO's 2009 SDRM list [28] embedded into the Stanford Calibrated Population Resistance algorithm Version 4.1beta [29] with the following caveat: Based on a revised threshold for polymorphisms of 0.2%, M46I and L protease mutations were removed from the SDRM list when performing analysis to be in keeping with revisions introduced at the time of the 2012 WHO HIVDR global report [13]. Exclusion of M46I/L effectively increases the specificity of the analysis although at the potential expense of reduced sensitivity. By reducing the prevalence threshold to differentiate a SDRM from a polymorphism, the proportion of false positives is reduced and the positive predictive value of the detection of PI resistance is increased.

Following WHO TDR threshold survey methods [17,18], a maximum of 47 sequences from each survey were ordered consecutively according to date of collection. TDR prevalence classifications were assigned as follows: if one or more drug class-specific SDRM was present in < 2 sequence, a prevalence of <5% was assigned for that specific drug class. If ≥2 sequences had one or more drug class-specific SDRM, a prevalence classification of 5 – 15% was assigned for that drug class, or >15% if ≥8 sequences had one or more drug class-specific SDRM.

Drug level testing

In 2012, to minimize inclusion of individuals with prior ARV drug exposure, drug level testing (DLT) was performed using a semi-quantitative liquid chromatography tandem mass spectrometry in order to screen serum samples for the presence of the following antiretroviral drugs: zidovudine (AZT), efavirenz (EFV), emtracitabine (FTC), lopinavir (LPV), nevirapine (NVP), and tenofovir (TDF). Specificity was ensured by the use of deuterated internal standards for all analytes. DLT was performed at the Division of Clinical Pathology, University of Cape Town. In the 2012 analyses (threshold survey and point prevalence methods), specimens with detectable levels of any ARV drug were excluded, as they were deemed to be from individuals receiving ART.

Statistical analysis

The 2010 and 2011 surveys used the WHO threshold survey method to generate drug class specific prevalence classifications [17,18]. In the 2012 survey, drug class-specific point prevalence estimates with Wilson 95% confidence intervals were constructed for each province. Weighting per province was

performed using the proportion of HIV positive primigravidae women aged <21 in each province recruited into the survey (regardless of genotype availability). After weighting, a national point prevalence and modified Wilson confidence intervals were calculated. All analyses were performed using STATA v12 [30].

RESULTS

Provincial TDR classifications from the 2010 ANSUR survey

In order to obtain ≤47 HIVDR genotypes for analysis, specimens from 76 consecutively enrolled participants from the Gauteng ANSUR survey were genotyped. The genotyping PCR amplification rate was 62% (Table 1). Within these 47 consecutively sequenced genotypes, one contained the NNRTI SDRM, K103N. No PI or NRTI SDRMs were detected. TDR in Gauteng in 2010 was therefore classified as <5% for all three drug classes.

From the KwaZulu-Natal specimen set, 64 consecutive specimens were genotyped to obtain 47 sequences (amplification success rate 73%). Within these 47 sequences, four had SDRMS: three had NRTI SDRMs (M184V, T69D, and M41L) and two contained NNRTI SDRMs (K103NS + G190A and K101E). No PI SDRM was detected. The prevalence classification of TDR for KwaZulu-Natal in 2010 was between 5 and 15% (moderate) for both the NRTI and NNRTI drug classes and <5% (low) for the PI drug class.

Provincial TDR classifications from the 2011 ANSUR survey with expansion to additional provinces

Using the same criteria and analysis of consecutively obtained genotypes as described for the 2010 survey, a total of 457 ANSUR specimens were genotyped: Eastern Cape (n=55), Free State (n=62), Gauteng (n=86), KwaZulu-Natal (n=192), and Western Cape (n=62) in order to obtain ≤47 sequences per province (Table 1). Genotypes from the Eastern Cape, Free State, and KwaZulu-Natal each had two sequences with NNRTI SDRM detected, yielding a provincial TDR prevalence classification of 5-15% for the NNRTI drug class in each of the three provinces. All five provinces had NRTI and PI TDR estimates of <5%, as did Gauteng and Western Cape for the NNRTI drug class. Overall, the K103N mutation was detected in five sequences and the G190A mutation in two sequences. Other SDRM detected were Y188L, P225H and Y181C (NNRTI), D67N and K219N (NRTI), and I47A (PI).

Estimating a national weighted TDR prevalence using the 2012 ANSUR survey

The ANSUR survey in 2012 enrolled 34,260 pregnant women of whom 29.5% were HIV-infected (Table 2a). Of these, 886 met the eligibility criteria for age and parity, of which 789 had sufficient volume of remnant specimen for further testing. There was a large variation in the numbers of specimens collected from each province that generally reflected the prevalence of HIV in each region. Thus, the largest

number of specimens were obtained from KwaZulu-Natal (n=304), while the lowest number was from the Northern Cape (n=7). A total of 551 specimens were successfully genotyped; of these, 3 were excluded because of unresolved phylogenetic linkage as were a further 16 that tested positive for the presence of antiretroviral drug(s). A final set of 532 sequences were then analyzed for the presence of SDRMs (Table 2b). For the NRTI and PI drug classes, the national point prevalence estimates of TDR were 1.1% (95% CI 0.5 – 2.4%) and 0.6% (95% CI 0.1 – 0.6%), respectively, with almost all provincial prevalence estimates below 5%. For the NNRTI drug class, the national prevalence estimate was 5.4% (95% CI 3.7 – 7.8%), with four of nine provinces having prevalence estimates greater than 5%.

A total of 36 specimens from eight of nine provinces had any detected SDRM (no resistance was detected in the seven specimens from the Northern Cape). 28 of 36 sequences had NNRTI SDRMs; the K103N mutation was detected in 20 (71%) and the V106M mutation in 8 (29%). Other NNRTI SDRMs detected included K101E (n=4), G190A (n=1) and Y188L (n=1). Of the NRTI SDRMs, M184IV was the most commonly detected (three sequences). Other detected NRTISDRMs were: M41L, L74V, K70R, and K219R.The following PI SDRMs were detected in three sequences: I85V, I47V..

Analysis of the 2012 ANSUR data using the threshold survey method

Sufficient numbers of sequences were obtained to analyze the first consecutive 47 sequences from Eastern Cape, Free State, Gauteng and KwaZulu-Natal only (Table 1). Using this approach, Eastern Cape and KwaZulu-Natal had low levels (<5%) of NNRTI TDR whilst Free State and Gauteng had moderate levels (5-15%).

Antiretroviral Drug level testing of 2012 ANSUR specimens

All 36 specimens with one or more detected SDRM and a randomly selected subset of 77 specimens that were not amplifiable were tested for the presence of antiretroviral drugs. The 16 specimens that were positive for drug were removed from TDR analyses: four were specimens with detectable SDRMS, and 12 were specimens that could not be amplified by PCR. Of the four specimens with detectable drug levels and SDRMs, all had detectable levels of NVP. Of the 12 specimens that failed to amplify by PCR, four were had detected levels of AZT, one had detected levels of NVP, three had both detected level of EFV and TDF, and three had detected levels of NVP and TDF.

DISCUSSION

Surveillance of transmitted HIV drug resistance spanning a three year period in South Africa was performed using two different methods following WHO recommendations. This study made use of remnant specimens obtained from young women epidemiologically predicted to be recently infected, collected through annual antenatal surveys conducted by the South African National Department of Health between 2010 and 2012. Using the point prevalence method, our results show that at the time of

the 2012 survey, the prevalence of transmitted NNRTI drug resistance was moderate (5-15%) in four of 9 provinces, with national estimates of 5.4% (3.7 – 7.8%). Levels of NRTI and PI resistance remained <5% in all provinces suggesting limited transmission of multi-class HIV drug resistance in South Africa at least up until 2012.

Using the minimum resource threshold survey method ($n \leq 47$ sequences) over the 2010 – 2012 period, the prevalence classification for the NNRTI drug class increased to moderate in 2012 in Gauteng Province and were moderate in KwaZulu-Natal in 2010 and 2011 but decreased to < 5% in 2012. Levels of TDR were moderate in Eastern Cape in 2011 and low in 2012; levels of TDR in Free State remained moderate in both years. We were unable to classify the prevalence of TDR in Western Cape in 2012 using this approach due to insufficient specimen numbers.

Two WHO-recommended methods were used in this analysis, spanning a period where the recommended approach was modified. The threshold survey method originally proposed by WHO is significantly less resource-intensive and not nationally representative and is intended to generate an alert that resistance transmission is occurring in a region warranting further investigation including possibly nationally representative surveillance and or possible public health interventions to minimize transmission of drug-resistant HIV. The updated 2012 point-prevalence method proposed analyzing a minimum of 200 sequences per area under surveillance. Data from four provinces in the 2012 survey were analyzed using both methods and are shown to be consistent, in that moderate NNRTI TDR prevalence classifications were made in in Free State and Gauteng, while low levels were detected in Eastern Cape and KwaZulu-Natal.

The detection of antiretroviral drugs in 16 specimens from young women epidemiologically predicted to be recently infected suggests that epidemiological criteria alone may be insufficient and unreliable in predicting ARV drug naiveté, in a country such as South Africa with a mature epidemic and a high level of access to ART. The detection of drug amongst analytes with detected SDRMs further suggests that mutations detected in these sequences could have been acquired by virtue of drug selective pressure rather than having been transmitted at time of initial infection. Selection of participants for studies of TDR should therefore include the use of testing algorithms for recent infection to exclude women who are chronically infected, or inclusion of routine DLT and/or thorough and reliable investigations as to whether the participant is accessing ART.

Despite its interest, the value of TDR surveillance beyond documenting transmission of drug resistant virus may be of limited public health and ART program utility. Moreover, TDR surveys are becoming more difficult to perform as universal treatment of HIV infection at diagnosis has become standard of care. Only prospective incidence cohorts are able to yield reliable TDR prevalence estimated, but are costly to perform and require the enrolment of large numbers of participants. For these reasons and to provide data reflective of the drug resistance prevalence in the populations initiating first-line ART, TDR surveys are no longer recommended by WHO [31]. TDR surveys have been replaced by nationally

representative surveys of pretreatment HIVDR amongst people starting or restarting ART regardless of prior exposure to ARV drugs [32].

The NNRTI mutations K103N and V106M were the most frequently detected SDRM in of South Africa's TDR surveys. These mutations confer high levels of resistance to the NNRTIs EFV and NVP, and are frequently detected in persons failing standard first-line combination antiretroviral therapy in South Africa [11,33–35], indicating that these mutations are stable and readily transmitted in the population. The frequency of the most commonly observed SDRMs in this analyses are similar to those reported globally, where mutations at positions 103, 101, 181 and 184 of reverse transcriptase are most commonly detected [13]. The higher proportion of V106M in specimens from southern Africa is associated with the high proportion of HIV-1 subtype C virus circulating in this region and the high use of efavirenz [36]. Notably, only one sequence from 2012 showed the K65R mutation, associated with TDF-based regimen failure.

Our Sanger-based sequencing assay does not detect low abundance drug resistance mutations present below ~ 15%. Thus, we may have underestimated the true prevalence of TDR by not identifying virus with SDRM present at less than 10 - 20%. However, evidence from currently published studies is not sufficiently consistent to define a threshold of clinical significance of low abundance NNRTI-resistant variants; in addition available evidence regarding NRTI and PI low abundance mutations suggests that they are not clinically relevant.

In conclusion, this analysis provides the first national prevalence estimates of TDR in South Africa and indicates that levels of TDR were moderate for the NNRTI drug class but low for NRTI and PI. Despite these low levels, the incidence of TDR in South Africa is expected to increase as ART coverage increases, mirroring trends observed in global incidence of TDR between 2003 and 2010 [14]. Notably, a third of all WHO-recommended TDR surveys conducted globally reported moderate levels of transmitted resistance to at least one drug class between 2007 and 2012 [13]. Thus, while our data are somewhat expected, they highlight a need for new approaches in the management of the HIV epidemic. As outlined in the Global Action Plan on HIVDR published by WHO in 2017 [32], efforts should be intensified at country and global levels to identify gaps in ART service delivery, which may be predictive of emergence of HIVDR, and corrective action taken to minimize unnecessary emergence and transmission of drug resistant virus. In addition, routine nationally representative surveillance of pretreatment resistance in people starting ART and acquired drug resistance in treated populations should be performed in South African to inform selection of optimal first- and second-line ART regimens in-country and to monitor trends in HIV drug resistance over time.

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Disclosures

The authors have no conflict of interests to declare.

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Table 1: Transmitted drug resistance (TDR) ANSUR surveys analyzed using the minimum resource method from 2010-2012

ANSUR survey year	Province	Number of Sequences	Amplification rate	Number with PI SDRM	Number with NRTI SDRM	Number with NNRTI SDRM	PI TDR classification	NRTI TDR classification	NNRTI TDR classification
2010	GP	47	62%			K103N	<5%	<5%	<5%
	KZN	47	76%		M184V T69D M41L	K103N+G 190A K101E	<5%	**5-15%**	**5-15%**
2011	EC	47	85%			K103N G190A	<5%	<5%	**5-15%**
	FS	47	76%	I47A		Y188L+P 225H K103N+G 190A	<5%	<5%	**5-15%**
	GP	47	55%		D67N+K2 19N	Y181C	<5%	<5%	<5%
	KZN	47	24%			K103N (n=2)	<5%	<5%	**5-15%**
	WC	47	76%			K103N	<5%	<5%	<5%
2012	EC	47	92%			K101E	<5%	<5%	<5%
	FS	47	77%			K103N+V 106M K103N (n=2)	<5%	<5%	**5-15%**
	GP	47	72%	I85V		K103N (n=5)	<5%	<5%	**5-15%**
	KZN	47	65%			K103N+V 106M	<5%	<5%	<5%

SDRM = Surveillance Drug Resistance Mutations (2009); PI = Protease Inhibitor; NRTI = Nucleoside Reverse Transcriptase inhibitors; NNRTI = Non-nucleoside Reverse Transcriptase Inhibitors
GP = Gauteng Province; KZN = KwaZulu-Natal Province; EC = Eastern Cape Province; FS = Free State Province; WC =Western Cape Province;

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Table 2a: Details of ANSUR specimens eligible for inclusion in 2012 TDR survey.

Province	Total number of specimens collected in ANSUR 2012	Provincial contribution to ANSUR	Provincial HIV prevalence estimate	Number of eligible specimens	Number of specimens not available for testing	Number removed - unresolved phylogenetic linkage	Number removed - DLT positive
Eastern Cape	4625	13.5%	29.1%	127	10	3	2
Free State	2325	6.8%	32.0%	75	3	0	1
Gauteng	6862	20.0%	29.9%	121	25	0	2
KwaZulu Natal	7011	20.5%	37.4%	327	15	0	8
Limpopo	3579	10.4%	22.3%	56	14	0	0
Mpumalanga	2201	6.4%	35.6%	70	8	0	3
North West	2457	7.2%	29.7%	61	14	0	0
Northern Cape	1190	3.5%	17.8%	14	7	0	0
Western Cape	4010	11.7%	16.9%	35	1	0	0
National	**34260**	**100.0%**	**29.5%**	**886**	**97**	**3**	**16**

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Table 2b: Transmitted drug resistance (TDR) prevalence estimates from the 2012 ANSUR survey performed in all 9 provinces

Province	Final number of specimens included in TDR analysis	Number of specimens amplifiable by genotyping PCR	Genotyping amplification rate	Number of sequences with PI mutations	PI Point Prevalence (95% CI)	Number of sequences with NRTI mutations	NRTI Point Prevalence (95% CI)	Number of sequences with NNRTI SDRM	NNRTI Point Prevalence
Eastern Cape	112	99	88.4%	0	0% (0 - 3.7)	0	0% (0 - 3.7)	3	3% (1.0 - 8.5)
Free State	71	54	76.1%	0	0% (0 - 6.6)	1	1.9% (0.3 - 9.8)	4	**7.4% (2.9 - 17.6)**
Gauteng	94	65	69.1%	1	1.5% (0.3 - 8.2)	0	0% (0 - 5.6)	6	**9.2% (4.3 - 18.7)**
KwaZulu Natal	304	196	64.5%	0	0% (0 - 1.9)	4	2% (0.8 - 5.1)	8	4.1% (2.1 - 7.8)
Limpopo	42	20	47.6%	0	0% (0 - 16.1)	0	0% (0 - 16.1)	2	**10% (2.8 - 30.1)**
Mpumalanga	59	45	76.3%	0	0% (0 - 7.9)	1	2.2% (0.4 - 11.6)	2	4.4% (1.2 - 14.8)
North West	47	21	44.7%	1	4.8% (0.8 – 22.7)	0	0% (0 - 15.5)	1	4.8% (0.8 - 22.7)
Northern Cape	7	4	57.1%	0	0% (0 - 49.0)	0	0% (0 - 49.0)	0	0% (0 - 49.0)
Western Cape	34	28	82.4%	0	0% (0 - 12.1)	0	0% (0 - 12.1)	2	**7.1% (2.0 - 22.6)**
National	**770**	**532**	**69.1%**	**3**	**0.5% (0.1 – 2.2)**	**6**	**1.1% (0.5 - 2.4)**	**28**	**5.4% (3.7 - 7.8)**

SDRM = Surveillance Drug Resistance Mutations (2009); PI = Protease Inhibitor; NRTI = Nucleoside Reverse Transcriptase inhibitors; NNRTI = Non-nucleoside Reverse Transcriptase Inhibitors

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Table 3: Transmitted HIV surveillance drug resistance mutations detected in the 2012 ANSUR survey.

Province	PI SDRM *	NRTI SDRM	NNRTI SDRM
Eastern Cape			K101E (n=3)
Free State		M41L	K103N + V106M (n=2) K103N (n=2)
Gauteng	I85V		K103N (n=6)
KwaZulu-Natal		M184I L74V M184V + K219R M184V	K103N (n=4) V106M G190A V106M + Y188L K103N + V106M
Limpopo			K103N + V106M V106M
Mpumalanga		K70R	K103N (n=2)
North West	I47V		K103N
Western Cape			K101E K103N + V106M

SDRM = Surveillance Drug Resistance Mutations (2009); PI = Protease Inhibitor; NRTI = Nucleoside Reverse Transcriptase inhibitors; NNRTI = Non-nucleoside Reverse Transcriptase Inhibitors. * In keeping with WHO 2012 global analysis on TDR, we have used the WHO 2009 SDRM list excluding mutations M46I and L. This guidance is based on a revised threshold of 0.2%, M46I and L. This effectively increases the specificity of the analysis although at the potential expense of reduced sensitivity. By reducing the prevalence threshold to differentiate a mutation from a polymorphism, the proportion of false-positives is likely to be reduced and the positive predictive value of the detection of PI resistance is likely to increase accordingly.