SURVEILLANCE OF TRANSMITTED HIV-1 DRUG RESISTANCE IN FIVE PROVINCES IN SOUTH AFRICA IN 2011

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Introduction

As part of its strategy for surveillance of HIV-1 drug resistance in resource-limited settings, the World Health Organization (WHO) recommends surveillance for transmitted drug resistance (TDR) among individuals assumed to be recently infected, such as pregnant women.[1] The proposed method analyses sequences of ≤47 remnant specimens from individuals consecutively identified as HIV-infected in order to categorize TDR as low (<5%), moderate (5-15%) or high (>15%).[2] In 2012, the WHO published a global HIV drug resistance report detailing increasing levels of transmitted resistance to non-nucleoside reverse transcriptase inhibitors (NNRTI) particularly in Africa. This increase was attributed to expanded antiretroviral therapy coverage.[3]

The Centre for HIV and STI, National Institute for Communicable Diseases (NICD), has been performing TDR surveys in pregnant women since 2002, using specimens collected as part of the annual antenatal survey (ANSUR) conducted by the National Department of Health. Previous reports in the November 2012 NICD Bulletin show that while TDR remained low (<5%) to all drug classes in Gauteng, moderate (5-15%) rates of transmitted NNRTI drug resistance were detected in KwaZulu-Natal in 2009 and 2010.[4,5]

In this report an updated dataset with results from the analysis of specimens collected in the 2011 ANSUR survey from Gauteng (GP) and KwaZulu-Natal (KZN) is given. In addition, the first analyses of data from the Orange Free State (OFS) and Eastern Cape (EC) provinces are presented. Previously, specimens collected in the Western Cape (WC) province in 2006 were successfully analyzed whilst specimens from a 2007 survey were also tested but with poor polymerase chain reaction (PCR) amplification rates leading to non-classifiable results. The DNA samples of specimens from a WC 2011 survey were successfully amplified and are also included in this analysis (table 1).

Methods

Specimen collection and testing

All participants were from the GP, KZN, OFS, EC and WC ANSUR surveys conducted in 2011. HIV-1 positive specimens from primigravid women age <21 years or <25 years (if required) were selected for genotypic analysis.[2] Genotypic resistance was defined as the presence of resistance mutations using the Stanford Calibrated Population Resistance (CPR) algorithm Version 6.0.[6,7] Sequences were ordered according to date of collection and prevalence classification assigned as per the recommended WHO method.[2] If no surveillance drug resistance mutations (SDRM) were found within the first 34 specimens, prevalence was classified as <5%. If resistance was detected, then 47 sequences were evaluated and if the number of sequences with relevant resistance mutations was between 2 or 8, the prevalence of TDR was classified as 5-15%. Ethical approval for drug resistance testing was obtained from the University of the Witwatersrand Human Research Ethics Committee.

Results

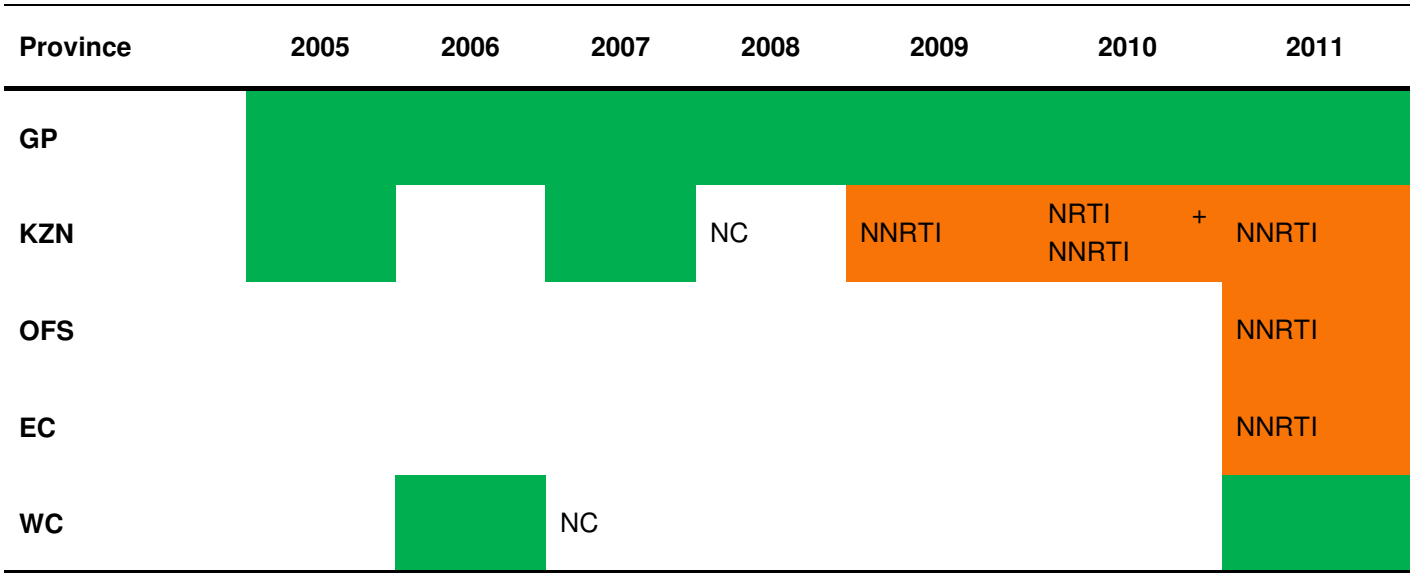
Classification of Threshold Survey (TS) sequence data

Around 100 specimens were collected each from the GP, WC, EC and OFS surveys, while 245 specimens were collected from the KZN 2011 survey due to previously poor PCR amplification rates. The TDR prevalence rates for 2011 by province are:

- GP2011: 2 sequences contained SDRM. One specimen had the M46I protease mutation, and a second contained the nucleoside reverse transcriptase inhibitor (NRTI) mutations D67N and K219N in addition to the NNRTI mutation Y181C. As the number of sequences with relevant resistance mutations to each drug class was one, the prevalence of TDR was low (<5%) for all drug classes.

- KZN2011: 2 specimens contained the NNRTI mutation K103N. The TDR prevalence rate for this province was low for the protease inhibitor (PI) and NRTI drug classes and 5-15% (moderate) for the NNRTI drug class.
- OFS2011: 2 specimens contained NNRTI mutations (Y188L and P225H in 1 sequence, and K103N and G190A in the second). One sequence contained the PI mutation I47A. The TDR rates for this province are <5% for PI and NRTI and 5-15% (moderate) for NNRTI.
- EC2011: 2 specimens contained NNRTI mutations (K103N and G190A). The TDR prevalence rate for this province was low for PI and NRTI, and moderate for NNRTI.
- WC2011: 1 specimen contained K103N, consequently the TDR rate was <5% (low) for all drug classes.

Table 1: Prevalence classification of transmitted drug resistance (TDR) in selected provinces of South Africa as per the WHO recommended method of using annual antenatal survey (ANSUR) specimens, 2005 – 2011.



Green: Low (<5%) prevalence classification of HIV TDR; Orange: moderate (5-15%) prevalence classification of TDR. NC: not classifiable. GP = Gauteng, KZN = KwaZulu-Natal, OFS = Orange Free State, EC = Eastern Cape, WC = Western Cape. NRTI = nucleoside reverse transcriptase inhibitor, NNRTI = non-nucleoside reverse transcriptase inhibitor.

Modifications to survey sampling strategy and data analysis

Following concerns in the survey design regarding the small number of specimens analyzed and the possibility that the analysis was over-estimating TDR levels, the WHO recently modified the TDR survey analysis sampling plan and strategy to collect and sequence all available remnant specimens from primagravid patients age <25; preferably <22 years, and to determine a point prevalence with 95% confidence intervals to estimate levels of TDR in a country. The data from 2011 was analyzed according to the new modifications (table 2).

In all five provinces the point prevalence TDR estimates were <5% for both the NRTI and NNRTI drug classes. However, due to the relatively low number of specimens analyzed, the confidence intervals were wide and reached the upper limit of ~10%. The point prevalence estimates for NNRTI TDR in KZN, OFS, EC and WC were higher than for GP and for the NRTI drug class, consistent with previous analysis using the previous method.

Discussion

In Gauteng province the levels of transmitted resistance continued to remain low (<5%) for all drug classes. However, levels of transmitted resistance were shown to be moderate (5-15%) for the NNRTI drug class in KZN, OFS and EC. Whilst calculating point prevalence by province has produced figures that are lower than

previously estimated, the confidence intervals indicate that the prevalence of TDR in all five provinces should be classified as low to moderate.

These surveys are limited by sub-optimal PCR amplification rates of specimens tested, probably due to inadequate storage of the serum specimens leading to sub-optimal preservation of viral RNA which is used for resistance testing. Although rates of PCR amplification have improved in recent years, particular care and attention to the preservation of specimens for resistance testing is encouraged.

The data presented here suggest that transmission of NNRTI resistant viruses is occurring in a number of provinces in South Africa. However, these data must be treated with caution and ongoing vigilance is required. Surveys such as these need to be interpreted in conjunction with systematic assessment of the antiretroviral therapy (ART) delivery program in order to minimize the selection of drug resistant viruses and subsequent transmission to newly infected individuals.

Moving forward, this analysis needs to be expanded to all provinces in South Africa. Plans are in place to sequence specimens collected from across the country in order to provide national and provincial TDR prevalence estimates.

Acknowledgements

The National Department of Health of South Africa is thanked for providing specimens from the ANSUR surveys for this study. Dr Sylvia Bertagnolio and Dr Michael Jordan of the WHO are thanked for assisting with data analysis and prevalence classification. This work was funded by the NICD, NHLS, and the National Institute for Allergy and Infectious Disease (NIAID): K23 AI074423-05.

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Table 2: Transmitted drug resistance (TDR) sequence data by province, South Africa, from the 2011 ANSUR surveys analyzed using previous and new WHO recommended methods.

Province	# sequences with NRTI mutations	# sequences with NNRTI mutations	NRTI threshold level	NNRTI threshold level	# sequences with NRTI mutations	# sequences with NNRTI mutations	Mutations detected	NRTI Point prevalence (95%CI)	NNRTI Point Prevalence (95%CI)
GP	1/47	1/47	<5%	<5%	1/54	1/54	D67N+K219N+ Y181C	1.9% (0.3 – 10.1%)	1.9% (0.3 - 10.1%)
KZN	0/47	2/47	<5%	5-15%	0/62	2/62	K103N; K103N	0	3.2% (0.9 – 11.0%)
OFS	0/47	2/47	<5%	5-15%	0/67	2/67	Y188L+P225H; K103N+G190A	0	3.0% (0.8 – 10.3%)
EC	0/47	2/47	<5%	5-15%	1/85	3/85	K103N; G190A; K65R+K103N	1.5% (0.3 – 8.0%)	3.5% (1.2 – 9.9%)
WC	0/47	1/47	<5%	<5%	2/79	3/79	K103N; M41L; K103N; K65R+Y181C	2.6% (0.7 – 9.0%)	3.9% (1.3 – 10.9%)
Prevalence classification using the previous denominator of 47 specimens: if the number of sequences with relevant resistance mutations is between 2 or 8, the prevalence of TDR is classified as 5-15%.					**Prevalence classification using the new, modified approach to sequence as many specimens as possible and estimate the point prevalence with 95% confidence intervals.**				

GP = Gauteng, KZN = KwaZulu-Natal, OFS = Orange Free State, EC = Eastern Cape, WC = Western Cape. = nucleoside reverse transcriptase inhibitor, NNRTI = non-nucleoside reverse transcriptase inhibitor.

Table 1: Provisional number of laboratory confirmed cases of diseases under surveillance reported to the NICD, South Africa, for the corresponding periods 1 January - 30 September 2012/2013*

Disease/Organism	1 Jan to 30 Sep, year	EC	FS	GA	KZ	LP	MP	NC	NW	WC	South Africa
Anthrax	2012	0	0	0	0	0	0	0	0	0	0
	2013	0	0	0	0	0	0	0	0	0	0
Botulism	2012	0	0	0	0	0	0	0	0	0	0
	2013	0	0	0	0	0	0	0	0	0	0
Cryptococcus spp.	2012	892	254	1579	1531	152	305	56	248	506	5523
	2013	563	194	1571	1363	111	266	40	202	483	4793
Haemophilus influenzae, invasive disease, all serotypes	2012	29	15	83	36	1	9	4	5	63	245
	2013	19	13	92	38	1	10	4	3	82	262
Haemophilus influenzae, invasive disease, < 5 years											
Serotype b	2012	2	5	14	3	1	3	2	2	9	41
	2013	3	1	9	4	0	0	0	0	4	21
Serotypes a,c,d,e,f	2012	1	0	3	0	0	1	0	0	5	10
	2013	0	1	5	0	0	0	1	0	6	13
Non-typeable (unencapsulated)	2012	0	1	18	3	0	0	0	0	7	29
	2013	0	1	11	2	0	0	1	1	14	30
No isolate available for serotyping	2012	6	2	9	5	0	3	1	1	4	31
	2013	1	4	24	9	0	6	1	0	11	56
Measles	2012	0	1	3	5	1	0	0	1	1	12
	2013	1	0	0	1	0	0	0	1	0	3
Neisseria meningitidis, invasive disease	2012	31	9	66	20	2	3	0	7	37	175
	2013	30	11	48	32	2	3	2	4	37	169
Novel Influenza A virus infections	2012	0	0	0	0	0	0	0	0	0	0
	2013	0	0	0	0	0	0	0	0	0	0
Plague	2012	0	0	0	0	0	0	0	0	0	0
	2013	0	0	0	0	0	0	0	0	0	0
Rabies	2012	1	1	0	4	3	1	0	0	0	10
	2013	0	2	0	1	3	1	0	0	0	7
Salmonella spp. (not typhi), invasive disease	2012	29	13	250	96	4	23	10	5	82	512
	2013	32	15	215	98	5	28	5	4	117	519
Salmonella spp. (not typhi), isolate from non-sterile site	2012	153	24	461	192	8	49	13	11	269	1180
	2013	154	58	734	232	9	103	13	42	398	1743
Salmonella typhi	2012	2	0	16	9	0	3	0	0	12	42
	2013	1	1	21	10	0	10	0	1	12	56
Shigella dysenteriae 1	2012	0	0	0	0	0	0	0	0	0	0
	2013	0	0	0	0	0	0	0	0	0	0
Shigella spp. (Non Sd1)	2012	193	43	447	171	3	19	18	5	309	1208
	2013	196	64	525	221	12	55	12	23	222	1330
Streptococcus pneumoniae, invasive disease, all ages	2012	236	164	1040	449	53	123	33	104	320	2522
	2013	223	132	742	349	42	86	62	90	352	2078
Streptococcus pneumoniae, invasive disease, < 5 years	2012	43	25	185	71	4	13	4	15	38	398
	2013	33	28	173	52	5	9	3	27	51	381
Vibrio cholerae O1	2012	0	0	0	0	0	0	0	0	0	0
	2013	0	0	0	0	1	0	0	0	0	1
Viral Haemorrhagic Fever (VHF)											
Crimean Congo Haemorrhagic Fever (CCHF)	2012	0	0	0	0	0	0	0	0	0	0
	2013	0	2	0	0	0	2	0	1	0	5
Other VHF (not CCHF)	2012	0	0	0	0	0	0	0	0	0	0
	2013	0	0	0	0	0	0	0	0	0	0

Footnotes

*Numbers are for cases of all ages unless otherwise specified. Data presented are provisional cases reported to date and are updated from figures reported in previous bulletins.
Provinces of South Africa: EC – Eastern Cape, FS – Free State, GA – Gauteng, KZ – KwaZulu-Natal, LP – Limpopo, MP – Mpumalanga, NC – Northern Cape, NW – North West, WC – Western Cape

0 = no cases reported

Table 2: Provisional laboratory indicators for NHLS and NICD, South Africa, for the corresponding periods 1 January - 30 September 2012/2013*

Programme and Indicator	1 Jan to 30 Sep, year	EC	FS	GA	KZ	LP	MP	NC	NW	WC	South Africa
Acute Flaccid Paralysis Surveillance											
Cases < 15 years of age from whom specimens received	2012	49	17	46	53	32	36	3	16	22	274
	2013	44	13	46	58	36	26	5	20	30	278

Footnotes

*Numbers are for all ages unless otherwise specified. Data presented are provisional numbers reported to date and are updated from figures reported in previous bulletins.

Provinces of South Africa: EC – Eastern Cape, FS – Free State, GA – Gauteng, KZ – KwaZulu-Natal, LP – Limpopo, MP – Mpumalanga, NC – Northern Cape, NW – North West, WC – Western Cape

Monitoring for the presence of polio in a country is based on AFP (acute flaccid paralysis) surveillance – the hallmark clinical expression of paralytic poliomyelitis. The clinical case definition of AFP is an acute onset of flaccid paralysis or paresis in any child under 15 years of age. AFP is a statutory notifiable disease and requires that 2 adequate stool specimens are taken as soon as possible, 24 to 48 hours apart, but within 14 days after onset of paralysis, for isolation and characterisation of polio virus. The differential diagnosis of AFP is wide, the most common cause of which is Guillain-Barre Syndrome. The incidence of AFP in a population has been studied in a number of developing countries and WHO have determined, as a result of these studies, that the criterion for adequate surveillance of AFP is 2 cases per 100 000 population of children less than 15 years of age (it was formerly 1 per 100,000 but this was thought to be inadequately sensitive).

The Communicable Diseases Surveillance Bulletin is published by the National Institute for Communicable Diseases (NICD) of the National Health Laboratory Services (NHLS), Private Bag X4, Sandringham, 2131, Johannesburg, South Africa.

Suggested citation: [Authors' names or National Institute for Communicable Diseases (if no author)]. [Article title]. Communicable Diseases Surveillance Bulletin 2013; 11(3): [page numbers].