

Antiretroviral drug resistance surveillance among drug-naïve HIV-1-infected individuals in Gauteng Province, South Africa in 2002 and 2004

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Background: Surveillance for transmitted HIV-1 drug resistance was conducted among drug-naïve HIV-1-infected pregnant women in South Africa, where single-dose nevirapine has been in use since 2001 and a national antiretroviral treatment programme started in 2004.

Methods: All subjects were from the Gauteng Province and were part of the 2002 and 2004 annual antenatal HIV seroprevalence survey conducted by the South African National Department of Health. All subjects met the inclusion criteria as set out by the World Health Organisation guidelines for HIV-1 transmitted drug resistance surveillance (women <22 years of age and in first pregnancy). Genotyping was performed on viral RNA by sequencing the protease and reverse transcriptase genes. Samples were also tested for the K103N mutation using a highly sensitive allele-specific real-time PCR assay (AS-PCR).

Results: Of 128 eligible participants from 2002, 65 (51%) samples were successfully amplified. None of them had evidence of resistance mutations by genotyping or by AS-PCR. Of 117 eligible participants from 2004, 48 (41%) samples were successfully amplified. Of these, one had T69D and one had the K70R resistance mutation, to give a total of 2/48 (4.2%) participants with evidence of resistance mutations by genotyping. One sample that was wild-type by genotyping was positive for K103N by AS-PCR. All samples clustered phylogenetically with HIV-1 subtype C, the predominant subtype circulating in South Africa.

Conclusions: Using the threshold survey, resistance prevalence overall and for each drug class in 2002 and 2004 was <5% for the Gauteng province of South Africa. The detection of a low frequency of resistance mutations in the 2004 survey suggests that surveillance should be conducted annually among untreated populations to determine if this increases with time.

Introduction

The transmission of drug-resistant HIV-1 variants is being increasingly reported in countries where antiretroviral therapy has been in use for some time, in particular the United States and Europe but also in developing countries, such as Brazil [1–3]. In South Africa, a country with an estimated 5.7 million HIV-infected individuals and almost 1,000 AIDS deaths every day [4], a Comprehensive HIV and AIDS Care, Management and Treatment Plan was initiated in April 2004. The first line regimen in use is stavudine, lamivudine and efavirenz or nevirapine with Kaletra[®] being used among infants and children. At the end of September 2006, there were an estimated 235,000 people (10% of them children) receiving antiretroviral therapy through this programme; an additional 80,000 people were being treated in the private

health care sector, where a variety of regimens are in use [5].

A national programme for the prevention of mother-to-child transmission (pMTCT) of HIV-1 was started in September 2001, which provides single-dose nevirapine (sd-nevirapine) to women at delivery and infants at birth [5]. Nevirapine is widely prescribed in resource-poor settings for this purpose as it is safe, affordable and easy to administer [6]. Since its inception, over 300,000 women and infants are estimated to have received sd-nevirapine through this programme. Although this drug has shown efficacy and continues to be used, the rapid selection of resistant variants has caused concern with calls for alternative and more effective regimens to replace sd-nevirapine. By standard genotyping, ~40% of South African women have been

found to harbour resistant variants shortly after receiving sd-nevirapine [7], although this percentage near doubles when more sensitive assays for the resistant variants K103N and Y181C are used [8–10].

The annual antenatal survey (ANSUR) conducted by the National Department of Health is an anonymous, unlinked cross-sectional survey which estimates HIV prevalence using blood samples taken from pregnant women attending public health sector antenatal clinics across all nine provinces in South Africa. This survey provides the best available estimates of HIV infection in the country [11]. Data show that HIV-1 prevalence has increased dramatically since 1991 from <1% to 30% in 2005. HIV-1 prevalence is highest among women in the 25–29 year age group and differs by province. Among women <20 years of age, which constitute ~20% of the ~16,000 samples collected, prevalence appears to have been stable at ~16% for the past 3 years [11]. As this group represents one of the target groups for World Health Organization (WHO) recommended surveillance, we initiated a retrospective HIV Drug Resistance Threshold Assessment Survey using the ANSUR samples [12].

Methods

All subjects were from the Gauteng Province and were part of the 2002 and 2004 ANSUR (Tables 1 and 2). In 2004, a total of 16,064 women at 399 clinics within South Africa participated in the survey. Of these 3,133 were from 68 ANC sites in the province of Gauteng with an HIV-1 prevalence of 32.4%. Similar data was not available for the 2002 ANSUR. The WHO guidelines for inclusion in the HIV Threshold Assessment Surveys for transmitted drug resistance are women <22 years of age and in first pregnancy [12]. A total of 128 eligible participants were selected from the 2002 ANSUR and 117 eligible participants were selected from the 2004 ANSUR.

Sample collection and HIV testing

Serum samples were collected and tested anonymously for HIV-1 antibodies by ELISA. HIV resistance genotyping was performed on HIV-positive serum samples stored at -70°C following serological testing. Ethical clearance for performing drug resistance testing on these samples was obtained from the University of the Witwatersrand Committee for Research on Human Subjects (Medical).

Genotyping

Sequencing of the HIV-1 *pol* gene was conducted using an in-house assay that has been certified by the Virology Quality Assessment Program (VQA) on three recent proficiency panels. Briefly, viral RNA was isolated from

plasma using the MagNa Pure automated system (Roche Diagnostics, Indianapolis, IN, USA), and a 1.7 kb fragment spanning the *pol* gene was amplified by nested PCR using the Thermoscript™ RT-PCR System. The first round PCR primers were G25REV (5'GCAA-GAGTTTTGGCTGAAGCAATGAG3') and IN3 (5'TCTATVCCATCTAAAAATAGTACTTTCCT-GATTCC3'). The second round primers were AV150 (5'GTGGAAAGGAAGGACACCAAATGAAAG3') and PolM4 (5'CTATTAGCTGCCCCATCTACATA3'). Amplification conditions for the first and second round of PCR were identical: 1 cycle at 94°C for 2 min, 10 cycles at 94°C for 10 s, 50°C for 30 s and 68°C for 2 min; 25 cycles at 94°C for 15 s, 50°C for 30 s, 68°C for 2 min plus a 20 s cycle elongation for each successive cycle with a final step at 68°C for 7 min. PCR products were sequenced (codons 1–99 of the protease and codons 1–350 of the reverse transcriptase) by using BigDyeTerminators and an ABI 310 DNA Sequencer (Applied Biosystems, Foster City, CA, USA).

Consensus sequences were aligned and manually edited using the Sequencher version 4.5 program (GeneCodes, Ann Arbor, MI, USA). Multiple alignments were performed using Clustal X. Reference sequences were downloaded from Los Alamos (www.hiv.lanl.gov). Phylogenetic analysis of nucleic acid sequences was performed with Mega version 3.1. A neighbour-joining tree was constructed with distances calculated using Kimura's two-parameter method. Genotypic resistance was defined as the presence of mutations associated with impaired drug susceptibility or virological response, as specified by the WHO surveillance list of mutations [13].

Allele-specific PCR

In addition to genotyping, samples were also tested for the K103N mutation using a highly sensitive allele-specific real-time PCR assay (AS-PCR) [8]. This method was developed to distinguish between the wild-type codons for lysine (AAA and AAG) and the mutant codons for asparagine (AAC and AAT) at position 103 of the reverse transcriptase. Synthetic plasmid mixtures showed a cut-off of detection of 0.2% for the minor variant [8].

Results

Stored serum samples from pregnant women attending antenatal clinics in the Gauteng Province of South Africa (where Johannesburg and Pretoria are located) during October of 2002 and 2004 were used to establish a drug resistance surveillance programme. The years 2002 and 2004 were selected as they represent time points prior to and after the start of the Comprehensive HIV and AIDS Care, Management

Table 1. Demographic data of women attending annual antenatal clinics in Gauteng in 2002

LabNo	Age, years	Race	Sample collection date	Region	Gravidity	Parity	Education grade	Partner age, years	Genotype	K103N AS-PCR
ANS6300	20	African	7 Oct 2002	A2	1	0	12	25	Wild type	Wild type
ANS5637	20	African	8 Oct 2002	A20	1	0	12	28	Wild type	Wild type
ANS6457	21	African	8 Oct 2002	B10	1	0	11	25	Wild type	Wild type
ANS6472	20	African	8 Oct 2002	B10	1	0	10	32	Wild type	Wild type
ANS6547	19	African	8 Oct 2002	A14	1	0	9	24	Wild type	Wild type
ANS6659	19	African	9 Oct 2002	B18	1	0	9	21	Wild type	Wild type
ANS6665	21	African	9 Oct 2002	A16	1	0	11	30	Wild type	Wild type
ANS6673	20	African	9 Oct 2002	B13	1	0	12	32	Wild type	Wild type
ANS6731	18	Unknown	9 Oct 2002	B27	1	0	9	20	Wild type	Wild type
ANS6882	19	African	9 Oct 2002	C14	1	0	10	26	Wild type	Wild type
ANS6899	19	African	9 Oct 2002	A25	1	0	11	20	Wild type	Wild type
ANS6917	18	African	9 Oct 2002	A15	1	0	7	25	Wild type	Wild type
ANS6987	18	African	9 Oct 2002	A11	1	0	12	22	Wild type	Wild type
ANS7048	18	African	10 Oct 2002	A1	1	0	9	28	Wild type	Wild type
ANS7098	19	African	10 Oct 2002	A4	1	0	12	25	Wild type	Wild type
ANS7170	19	Unknown	10 Oct 2002	B27	1	0	7	30	Wild type	Wild type
ANS7528	21	African	11 Oct 2002	B4	1	0	12	23	Wild type	Wild type
ANS7458	20	African	12 Oct 2002	A9	1	0	11	32	Wild type	Wild type
ANS7621	19	African	12 Oct 2002	A6	1	0	11	32	Wild type	Wild type
ANS7623	19	African	12 Oct 2002	A6	1	0	12	23	Wild type	Wild type
ANS7629	18	African	12 Oct 2002	B24	1	0	11	35	Wild type	Wild type
ANS7636	21	African	12 Oct 2002	23	1	0	10	23	Wild type	Wild type
ANS7648	18	African	12 Oct 2002	B22	1	0	9	19	Wild type	Wild type
ANS7737	18	African	12 Oct 2002	A25	1	0	11	20	Wild type	Wild type
ANS7794	18	African	14 Oct 2002	C8	1	0	11	21	Wild type	Wild type
ANS7809	21	African	14 Oct 2002	C7	1	0	10	24	Wild type	Wild type
ANS7832	18	African	14 Oct 2002	A2	1	0	10	21	Wild type	Wild type
ANS7839	19	African	14 Oct 2002	A2	1	0	11	28	Wild type	Wild type
ANS7870	21	African	14 Oct 2002	A3	1	0	12	27	Wild type	Wild type
ANS8018	21	African	14 Oct 2002	B16	1	0	11	28	Wild type	Wild type
ANS8175	21	African	14 Oct 2002	B8	1	0	12	29	Wild type	Wild type
ANS8199	19	African	14 Oct 2002	C3	1	0	11	28	Wild type	Wild type
ANS8584	19	African	14 Oct 2002	B1	1	0	12	26	Wild type	Wild type
ANS7931*	18	African	15 Oct 2002	A26	1	0	8	Unknown	Wild type	Wild type
ANS8209	19	African	15 Oct 2002	C11	1	0	12	26	Wild type	Wild type
ANS8219	20	African	15 Oct 2002	C11	1	0	12	22	Wild type	Wild type
ANS8249	21	African	15 Oct 2002	A19	1	0	11	27	Wild type	Wild type
ANS8302	19	African	15 Oct 2002	A13	1	0	11	26	Wild type	Wild type
ANS8308	20	African	15 Oct 2002	A10	1	0	11	24	Wild type	Wild type
ANS8310	21	African	15 Oct 2002	A15	1	0	12	26	Wild type	Wild type
ANS8317	19	African	15 Oct 2002	A3	1	0	11	26	Wild type	Wild type
ANS8432	19	African	15 Oct 2002	C4	1	0	8	23	Wild type	Wild type
ANS8470	21	African	15 Oct 2002	A2	1	0	10	28	Wild type	Wild type
ANS8495	20	African	15 Oct 2002	A5	1	0	5	31	Wild type	Wild type
ANS8546	21	African	16 Oct 2002	A15	1	0	12	24	Wild type	Wild type
ANS8734	20	African	16 Oct 2002	C4	1	0	6	23	Wild type	Wild type
ANS8762	21	African	17 Oct 2002	B4	1	0	10	24	Wild type	Wild type
ANS8812	20	African	17 Oct 2002	C3	1	0	11	24	Wild type	Wild type
ANS8891	20	Unknown	17 Oct 2002	B27	1	0	12	24	Wild type	Wild type
ANS9086	18	African	18 Oct 2002	A24	1	0	7	21	Wild type	Wild type
ANS9175	20	African	21 Oct 2002	B13	1	0	10	30	Wild type	Wild type
ANS9212	21	African	21 Oct 2002	C4	1	0	12	27	Wild type	Wild type
ANS9265	20	African	22 Oct 2002	B6	1	0	12	34	Wild type	Wild type

Regions: A, City of Johannesburg Metro and West Rand; B, Ekurhuleni (East Rand) and Sedibeng; C, Tshwane Metro. The number represents a different clinic in each of the regions. AS-PCR, allele-specific real-time PCR; LabNo, laboratory number given to sample. *The 34th consecutive sample represents a cut-off for assessing threshold levels.

Table 1. Continued

LabNo	Age, years	Race	Sample collection date	Region	Gravidity	Parity	Education grade	Partner age, years	Genotype	K103N AS-PCR
ANS9273	19	African	22 Oct 2002	A17	1	0	10	28	Wild type	Wild type
ANS9360	19	African	23 Oct 2002	B22	1	0	12	22	Wild type	Wild type
ANS9374	18	African	23 Oct 2002	B7	1	0	6	31	Wild type	Wild type
ANS9389	18	African	23 Oct 2002	C14	1	0	11	22	Wild type	Wild type
ANS9472	20	African	23 Oct 2002	C8	1	0	11	19	Wild type	Wild type
ANS9525	19	African	24 Oct 2002	B1	1	0	10	Unknown	Wild type	Wild type
ANS9552	19	African	25 Oct 2002	B28	1	0	12	24	Wild type	Wild type
ANS9577	20	African	28 Oct 2002	A1	1	0	11	23	Wild type	Wild type
ANS9621	18	African	29 Oct 2002	A17	1	0	12	24	Wild type	Wild type
ANS9624	21	African	29 Oct 2002	A17	1	0	12	24	Wild type	Wild type
ANS9633	21	African	29 Oct 2002	A5	1	0	12	35	Wild type	Wild type
ANS9595	18	African	1 Nov 2002	A19	1	0	10	22	Wild type	Wild type

and Treatment Plan, and also span the time when the pMTCT program was fully operational in this province. Consecutive samples were retrieved and subjected to PCR amplification of the *pol* gene. All samples were from black African women, ≤ 21 years of age with an average of 10 years of schooling (Tables 1 and 2). The average age of the partners was at least 7 years older.

Of 128 eligible participants from 2002, 65 (51%) samples were successfully amplified. None of the samples from 2002 had evidence of resistance mutations on genotyping or by AS-PCR for K103N (Table 1). Of 117 eligible participants from 2004, 48 (41%) samples were successfully amplified. Of these, one had T69D and a further one had the K70R resistance mutation, giving a total of 2 (4.2%) samples with evidence of resistance mutations on genotyping (Table 2). One sample (2.1%) that was wild type by genotyping was positive for K103N by AS-PCR. All samples clustered phylogenetically with HIV-1 subtype C, the predominant subtype circulating in South Africa (data not shown). There was no evidence for clustering based on year of collection, region or clinic that samples were collected from.

The data from 2004 was analysed using the WHO threshold analysis classification of HIV drug resistance prevalence using standard genotyping in specimens from primagravidas. Among the first 34 consecutive specimens, there was none with mutations associated with resistance in any drug class (Table 2), thus the overall prevalence of transmitted resistance, and the prevalence of resistance to each drug class, was classified as $<5\%$ in this population. In addition to the genotyping we also used the WHO threshold analysis classification of K103N prevalence using the point-mutation assay in primagravidas.

Among 34 consecutive specimens from 2004, there was one specimen with K103N; however, there were no additional K103N positives in the succeeding 10 specimens (Table 2). This is less than the lower limit of 2 for 44 specimens; therefore, prevalence of K103N was also classified as $<5\%$ using this more sensitive mutation-specific assay.

Discussion

We have successfully incorporated HIV drug resistance surveillance into the annual antenatal HIV seroprevalence survey (ANSUR) conducted by the South African National Department of Health. This study focused on Gauteng which is the largest Province and has a predominantly urban population. Using the WHO threshold survey the classification of resistance prevalence overall and for each drug class was $<5\%$ in both 2002 and 2004. No resistance was found among 2002 samples probably reflecting the low treatment rates at this time. In 2004, 4.2% of samples had resistance which, while still below the threshold level, might signal the start of the emergence of transmitted resistance. It is therefore crucial that this surveillance is conducted annually to continue monitoring trends, particularly in view of the fact that treatment has been available in the public sector since 2004 and is set to expand significantly in the coming years.

One of the limitations of the study is the low frequency of PCR amplification of samples from 2002 (51%) and 2004 (41%). These serum samples were primarily collected for serology and not for HIV-1 drug resistance testing; thus, they were not adequately stored for optimal preservation of viral RNA. It is also possible that low viral levels in this

Table 2. Demographic data of women attending annual antenatal clinics in Gauteng in 2004

LabNo	Age, years	Race	Sample collection date	Region	Gravidity	Parity	Education grade	Partner age, years	Genotype	K103N AS-PCR
ANS0226	21	African	5 Oct 2004	A3	1	0	10	26	Wild type	Wild type
ANS1517	20	African	6 Oct 2004	B6	1	0	11	31	Wild type	Wild type
ANS2279	20	African	6 Oct 2004	B18	1	0	5	21	Wild type	Wild type
ANS0238	21	African	10 Oct 2004	A4	1	0	12	31	Wild type	Wild type
ANS0138	18	African	10 Oct 2004	A2	1	0	11	24	Wild type	Wild type
ANS0180	19	African	10 Oct 2004	A2	1	0	12	22	Wild type	Wild type
ANS0192	18	African	10 Oct 2004	A3	1	0	11	20	Wild type	Wild type
ANS0469	20	African	10 Oct 2004	A7	1	0	11	23	Wild type	Wild type
ANS0641	18	African	10 Oct 2004	A11	1	0	10	23	Wild type	Wild type
ANS0745	21	African	10 Oct 2004	A12	1	0	12	26	Wild type	Wild type
ANS1027	21	African	10 Oct 2004	A20	1	0	11	25	Wild type	Wild type
ANS1058	20	African	10 Oct 2004	A20	1	0	12	25	Wild type	Wild type
ANS1338	19	African	10 Oct 2004	B1	1	0	12	19	Wild type	Wild type
ANS1437	21	African	10 Oct 2004	B2	1	0	12	24	Wild type	K103N
ANS2212	21	African	10 Oct 2004	B17	1	0	12	25	Wild type	Wild type
ANS0969	20	African	14 Oct 2004	A18	1	0	12	30	Wild type	Wild type
ANS1293	18	African	14 Oct 2004	A24	1	0	10	29	Wild type	Wild type
ANS1323	19	African	14 Oct 2004	A26	1	0	10	25	Wild type	Wild type
ANS1457	20	African	14 Oct 2004	B4	1	0	12	21	Wild type	Wild type
ANS2440	19	African	14 Oct 2004	B20	1	0	12	25	Wild type	Wild type
ANS0009	21	African	15 Oct 2004	A1	1	0	10	25	Wild type	Wild type
ANS0011	20	African	15 Oct 2004	A1	1	0	12	22	Wild type	Wild type
ANS0926	20	African	15 Oct 2004	A16	1	0	11	25	Wild type	Wild type
ANS1076	21	African	15 Oct 2004	A21	1	0	11	32	Wild type	Wild type
ANS1300	21	African	15 Oct 2004	A24	1	0	11	20	Wild type	Wild type
ANS0309	21	African	20 Oct 2004	A5	1	0	12	26	Wild type	Wild type
ANS1583	21	African	20 Oct 2004	B7	1	0	8	35	Wild type	Wild type
ANS1137	19	African	21 Oct 2004	A22	1	0	12	23	Wild type	Wild type
ANS0681	20	African	22 Oct 2004	A11	1	0	12	29	Wild type	Wild type
ANS3060	20	African	22 Oct 2004	C12	1	0	12	28	Wild type	Wild type
ANS1166	18	African	26 Oct 2004	A22	1	0	6	21	Wild type	Wild type
ANS0091	20	African	29 Oct 2004	A2	1	0	11	29	Wild type	Wild type
ANS1320	19	African	29 Oct 2004	A24	1	0	11	32	Wild type	Wild type
ANS2562*	19	African	29 Oct 2004	B21	1	0	11	21	Wild type	Wild type
ANS0946	18	African	2 Nov 2004	A17	1	0	8	27	K70R	Wild type
ANS1193	18	African	2 Nov 2004	A22	1	0	9	31	Wild type	Wild type
ANS1201	19	African	2 Nov 2004	A22	1	0	10	37	Wild type	Wild type
ANS2025	21	African	2 Nov 2004	B15	1	0	12	28	Wild type	Wild type
ANS2948	20	African	4 Nov 2004	C6	1	0	12	29	Wild type	Wild type
ANS1228	20	African	23 Nov 2004	A22	1	0	10	24	T69D	Wild type
ANS2556	18	African	23 Nov 2004	B21	1	0	11	Unknown	Wild type	Wild type
ANS3123	19	African	23 Nov 2004	C15	1	0	12	25	Wild type	Wild type
ANS3095	20	African	27 Nov 2004	C13	1	0	10	26	Wild type	Wild type
ANS0824*	20	African	10 Dec 2004	A15	1	0	7	29	Wild type	Wild type
ANS0873	20	African	10 Dec 2004	A15	1	0	8	26	Wild type	Wild type
ANS0886	19	African	10 Dec 2004	A15	1	0	11	26	Wild type	Wild type
ANS0888	20	African	10 Dec 2004	A15	1	0	11	26	Wild type	Wild type
ANS1068	21	African	10 Dec 2004	A21	1	0	12	24	Wild type	Wild type

Regions: A, City of Johannesburg Metro and West Rand; B, Ekurhuleni (East Rand) and Sedibeng; C, Tshwane Metro. The number represents a different clinic in each of the regions. AS-PCR, allele-specific real-time PCR; LabNo, laboratory number given to sample. *The 34th and 44th consecutive sample represent cut-offs for assessing threshold levels.

largely asymptomatic group of individuals may have compromised amplification rates. Nevertheless, the threshold survey is based on the number of genotypes obtained and does not take into account amplification failures, thus low amplification rates are unlikely to have caused any significant bias.

The three mutations detected in the survey are all considered primary mutations able to confer resistance to antiretroviral drugs and are among those considered significant for transmitted resistance [13]. T69D causes low level resistance to all nucleoside reverse transcriptase inhibitors and K70R causes low level resistance to zidovudine and stavudine. K103N is selected by nevirapine and efavirenz and causes high-level resistance to both drugs. As these mutations do not occur among untreated persons these individuals were either exposed to antiretroviral drugs or were infected with a resistant strain. It is not possible to distinguish between these two options using this cohort.

When the 2004 survey was performed, 12 women who were not true primagravidas were inadvertently included among the samples sent for genotyping. Interestingly, four of these women had K103N by genotyping, one of which also contained the M184V mutation. All four women were also positive for K103N by AS-PCR (data not shown). As K103N is the major mutation found among women following exposure to sd-nevirapine and is known to persist for over 6 months in some women [14], it is highly likely that these women received sd-nevirapine as part of a pMTCT during their previous pregnancy. This demonstrates the advantages of using primagravidas as a criterion, as selecting women in their first pregnancy excludes those with persistent mutations from the survey.

The use of the K103N AS-PCR identified one sample that was below the limit of detection by population-based sequencing. Thus, AS-PCR may represent a suitable screening method for drug resistance surveillance. The ability to use point-mutation assays for surveillance would greatly increase the number of samples that could be tested and significantly decrease the costs of surveillance. However, implementation would depend on the availability of allele-specific assays for all major mutations and the frequency of these mutations among individuals with transmitted resistance.

In this study we focused on only one province in South Africa, but we plan to expand our surveillance to include other provinces such as the Western Cape, where treatment has been available since the late 1990s, and to KwaZulu-Natal, which had the highest HIV prevalence among pregnant women in 2005 [11]. Given that there are currently over 300,000

HIV-1-infected people receiving antiretroviral therapy in South Africa and that the programme is set to expand over the next 5 years, the opportunities for patients to fail therapy and develop resistant strains will increase. It will be important to continue monitoring recently infected drug-naïve individuals to determine if resistant strains are being transmitted. Such information will be required to guide both prevention and treatment programmes.

Acknowledgements

This study was sponsored by the CDC Co-operative Agreement (grant number U62/CCU022901-1), the International Atomic Energy Agency (IAEA grant number RAF/6/029) and the WHO Regional Office for Africa. International Medical Press is acknowledged for their support with publishing this manuscript.

Disclosure statement

The authors declare that they have no competing interests.

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Accepted for publication 28 May 2007