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INTRODUCTION

The transmission of drug-resistant HIV-1 variants is being increasingly reported in countries where anti-retroviral therapy (ART) has been in use for some time, in particular the United States and Europe but also in developing countries, such as Brazil. In South Africa, a country with an estimated 5.7 million HIV-infected individuals and almost 1,000 AIDS deaths every day, a national treatment program, the Comprehensive HIV and AIDS Care, Management and Treatment Plan, was initiated in April 2004. The first line regimen in use is stavudine (d4T), lamivudine (3TC) and efavirenz (EFV) or nevirapine (NVP) with kaleta being used among infants and children. At the end of September 2006, there were 273 Operational Health facilities which were fully functional and providing ART services to an estimated 235,000 people, 10% of them children. An additional 80,000 people are on antiretroviral therapy in the private health care sector, where a variety of regimens are in use.

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 9 provinces in South Africa. This survey provides the best available estimates of HIV infection in the country. Data show that HIV-1 prevalence has increased dramatically since 1991 when prevalence was less than 1% to 30% in 2005 (Figure 1). To date, there have been no reports on primary HIV-1 resistance from South Africa. We therefore have begun a retrospective study using the ANSUR samples to conduct a drug resistance threshold assessment survey.

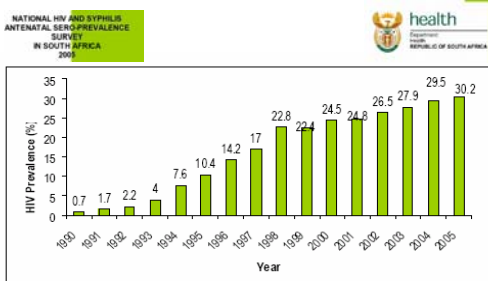


Figure 1 National HIV prevalence trends among antenatal clinic attendees in South Africa: 1990 - 2005

MATERIALS AND METHODS

All subjects were from the Gauteng Province (Figure 2) and were part of the 2002 and 2004 ANSUR and all subjects met the inclusion criteria as set out by the WHO guidelines for HIV-1 transmitted drug resistance surveillance (women <21 years of age and in first pregnancy). Samples were from black African women, 21 years or younger with an average of 10 years of schooling (Tables 1 and 2). The average age of the partners was at least 7 years older. 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). 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).



Figure 2: Regions in Gauteng where samples collected for the annual ante-natal HIV seroprevalence survey. Regions: A= City of Johannesburg Metro and West Rand; B= Ekurhuleni (East Rand) and Sedibeng; C= Tshwane Metro.

Table 1: Demographic data of women attending ANC in the Gauteng region in 2002

Region	Number	Average age	Gravidity	Parity	Average Education Grade	Average Partner Age
A	33	17	1	0	10	25
B	20	19	1	0	10	27
C	12	19	1	0	10	24

Table 2: Demographic data of women attending ANC in the Gauteng region in 2004

Region	Number	Average age	Gravidity	Parity	Average Education Grade	Average Partner Age
A	33	21	1	0	10	26
B	11	18	1	0	11	25
C	4	20	1	0	12	27

RESULTS

Of 128 eligible participants from 2002, 65 (51%) were successfully amplified. None of the samples from 2002 had evidence of resistance mutations on genotyping or by AS-PCR for K103N. Of 117 eligible participants from 2004, 48 (41%) samples were successfully amplified. Of these, 1 had T69D and a further 1 had the K70R resistance mutation, giving a total of 2 (4.2%) samples with evidence of resistance mutations on genotyping. One sample (2.1%) was positive for K103N by AS-PCR. All samples clustered phylogenetically with HIV-1 subtype C, the predominant subtype circulating in South Africa. There was no evidence for clustering based on year of collection, region or clinic that samples were collected from. These data from 2004 was analyzed using the WHO threshold analysis classification of HIVDR prevalence using standard genotyping in specimens from primigravidas. Among the first 34 consecutive specimens there were no with mutations associated with resistance in any drug class, 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 primigravidas. Among 44 consecutive specimens from 2004, there was one specimen with K103N. 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. When the 2004 survey was performed, 12 women who were not true primigravidas were inadvertently included among the samples sent for genotyping. These data were excluded from the above analysis but are reported here (Table 3). Interestingly, 4 of these women had K103N by genotyping, one of which also contained the M184V mutation. All 4 women were also positive for K103N by AS-PCR.

Table 3: Demographic data of the 12 non-primigravida women attending ANC in the Gauteng region in 2004

Region	Age	Gravidity	Parity	Education Grade	Partner Age	Genotype	K103N AS-PCR
A	19	2	1	7	24	Wildtype	Wildtype
A	20	2	1	11	26	Wildtype	Wildtype
A	19	2	1	10	22	K103N	K103N
A	20	2	1	12	27	Wildtype	Wildtype
A	21	2	1	4	35	K103N	K103N
B	20	2	1	9	22	Wildtype	Wildtype
B	20	2	1	12	28	K103N, M184V	K103N
B	20	2	1	10	25	K103N	K103N
C	19	2	1	3	17	Wildtype	Wildtype
C	19	2	1	12	23	Wildtype	Wildtype
C	20	2	1	10	28	Wildtype	Wildtype
C	20	2	1	7	35	Wildtype	Wildtype

CONCLUSIONS

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.

While the ANSUR serological survey provides an excellent opportunity for conducting resistance surveillance, extra care needs to be taken in future to store samples for RNA testing in order to improve the PCR amplification rates.

The 4 mutations detected in the survey are all considered primary mutations able to confer resistance to anti-retroviral drugs and are among those considered significant for transmitted resistance.

The presence of K103N in 4 of 12 women who were not true primigravidas demonstrates the advantages of using this selection criterion for the threshold survey.

The K103N AS-PCR may represent a suitable screening method which could greatly increase the number of samples that could be tested and significantly decrease the costs of surveillance

ACKNOWLEDGMENTS AND REFERENCES

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