

Surveillance of transmitted HIV drug resistance in the Manzini–Mbabane corridor, Swaziland, in 2006

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Background: In resource-limited settings where anti-retroviral treatment (ART) is being scaled-up, the World Health Organization (WHO) recommends the surveillance of transmitted HIV drug resistance (HIVDR). We used the WHO's HIVDR threshold survey method to assess transmitted HIVDR in three antenatal clinic (ANC) sites along the corridor between the two most populous cities in Swaziland, where ART was introduced in 2003.

Methods: From July–August 2006, remnant sera were aliquoted from HIV serosurvey specimens collected from 70 primagravidas <25 years old attending ANC during the national HIV serosurvey. Genotyping was performed at the National Institute for Communicable Diseases, South Africa. Transmitted resistance was defined by the WHO's surveillance list of mutations. HIVDR prevalence was categorized using the WHO's threshold survey binomial sequential sampling method.

Results: Among the 70 eligible specimens, 61 were sequenced – 60 (98%) were identified as subtype C and one as subtype B. No major nucleoside or non-nucleoside reverse transcriptase inhibitor mutations occurred among the first 34 consecutive specimens, which supported a transmitted resistance categorization to these drug classes as <5%. One protease inhibitor mutation, M46I, was seen among the first 44 specimens, supporting a categorization of PI resistance as <5%.

Conclusion: Our survey indicates that prevalence of transmitted HIVDR among recently infected pregnant women along the Manzini–Mbabane corridor is low (<5%). Surveys will be carried out in this area biannually and may be extended to other areas. Surveys for transmitted resistance make up one element among a spectrum of activities to assess and support minimization of HIVDR.

Introduction

The kingdom of Swaziland, situated in Southern Africa, is a small (17,364 km²) land-locked country with an estimated population of 1,182,500 in 2006, of which approximately 76% reside in rural areas. The country shares borders with South Africa and Mozambique.

According to the sentinel surveillance conducted in 2006 among pregnant women attending antenatal clinic (ANC) sites, the HIV prevalence was 39.2% compared with 42.6% in 2004. The most affected age group was the 25–29 year-olds with 48.0% of HIV prevalence, followed by the 30–34 year-olds with 45.8%. The

15–19 year-old age group has shown a decrease in HIV prevalence in two successive sentinel surveillances: 32.5% in 2002, 29.3% in 2004 and 26.0% in 2006.

At present, it is estimated that about 210,000–230,000 people in Swaziland are living with HIV or AIDS, approximately 56% are women and 44% are men. At present, it is estimated that about 34,000 people living with HIV/AIDS (PLWHA) are in need of antiretroviral treatment (ART). National guidelines published in 2006 recommend a first-line ART regimen consisting of zidovudine (AZT), lamivudine

(3TC) and Nevirapine (NVP); if AZT and/or NVP is contraindicated, stavudine (d4T) or efavirenz (EFV) may be substituted respectively for AZT and NVP. Protease inhibitors are reserved for second-line use.

In Swaziland, most patients whose first-line regimen fails will have been on AZT or d4T + 3TC + NVP or EFV. Second-line ART is currently rare. Guidelines from 2006 recommend lopinavir/ritonavir (LPV/r) and two new NRTIs.

In Swaziland, only 50% of health facilities belong to the government, 22% belong to religious missions, 17% belong to private institutions and 9.7% belong to industrial companies. All participate in ART provision. At present, 22 facilities are providing ART, which include four government hospitals, two mission hospitals, five governmental health centres, one governmental public health unit and 10 private clinics. All of the above health facilities receive anti-retroviral drugs (ARVs) free of charge from the government and follow the national guidelines for ARV treatment.

Swaziland initiated the ART programme in December 2003 and the national target, as defined by the Swaziland government, was 13,000 HIV/AIDS patients on ART by the end of 2005 ('3 by 5' target). As a consequence, in addition to the establishment of a multisectoral Technical Working Group on ART, the Ministry of Health and Social Welfare developed a three-phase action plan to roll out ART. The first phase concerned the establishment of ART centres in major hospitals in the country, the second comprised the expansion of the ART service to all health centres and some private company clinics, and the third phase was to expand the service to all private clinics as well as public clinics.

Plans were developed and coordinated through the Swaziland National AIDS programme, which coordinates all HIV/AIDS activities in the health sector, including the ART programme. At the end of 2006, 17,160 PLWHA were receiving treatment in different ART centres. The annual ART report for 2005 and the first quarter report for 2006 indicate that the percentage of ART patients on a first-line regimen in all ART centres was 98.8%. Even in the one ART centre that had the highest number of patients on the second line regimen, the percentage of patients on a first-line regimen was 96.7%.

The number of patients ever enrolled in the national ART programme at the end of 2006 was estimated at 23,371. Taking into account the number of patients currently on treatment, the number of individuals who have died, defaulted or have become otherwise lost to follow-up can be estimated at 6,211, which represents about 26.6% of patients enrolled in an ART programme. At the end of 2007, approximately 23,000

PLWHA are expected to have accessed ART and to be continuing on treatment.

After noting that the number of patients beginning and then ceasing ART because of death or default was unusually high, Swaziland decided to pay particular attention to HIV drug resistance (HIVDR). It was decided that a system should be put in place whose primary task would be to ensure monitoring and surveillance of HIVDR in the country, so that timely necessary measures could be taken if required. The National Committee on HIV Drug Resistance was established, and comprises representatives from the Ministry of Health and Social Welfare, particularly the Monitoring and Evaluation unit, Central Medical Stores, Reproductive Health Unit and Swaziland National AIDS Programme. Also represented in the committee were the ART centres, the National Emergency Response Council on HIV/AIDS, the University of Swaziland, UN agencies (mainly the WHO and the United Nations International Children's Emergency Fund) and non-governmental organizations including community-based organizations.

The first initiative undertaken by the HIV Drug Resistance Committee was to review the WHO's generic HIVDR threshold survey protocol [1,2] for surveillance of transmitted HIVDR and adapt it to the Swaziland situation. An HIVDR threshold survey was performed in 2006 and the results are reported here.

Methods

Geographic area

Manzini is the most populated city in Swaziland and Mbabane (Swaziland's capital) is the second. ART has been available in these cities since 2003. The two cities are linked by a highway and are a 20–30 min drive apart. Individuals who live in one city frequently work or travel (for leisure activities or healthcare) to the other city or along the corridor between the two cities. Because of the relatively long history of ART use along this corridor, it was decided to perform the first HIVDR threshold survey in this area.

Sites

In accordance with the strategy recommended by the WHO for surveillance of transmitted HIV drug resistance, it was decided to use remnant specimens from HIV serosurveillance taking place in ANC in the relevant geographical area. The HIV serosurveys take place every 2 years using an unlinked anonymous approach. Based on the previous survey, it was estimated that eligible specimen numbers would be sufficient to supply the 60–70 specimens the WHO recommends should be collected in order to ensure 47 suitable specimens for analysis. The HIVDR threshold survey was conducted

during July–August 2006 in three clinics located in the Manzini–Mbabane corridor (Mbabane Public Health Unit, King Sobuza II clinic and FLAS-Manzini Youth Clinic).

Eligibility criteria and specimen collection

As recommended by the WHO [2], eligible specimens were defined as specimens from HIV-seropositive primagravidas <25 years of age. Remnant sera from routine syphilis tests were tested for HIV as part of the HIV serosurvey. The HIVDR threshold survey used the first consecutively collected HIV serosurveillance specimens meeting the eligibility criteria.

Ethics committee approval

The national ethics committee approved the genotyping of remnant eligible specimens as an amendment to the unlinked anonymous HIV serosurvey protocol.

Laboratory methods

Sera were aliquoted from remnant serosurvey specimens and frozen at the Swaziland National Reference Laboratory. After the 70 eligible specimens had been collected, the frozen sera were shipped on dry ice to South Africa (National Institute for Communicable Diseases, AIDS Virus Research Unit, Johannesburg) for HIVDR genotyping as described in Pillay *et al.* in this supplement [3].

Data analysis

We used the WHO HIVDR threshold survey analysis method to categorize transmitted HIVDR to NRTIs, NNRTIs and PIs. The HIVDR threshold survey analysis supports classification of HIVDR to all relevant drugs and drug classes as <5%, 5–15% and >15%, using a maximum of 47 specimens. Sequences are analysed in the order of specimen collection. The analysis is stopped and a classification is made for a specific drug or drug class when the total number of specimens with relevant mutations falls below a lower limit or above an upper limit for the number of sequences analysed. Prevalence estimates and confidence intervals cannot be calculated using this method. A full description of the method and the principles behind the classification can be found in two articles in this supplement [1,2].

Results

Specimens from 70 pregnant women aged 14–24 years were collected for the HIVDR threshold survey. One woman (1.4%) was <15 years, 22 women (31.5%) were aged between 15 and 19 years, and 47 women (67%) were aged between 20 and 24 years.

Seven specimens (10%) could not be amplified and two PCR products could not be sequenced (3%). These

samples were drawn from women who were aged between 18 and 22 years.

For the 61 specimens that were amplified and sequenced, 60 (98%) were identified as subtype C and the remaining one as subtype B.

Based on the analysis of the first 34 consecutively collected specimens in which no NRTI or NNRTI mutations from the WHO's list of mutations for surveillance of transmitted HIVDR occurred, the prevalence of resistance to NRTIs and NNRTIs could be classified as <5% (the lower limit for the number of sequences with mutations among the first 34 specimens is one; see Table 1). Among the first 44 sequences analysed, the 20th sequence included a PI mutation from the WHO list, M46I. Because the lower limit for the number of sequences with relevant mutations among the first 44 sequences is two, the transmitted PI resistance was classified as <5%.

There were no other NRTI or NNRTI mutations included in the WHO's surveillance list of transmitted resistance among the sequences not used in the analysis. The protease mutation I47V, which is in the WHO list, was seen in a sequence not among the first 44 sequences. This does not change the classification.

Discussion

The classification of transmitted resistance to all relevant drug classes of <5% is understandable, given that ART had only been available in the area surveyed for three years at the time of the survey and detectable levels of HIVDR transmission are unlikely on a population basis in this situation, according to statistical models [4].

One sequence among the 44 analysed had the PI mutation M46I. This mutation is only provisionally included in the WHO surveillance list because it occurs with a prevalence >0.5% among specimens in at least one non-B subtype in the Stanford database [3,5], and is somewhat polymorphic in several non-B subtypes. The PI mutation I47V, seen in a sequence collected after the first 44, is also provisionally included in the list because of potential polymorphism among non-B subtypes. Given the very low level of PI use in Swaziland, it is more likely that the mutations among these sequences are polymorphic rather than evidence of transmitted resistance. Numerous minor polymorphic protease mutations that were not considered to be associated with transmitted resistance were detected.

Only polymorphic NRTI and NNRTI mutations were seen. Three specimens had the NRTI polymorphism V118I. This mutation occurs naturally at >2.0% in most subtypes and is not considered evidence of transmitted resistance. Polymorphic mutations (K103R and V179DV) were seen at two resistance-related positions in

Table 1. Swaziland HIVDR threshold survey 2006: classification of transmitted HIVDR prevalence

Specimen number genotyped	Lower limit	Running total of specimens with HIVDR mutations A/B/C/D	Upper limit
MMC1	ND	0/0/0/0	ND
MMC2	ND	0/0/0/0	ND
MMC3	ND	0/0/0/0	ND
MMC4	ND	0/0/0/0	ND
MMC5	ND	0/0/0/0	ND
MMC6	ND	0/0/0/0	ND
MMC7	ND	0/0/0/0	ND
MMC8	ND	0/0/0/0	ND
MMC9	ND	0/0/0/0	ND
MMC10	ND	0/0/0/0	ND
MMC11	ND	0/0/0/0	ND
MMC12	ND	0/0/0/0	ND
MMC13	ND	0/0/0/0	ND
MMC14	ND	0/0/0/0	5
MMC15	ND	0/0/0/0	5
MMC16	ND	0/0/0/0	5
MMC17	ND	0/0/0/0	5
MMC18	ND	0/0/0/0	5
MMC19	ND	0/0/0/0	5
MMC20	ND	1/0/0/1	5
MMC21	ND	1/0/0/1	5
MMC22	ND	1/0/0/1	5
MMC23	ND	1/0/0/1	5
MMC24	ND	1/0/0/1	5
MMC25	ND	1/0/0/1	6
MMC26	ND	1/0/0/1	6
MMC27	ND	1/0/0/1	6
MMC28	ND	1/0/0/1	6
MMC29	ND	1/0/0/1	6
MMC30	ND	1/0/0/1	6
MMC31	ND	1/0/0/1	6
MMC32	ND	1/0/0/1	6
MMC33	ND	1/0/0/1	6
MMC34	1	1/0/0/1	6
MMC35	1	1/ /1	7
MMC36	1	1/ /1	7
MMC37	1	1/ /1	7
MMC38	1	1/ /1	7
MMC39	1	1/ /1	7
MMC40	1	1/ /1	7
MMC41	1	1/ /1	7
MMC42	1	1/ /1	7
MMC43	1	1/ /1	7
MMC44	2	1/ /1	7

This table summarizes the analyses for overall HIV drug resistance (HIVDR) and for resistance to each of the three relevant drug classes (nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors and protease inhibitors). Mutations associated with transmitted HIVDR are evaluated in genetic sequences representing the relevant areas of the *pol* region of the HIV genome amplified from eligible specimens. Analysis of sequences stops when a classification of HIVDR prevalence has been made. Fewer sequences were required for the classification than were genotyped in the survey. A classification of HIVDR prevalence is made when the running total of sequences found with mutations associated with transmitted HIVDR is less than the lower limit or greater than the upper limit on the same horizontal line. HIVDR prevalence can be classified either as <5% (if the running total of sequences with HIVDR mutations is less than the lower limit) or >15% (if the running total of sequences with HIVDR mutations is greater than the upper limit). If neither of these conditions has occurred after the 47th specimen has been genotyped, prevalence is classified as 5–15%. Categorizations of transmitted HIVDR among primigravidas <25 years of age along the Manzini–Mbani corridor is <5% for each of the three relevant drug classes, and for all drug classes. The chart was developed using millions of computer simulations comparing classifications of prevalence based on small numbers of consecutively collected sequences from surveys of transmitted resistance to precise estimates of prevalence using appropriately large numbers from the same surveys. (A) number of sequences with relevant resistance mutations to any drug class; (B) number of sequences with relevant nucleotide or nucleoside reverse transcriptase resistance mutations; (C) number of sequences with relevant non-nucleoside reverse transcriptase resistance mutations; (D) number of sequences with relevant protease inhibitor resistance mutations. ND, not determined.

the reverse transcriptase region, but neither is associated with resistance.

The results of this first survey are reassuring, but assessment must continue. The Ministry of Health and Social Welfare has decided to carry out an HIVDR threshold survey every 2 years in the Mbabane–Manzini corridor using the same methodology; surveys may also be extended to other areas of the country. If resistance is emerging during treatment in an unnecessarily high percentage of patients, it is important to detect and take necessary action to prevent this phenomenon. To better characterize resistance emerging during treatment, surveys to monitor HIVDR emerging at sentinel ART sites [6] will also be performed regularly, beginning in two sentinel sites in 2007–2008 and expanding to representative sites throughout the country. The surveys will also assess baseline resistance before first-line ART starts, which any resistance mutations detected may be associated either with transmitted resistance or with previous ARV drug experience (reported or unreported). Along with surveys of transmitted resistance, the baseline assessment will provide information to evaluate the potential effectiveness of Swaziland's first-line regimens on a population basis. The evaluation of drug resistance emerging during ART will support assessment of the suitability of the Swaziland's second-line regimen.

The Ministry of Health and Social Welfare has also initiated an early warning system with five of the WHO HIVDR early warning indicators [7] to support ART sites in operating to limit HIVDR. The indicators listed in Box 1 will be assessed initially at

representative sites, expanding to all ART sites in the country. Additional, more detailed assessments may be developed if an indicator outcome indicates an ART programme problem that should be addressed in order to minimize HIVDR emergence.

Lastly, to create a favourable environment for HIVDR prevention in Swaziland, the Ministry of Health and Social Welfare has defined the specific activities to combat HIVDR listed in Box 2.

Assessment and prevention of HIVDR is an important aspect of ART scale-up in Swaziland. Surveys for transmitted drug resistance contribute to a range of activities to support HIVDR prevention and ensure that the standard ART regimens continue to be effective.

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Disclosure statement

The authors confirm that they have no competing interests.

Box 1. HIV drug resistance early warning indicators to be monitored in Swaziland's ART sites

- Percentage of patients starting first-line ART who are prescribed an appropriate regimen.
 - Percentage of patients lost to follow-up during the first year of ART.
 - Percentage of patients still on first-line ART 12 months after ART start.
 - Percentage of patients picking up their ARV drugs on time.
 - Number of days in a year on which ARV drug supply was interrupted.
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ART, antiretroviral therapy.

Box 2. HIV drug resistance prevention activities developed by the Ministry of Health and Social Welfare for Swaziland

- Improvement of patient management and follow-up at ART sites.
 - Improvement of drug supply management at ART sites.
 - Use of treatment supporters in collaboration with PLWHA to better support adherence and minimize loss to follow-up at ART sites.
 - Roll-out of treatment literacy activities in collaboration with PLWHA.
 - Routine assessment of adherence among patients on ART.
 - Improvement of collaboration with traditional healers through development and implementation of a collaborative framework to promote appropriate use of ART and support adherence.
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ART, antiretroviral therapy; PLWHA, people living with HIV/AIDS.

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