UNIVERSITY OF AMSTERDAM

UvA-DARE (Digital Academic Repository)

# HIV drug resistance among adults and children in sub-Saharan Africa

Sigaloff, K.C.E.

**Publication date**
2013

Link to publication

**Citation for published version (APA):**
Sigaloff, K. C. E. (2013). *HIV drug resistance among adults and children in sub-Saharan Africa*. [Thesis, fully internal, Universiteit van Amsterdam].

**General rights**
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

**Disclaimer/Complaints regulations**
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: https://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, P.O. Box 19185, 1000 GD Amsterdam, The Netherlands. You will be contacted as soon as possible.

*UvA-DARE is a service provided by the library of the University of Amsterdam (https://dare.uva.nl)*

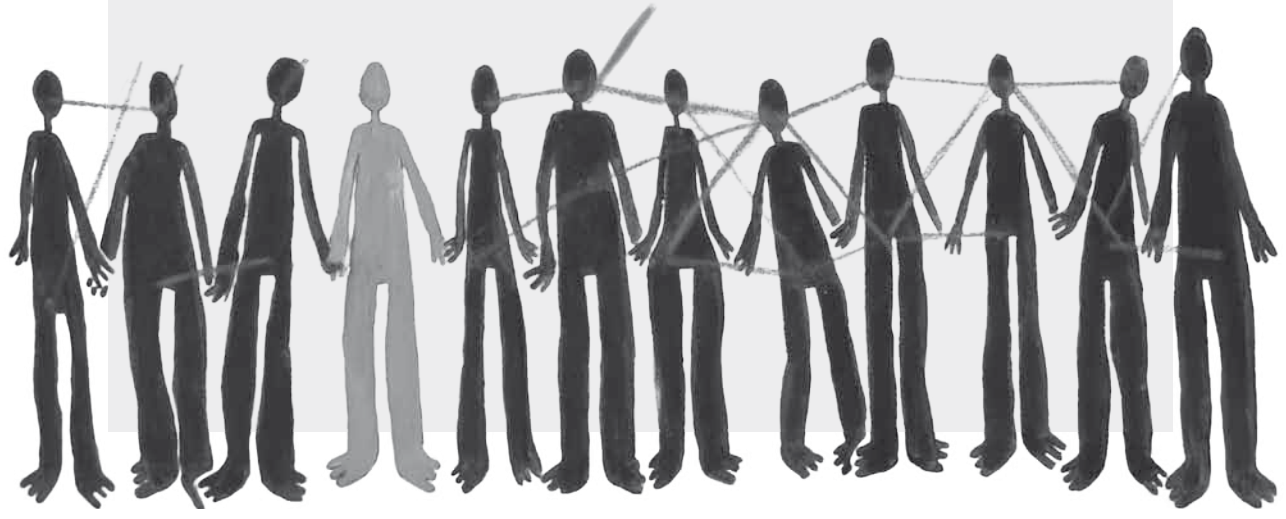
Download date:13 Nov 2026

# Chapter 3

## High prevalence of transmitted antiretroviral drug resistance among newly HIV-1 diagnosed adults in Mombasa, Kenya

Kim C.E. Sigaloff, Kishor Mandaliya, Raph L. Hamers, Francis Otieno, Irene M. Jao, Frederick Lyagoba, Brian Magambo, Anne Kapaata, Nicaise Ndembi, and Tobias F. Rinke de Wit

*AIDS Research and Human Retroviruses 2012; 28(9): 1033-1037*



## ABSTRACT

In view of the recent antiretroviral therapy (ART) scale-up in Kenya, surveillance of transmitted HIV drug resistance (TDR) is important. A cross-sectional survey was conducted among newly HIV-1 diagnosed, antiretroviral-naive adults in Mombasa, Kenya. Surveillance drug resistance mutations (SDRMs) were identified according to the 2009 WHO list. HIV-1 subtypes were determined using REGA and SCUEAL subtyping tools. Genotypic test results were obtained for 68 of 81 participants, and SDRMs were identified in 9 samples. Resistance to non-nucleoside reverse transcriptase inhibitors (K103N) occurred in five participants, yielding a TDR prevalence of 7.4% (95% confidence interval 2.4–16.3%). Frequencies of HIV-1 subtypes were A (70.6%), C (5.9%), D (2.9%), and unique recombinant forms (20.6%). The TDR prevalence found in this survey is higher than previously reported in different regions in Kenya. These findings justify increased vigilance with respect to TDR surveillance in African regions where ART programs are scaled-up in order to inform treatment guidelines.

## INTRODUCTION

With expanded access to combination antiretroviral therapy (ART) in many countries in sub-Saharan Africa, the prevalence of HIV drug resistance is likely to increase. Viral resistance can develop through selective pressure while receiving ART and can subsequently spread to newly infected individuals. The latter is referred to as primary or transmitted drug resistance (TDR).[1,2] TDR increases the risk of virological failure,[3,4] which may have a substantial impact in areas where options for first-line therapy are limited. Studies from industrialized countries have shown an increased incidence of TDR with time after the introduction of ART.[5–7] In Africa, studies conducted during the early scale-up of ART reported low levels of TDR.[8–10] Recently accruing evidence, however, points to increasing TDR among individuals with recent HIV infection in East Africa.[11–13]

In Kenya, national ART roll-out started in late 2003, and by 2010 an estimated 50% of adults in need of ART were receiving treatment, which is among the higher coverage rates for sub-Sahara African countries.[14] Data on TDR from Kenya are limited, pointing toward levels of 0–10% in different regions and time periods.[11,15,16] Because the prevalence of transmitted resistance mutations can influence policy and guide treatment choices in the absence of individual resistance testing, the World Health Organization (WHO) has provided guidelines for TDR surveillance in developing countries.[1,9] We report the results of a survey evaluating the prevalence of TDR among newly HIV-1 diagnosed individuals attending voluntary counseling and testing (VCT) sites in Mombasa, Kenya.

## MATERIALS AND METHODS

### Study design and population

As part of the PharmAccess African Studies to Evaluate Resistance-Surveillance (PASER-S) program, a cross-sectional survey was conducted among clients attending four VCT sites in Mombasa, Kenya: Coast Provincial General Hospital, Likoni Sub-District Hospital, Kisauni Health Centre, and Magongo Health Centre. The institutional review boards at the Kenyatta National Hospital and the Academic Medical Center of the University of Amsterdam approved the study. Eligibility criteria as defined by the WHO were used to identify individuals who were likely to have been recently infected: newly diagnosed with HIV-1 and aged between 18 and 25 years, or laboratory evidence of recent HIV-1 infection (defined as a confirmed positive antibody test with a negative antibody test in the past 12 months, or an indeterminate/ negative antibody test with detectable HIV-RNA or p24 antigen).[9] Exclusion criteria were any previous antiretroviral use, WHO clinical stage 4 event, or previous pregnancy. All participants provided written informed

consent prior to enrollment. During the enrollment period of maximum 12 months, the VCT clients were screened and sequentially enrolled.

### Population genotype analysis

HIV-RNA and genotypic testing was performed as previously described.[12] Final sequences were checked before inclusion using ViroScore Suite v8.1 (ABL, Paris, France). Neighbor-joining phylogenetic trees were constructed using MEGA 5.05,[17] in order to rule out cross-contamination. TDR was analyzed in sequences with > 1020 base pairs. Surveillance drug resistance mutations (SDRMs) were identified according to the 2009 WHO list for surveillance of TDR,[1] using the Stanford calibrated population resistance analysis tool (version 5.0).[18] Subtype classification and recombinant patterns were determined using REGA[19] and SCUEAL[20] subtyping tools.

### Statistical methods

The survey sample size was estimated from the hypothesis that TDR prevalence increased with time (from 0 to 10%). To detect such an increase with 80% power using a two-sided significance level of 0.05, the required number of HIV sequences per geographic area was 71. Assuming 20% assay failures, the target sample was 85 individuals. The proportions of sequences containing at least one SDRM were calculated by each of three drug classes: nucleoside reverse transcriptase inhibitors (NRTIs), NNRTIs, and protease inhibitors (PIs). TDR prevalence was estimated with a 95% confidence interval (CI) based on the binomial distribution. As a secondary analysis, the WHO-recommended truncated sampling technique was used to categorize TDR prevalence as low ( < 5%), moderate (5–15%), or high ( > 15%) for each of the three drug classes, based on the testing of the first 47 sequences.[21] Categorical data were compared using the chi-square test and continuous data with the Wilcoxon rank-sum or Student's t-test. All analyses were performed using Stata version 10 (StataCorp LP, College Station, TX).

## RESULTS

### Participant characteristics

Study enrollment took place from May 2009 to March 2010 at four VCT centers in Mombasa, Kenya. A total of 241 individuals were screened, of whom 93 (38.6%) were enrolled. Protocol violations were noted in 12 individuals (8 had a previous pregnancy and 4 had no laboratory evidence of recent infection). Finally, 81 participants (11 from Coast Provincial General Hospital, 51 from Likoni Sub-District Hospital, 11 from Kisauni Health Centre, and 8 from Magongo Health Centre AIC) were included in the analysis. Sixty-one participants were included based on the age criterion and 20 participants had

a new confirmed HIV-1 diagnosis after a recent negative test (median 6.7 months ago, range 1.5–12 months). The median age was 23.4 years (IQR 21.6–24.9). Except for age, participant characteristics were similar for those included based on the age criterion and those with laboratory evidence of recent infection (Table 1). The median age at sexual debut was 16 years (IQR 14–18); all participants reported that they had engaged in unprotected sex in the 3 years prior to study enrolment. Among participants with a steady sexual partner, 40 (49.4%) were unaware of their partner's HIV status.

## Genotypic profiles

Of 76 specimens with HIV-RNA > 1000 copies/ml, 72 were successfully genotyped and 4 failed to amplify or generated a poor sequence. Additionally, four sequences were excluded from analysis because they contained less than 1020 base pairs. An SDRM was

**Table 1.** Participant characteristics by criteria to identify recent infection

| | Total | Age 18-24 | Lab evidence | *p* |
|---|---|---|---|---|
| Participants | 81 | 61 | 20 | |
| Sex | | | | 0.678 |
| Female | 71 (87.7) | 54 (88.5) | 17 (85.0) | |
| Male | 10 (12.4) | 7 (11.5) | 3 (15.0) | |
| Age - median yrs (IQR) | 23.4 (21.6-24.9) | 21.4 (22.6-23.5) | 28.2 (31.5-34.2) | <0.001 |
| Kenyan nationality | 79 (97.5) | 60 (98.4) | 19 (95.0) | 0.401 |
| Marital status | | | | 0.210 |
| Married/cohabiting | 65 (80.3) | 48 (78.7) | 17 (85.0) | |
| Divorced/separated | 3 (3.7) | 2 (3.3) | 1 (5.0) | |
| Widowed | 4 (4.9) | 2 (3.3) | 2 (10.0) | |
| Never married/single | 9 (11.1) | 9 (14.8) | - | |
| Education level | | | | 0.518 |
| None/illiterate | 3 (3.8) | 2 (3.3) | 1 (5.3) | |
| Primary school | 46 (57.5) | 35 (57.4) | 11 (57.9) | |
| Secondary school | 25 (31.3) | 18 (29.5) | 7 (36.8) | |
| Higher education | 6 (7.5) | 6 (9.8) | - | |
| Main occupation | | | | 0.860 |
| None/at home | 48 (59.3) | 35 (57.4) | 13 (65.0) | |
| Employed | 31 (38.3) | 24 (39.3) | 7 (35.0) | |
| Student | 2 (2.5) | 2 (3.3) | - | |
| Hemoglobin – median g/dL (IQR) | 10.9 (8.7-12.3) | 10.9 (8.7-12.3) | 10.9 (9.2-12.3) | 0.705 |
| CD4 cell count – median cells/μL (IQR) | 400 (239-564) | 404 (246-589) | 325.5 (230-550) | 0.547 |
| HIV RNA – median $\log_{10}$ c/ml (IQR) | 4.6 (4.2- 5.1) | 4.6 (4.3-5.1) | 4.6 (3.5-5.1) | 0.440 |

Data represent n (%) unless otherwise specified. IQR, interquartile range.

identified in 9 (13.2%, 95% CI 6.2-23.6%) of the 68 valid sequences. The respective TDR prevalence was 1.5% (95% CI 0.04–7.9%) for NRTIs, 7.4% (95% CI 2.4–16.3%) for NNRTIs, and 4.4% (95% CI 0.9–12.4%) for PIs. We observed five different SDRMs: K70R and K103N in reverse transcriptase and I85V, N88D and L90M in protease. TDR was confined to a single drug class in all sequences. Table 2 summarizes the characteristics of the nine participants who harbored an SDRM. Using the WHO-recommended truncated sequential sampling technique, 5 of the first 47 sequences harbored any SDRM (moderate prevalence), of which 3 were NNRTI-associated (moderate prevalence), 2 were protease inhibitor-associated (low prevalence), and none was NRTI-associated (low prevalence). HIV-1 subtype A (n = 48, 70.6%) was observed most frequently, followed by unique recombinant forms (n = 14, 20.6%), C (n = 4, 5.9%), and D (n = 2, 2.9%).

## DISCUSSION

This survey among 68 newly HIV diagnosed VCT clients in Mombasa, Kenya, demonstrated an estimated TDR prevalence of 7.4% for NNRTIs, 4.4% for PIs, and 1.5% for NRTIs; no multiclass resistance was detected. The TDR prevalence corresponded with the WHO-recommended truncated sampling technique, yielding "moderate" prevalence overall for the NNRTI drug class and "low" prevalence for the NRTI and PI drug classes. To our knowledge, our survey is among the first to systematically quantify TDR in the coastal region of Kenya. Our findings support the hypothesis that a rise in TDR is occurring with increasing antiretroviral drug exposure.

Few studies of primary HIV drug resistance have been conducted in Kenya. The PASER-Monitoring study among chronically infected, ARV-naive adults revealed an HIV drug resistance prevalence of 4.9% in Mombasa and 4.5% in Nairobi.[16] A study conducted in Nairobi revealed reverse transcriptase mutations in 7.5% of 53 ARV-naive patients initiating ART.[15] The International AIDS Vaccine Initiative cohort of newly infected individuals found a TDR prevalence of 3.1% among 64 participants tested at three sites in Kenya (Nairobi, Mtwapa, and Kilifi).[11] A trend toward increasing prevalence over time was found, with a prevalence of 10% in 2009, but numbers of participants per time period were too small to draw significant conclusions.

To date, most surveys conducted in sub-Saharan Africa using WHO methodology have reported low levels of TDR.[8–10,22,23] By contrast, recent surveys conducted in Kampala,[12] Dar es Salaam,[13] and Yaounde[24] detected higher TDR rates (9–12%) compared to earlier estimates in the same regions, suggesting a trend of increasing TDR. Our study could unfortunately not be compared to an earlier survey in Mombasa. However, in the context

**Table 2.** Characteristics of the participants who harbored a drug resistance mutation.

| ID | Date of enrolment | Inclusion criterion* | Age (years) | Sex | CD4 count (cells/µl) | HIV-1 RNA load ($log_{10}$ c/ml) | Viral subtype | DRMs | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | NRTI | NNRTI | PI |
| KECGP041 | 29-01-2010 | Lab evidence | 35 | M | 369 | 4.6 | A1 | | | I85V |
| KEKSN408 | 23-10-2009 | Lab evidence | 33 | M | 508 | 2.7 | A1/D | | K103N | |
| KELKN209 | 25-01-2010 | Age | 21 | F | 476 | 4.5 | A1 | | K103N | |
| KELKN227 | 23-02-2010 | Age | 23 | M | 87 | 6.1 | A1//C/D | | | N88D |
| KELKN252 | 05-03-2010 | Lab evidence | 30 | M | 564 | 4.9 | A1/D | K70R | | |
| KELKN256 | 11-03-2010 | Lab evidence | 36 | F | 814 | 3.6 | A/D | | | L90M |
| KELKN260 | 11-03-2010 | Lab evidence | 32 | F | 497 | 4.4 | A1 | | K103N | |
| KELKN262 | 12-03-2010 | Age | 23 | F | 487 | 5.3 | A2/D | | K103N | |
| KEMGO602 | 02-06-2009 | Age | 24 | F | 374 | 4.6 | A1 | | K103N | |

DRM, drug resistance mutation; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor. *Study eligibility was based on age (newly diagnosed with HIV and aged between 18 and 25 years) or on laboratory evidence of recent HIV infection (defined as a confirmed positive antibody test with a negative antibody test in the past 12 months, or an indeterminate/negative antibody test with detectable HIV-RNA or p24 antigen).

of prior studies in East-Africa and the available data from chronically infected patients, our data likely represent a rise in TDR among newly infected individuals.

Subtype analysis revealed a predominance of HIV subtype A (70.6%), but also many unique recombinant forms (20.6%). This corroborates a recent study reporting high subtype recombination in a coastal town in Kenya.[25] Our study participants were recruited from the urban setting of Mombasa, a major port with worldwide shipping links, and therefore importation of new HIV-1 strains into the area is likely. These specific characteristics of Mombasa should also be taken into account when interpreting the observed TDR rate, as it may not be extrapolated to more rural, isolated settings in Kenya.

The presence of transmitted resistance mutations has important implications for clinical management of patients in whom ART is indicated, as their risk of virological failure is increased.[3,4] In our study, NNRTI-associated mutations were most common, which is consistent with the widespread use of this drug class for first-line ART and for prophylaxis to prevent vertical transmission. NNRTIs have a low genetic barrier for the development of resistance[26] and mutations may persist for a long time.[27] As NNRTIs are the cornerstone of standard first-line ART in sub-Saharan Africa, reduced susceptibility to this drug class is especially worrisome in view of the limited availability of alternative drug options.

Our study has the following limitations. The WHO-recommended proxy criteria for recent infection were used, which have been suggested to agree poorly with laboratory-based methods to detect recent infection.[22] However, our study included a relatively high number of participants with laboratory evidence of seroconversion and their characteristics, apart from age, did not differ significantly with the participants selected by proxy criteria. Furthermore, although the study specifically selected ARV-naive participants, any unknown or undisclosed prior exposure cannot be ruled out completely.

In conclusion, 7 years after large-scale ART introduction in Kenya, this survey demonstrated a high rate of TDR among newly HIV diagnosed persons in Mombasa. These findings have important clinical consequences as TDR can impair the response to standard first-line ART. Increased vigilance is warranted and TDR surveys should be repeated at regular intervals in order to inform treatment guidelines and drug choices. Surveillance of TDR will help to avoid unnecessary costs of using less-effective first-line regimens and consequent regimen failure. The implementation of an affordable, simplified HIV drug resistance test could help to make TDR threshold surveys more routinely applicable in field conditions in African countries.

## ACKNOWLEDGEMENTS

The authors are grateful to all survey participants, the support staff at the International Center for Reproductive Health in Mombasa, Kenya, the Medical Research Council – Uganda Virus Research Institute (MRC-UVRI) in Uganda and at PharmAccess Foundation in The Netherlands. The PharmAccess African Studies to Evaluate Resistance (PASER) is part of the LAASER program (Linking African and Asian Societies for an Enhanced Response to HIV/AIDS), a partnership of Stichting Aids Fonds, The Foundation for AIDS Research (amfAR)— TREAT Asia, PharmAccess Foundation, and International Civil Society Support.

### Funding

The PharmAccess African Studies to Evaluate Resistance (PASER) is an initiative of PharmAccess Foundation, with financial support provided by the Ministry of Foreign Affairs of The Netherlands through a partnership with Stichting Aids Fonds (grant 12454). The funders had no role in the study design, data collection, data analysis, data interpretation, decision to publish or writing of the report.

## REFERENCES


1. Bennett DE, Camacho RJ, Otelea D, et al.: Drug resistance mutations for surveillance of transmitted HIV-1 drug resistance: 2009 update. PloS One 2009;4(3):e4724.
2. Shafer RW, Rhee SY, Pillay D, et al.: HIV-1 protease and reverse transcriptase mutations for drug resistance surveillance. AIDS 2007;21(2):215–223.
3. Wittkop L, Gunthard HF, de Wolf F, et al.: Effect of transmitted drug resistance on virological and immunological response to initial combination antiretroviral therapy for HIV (EuroCoord-CHAIN joint project): A European multicohort study. Lancet Infect Dis 2011;11(5):363–371.
4. Hamers RL, Schuurman R, and Sigaloff KC: Effect of pre-treatment HIV-1 drug-resistance on immunological, virological and drug-resistance outcomes of first-line antiretroviral treatment: Multicentre cohort in six African countries. Lancet Infect Dis 2011;online.
5. Jain V, Liegler T, Vittinghoff E, et al.: Transmitted drug resistance in persons with acute/early HIV-1 in San Francisco, 2002–2009. PloS One 2010;5(12):e15510.
6. Grant RM, Hecht FM, Warmerdam M, et al.: Time trends in primary HIV-1 drug resistance among recently infected persons. JAMA 2002;288(2):181–188.
7. Wensing AM, van de Vijver D, Angarano G, et al.: Prevalence of drug-resistant HIV-1 variants in untreated individuals in Europe: implications for clinical management. J Infect Dis 2005;192(6):958–966.
8. Ndembi N, Lyagoba F, Nanteza B, et al.: Transmitted antiretroviral drug resistance surveillance among newly HIV type 1-diagnosed women attending an antenatal clinic in Entebbe, Uganda. AIDS Res Hum Retroviruses 2008;24(6):889–895.
9. Bennett DE, Myatt M, Bertagnolio S, Sutherland D, and Gilks CF: Recommendations for surveillance of transmitted HIV drug resistance in countries scaling up antiretroviral treatment. Antivir Ther 2008;13(2):25–36.
10. Somi GR, Kibuka T, Diallo K, et al.: Surveillance of transmitted HIV drug resistance among women attending antenatal clinics in Dar es Salaam, Tanzania. Antivir Ther 2008;13(2):77–82.
11. Price MA, Wallis CL, Lakhi S, et al.: Transmitted HIV type 1 drug resistance among individuals with recent HIV infection in East and Southern Africa. AIDS Res Hum Retroviruses 2010;27(1):5–12.
12. Ndembi N, Hamers RL, Sigaloff KC, et al.: Transmitted antiretroviral drug resistance among newly HIV-1 diagnosed young individuals in Kampala. AIDS 2011;25(7):905–910.
13. Mosha F, Urassa W, Aboud S, et al.: Prevalence of genotypic resistance to antiretroviral drugs in treatment-naive youths infected with diverse HIV type 1 subtypes and recombinant forms in Dar es Salaam, Tanzania. AIDS Res Hum Retroviruses 2011;27(4):377–382.
14. UNAIDS: Global report: UNAIDS report on the global AIDS epidemic 2010. Available at: http://www.unaids.org/globalreport/Global_report.htm. Accessed April 21, 2011.
15. Lihana RW, Khamadi SA, Lubano K, et al.: HIV type 1subtype diversity and drug resistance among HIV type 1-infected Kenyan patients initiating antiretroviral therapy. AIDS Res Hum Retroviruses 2009;25(12):1211–1217.
16. Hamers RL, Wallis CL, Kityo C, et al.: HIV-1 drug resistance in antiretroviral-naive individuals in sub-Saharan Africa after rollout of antiretroviral therapy: A multicentre observational study. Lancet Infect Dis 2011;11:750–759.
17. Tamura K, Dudley J, Nei M, and Kumar S: MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol 2007;24(8):1596–1599.
18. Gifford RJ, Liu TF, Rhee SY, et al.: The calibrated population resistance tool: Standardized genotypic estimation of transmitted HIV-1 drug resistance. Bioinformatics 2009;25(9): 1197–1198.

19. de Oliveira T, Deforche K, Cassol S, et al.: An automated genotyping system for analysis of HIV-1 and other microbial sequences. Bioinformatics 2005;21(19):3797–3800.
20. Kosakovsky Pond SL, Posada D, Gravenor MB, Woelk CH, and Frost SD: Automated phylogenetic detection of recombination using a genetic algorithm. Mol Biol Evol 2006; 23(10):1891–1901.
21. Myatt M and Bennett DE: A novel sequential sampling technique for the surveillance of transmitted HIV drug resistance by cross-sectional survey for use in low resource settings. Antivir Ther 2008;13(2):37–48.
22. Bussmann H, de la Hoz Gomez F, Roels TH, et al.: Prevalence of transmitted HIV drug resistance in Botswana: Lessons learned from the HIVDR-Threshold Survey conducted among women presenting for routine antenatal care as part of the 2007 national sentinel survey. AIDS Res Hum Retroviruses 2010;27(4):365–372.
23. Parboosing R, Naidoo A, Gordon M, Taylor M, and Vella V: Resistance to antiretroviral drugs in newly diagnosed, young treatment-naive HIV-positive pregnant women in the province of KwaZulu-Natal, South Africa. J Med Virol 2011; 83(9):1508–1513.
24. Aghokeng AF, Kouanfack C, Laurent C, et al.: Scale-up of antiretroviral treatment in Cameroon is accompanied by increasing HIV-1 drug resistance mutations in drug-naive patients. AIDS 2011;25(17):2183–2188.
25. Hue S, Hassan AS, Nabwera H, et al.: HIV type 1 in a rural coastal town in Kenya shows multiple introductions with many subtypes and much recombination. AIDS Res Hum Retroviruses 2011; online.
26. Richman DD, Havlir D, Corbeil J, et al.: Nevirapine resistance mutations of human immunodeficiency virus type 1 selected during therapy. J Virol 1994;68(3):1660–1666.
27. Little SJ, Frost SD, Wong JK, et al.: Persistence of transmitted drug resistance among subjects with primary human immunodeficiency virus infection. J Virol 2008;82(11):5510–5518.