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During 2007–2008, surveillance of transmitted human immunodeficiency virus (HIV) drug resistance (TDR) was performed following World Health Organization guidance among clients with newly diagnosed HIV infection attending voluntary counseling and testing (VCT) sites in Ho Chi Minh City (HCMC), Vietnam. Moderate (5%–15%) TDR to nonnucleoside reverse-transcriptase inhibitors (NNRTIs) was observed among VCT clients aged 18–21 years. Follow-up surveillance of TDR in HCMC and other geographic regions of Vietnam is warranted. Data generated will guide the national HIV drug resistance surveillance strategy and support selection of current and future first-line antiretroviral therapy and HIV prevention programs.

As of 2010, Vietnam had an estimated human immunodeficiency virus (HIV) prevalence of 0.29% and 254,000 individuals living with HIV [1]. The epidemic is concentrated in most-at-risk populations, with an estimated prevalence of 18.4% in injection drug users and 3.7% in commercial sex workers in 2009 [2].

As of April 2010 [3], Ho Chi Minh City (HCMC) reported the most cumulative cases of HIV infection (n = 49,552), AIDS (n = 25,711), and AIDS-related deaths (n = 8,175) in Vietnam, with a reported HIV prevalence of 568/100,000 compared with a national prevalence of 187/100,000 [4]. Notably, HCMC accounts for 50% of HIV patients receiving antiretroviral therapy (ART) in Vietnam; as of April 2010, 22,218 patients were receiving HIV care and treatment services at 20 clinics citywide, including 15,796 patients receiving ART [3].

Data from HCMC, including historical reports of suboptimal treatment of many patients, have led to concern among ART program planners regarding possible transmission of HIV drug-resistant virus to newly infected individuals [5–8]. Additionally, anecdotal reports indicate that some individuals receiving ART continue to inject drugs; therefore, it is theoretically possible that if these individuals have drug-resistant HIV, it may be transmitted to previously HIV-uninfected individuals. As a result of these concerns and in support of routine programmatic monitoring of ART scale-up, the United States President’s Emergency Plan for AIDS Relief (PEPFAR) Vietnam funded a collaboration between the National Institute for Hygiene and Epidemiology, the HCMC AIDS Committee, and the US Centers for Disease Control and Prevention (CDC) to conduct surveillance of transmitted HIV drug resistance (TDR) to inform program managers about issues related to
ART scale-up and to provide information for planning future HIV drug resistance (HIVDR) surveillance activities.

METHODS

Transmitted HIV drug resistance surveillance was performed in HCMC from October 2007 to February 2008 using the World Health Organization (WHO) TDR survey method [9], which classifies TDR in specific geographic areas of a country where it is likely to be observed first. The method uses truncated sequential sampling to classify TDR prevalence to specific drug classes as low (<5%), moderate (5%–15%), or high (>15%) using ≤47 consecutively collected specimens.

Participant Inclusion Criteria

Recruited clients were attending voluntary counseling and testing (VCT) sites, were aged 18–21 years, and reported antiretroviral drug (ARV) naivety on a questionnaire. Age and ARV naivety were criteria used to maximize the likelihood that individuals included in the analyses were recently infected, thus maximizing the probability that detected HIVDR was in fact transmitted. The survey received nonresearch de-

Site Selection

Six VCT sites in HCMC (VCT sites in Districts 1, 2, 4, 8, and 10 and in Binh Thanh), funded by PEPFAR in collaboration with the HCMC AIDS Committee, were selected because of their capacity to provide an adequate number of eligible clients and for convenience in specimen collection and transportation.

Specimen Collection Procedures

At the 6 VCT sites, routine procedures included pretest counseling and verbal consent for HIV testing. All testing was anonymous but linked through a unique client code. During the period of this survey, in addition to the routine VCT questionnaire, clients who were aged 18–21 years were asked if they had been exposed to ARV anytime in the past using pictures of ARV pills as an aid. If a client reported ARV naivety, VCT counselors asked the client to participate in the TDR survey using an amended consent. If the client agreed, 5 mL of blood was collected. Both plasma and dried blood spot (DBS) specimens were obtained. The DBS specimens were collected, stored, processed, and transported per WHO guidance. The DBS and whole-blood specimens were transported to the HIV laboratory at the HCMC Preventive Medicine Center, where plasma was prepared and aliquotted into 3 tubes of 0.5 mL each within 72 hours after blood collection. Plasma and DBS specimens were stored at −80°C until shipment on dry ice to the WHO-accredited HIVDR testing laboratory at the CDC in Atlanta, Georgia.

HIVDR Testing

Genotyping was performed using a broadly sensitive assay [10], and specimens were genotyped according to the order of the date and time of the blood draw. Transmitted HIV drug resistance mutations were determined using the 2009 WHO TDR mutations list embedded within the Stanford HIVDR Calibrated Population Resistance tool [11, 12].

RESULTS

Survey Population

From 4 October 2007 to 20 February 2008, 4466 clients received services at the 6 VCT sites, including 4341 who were tested for HIV. Of the clients who were tested, 679 (15.6%) were aged between 18–21 years and thus eligible to participate. Among the 679 eligible participants, 539 (79%) agreed to participate, and 91 of those 539 were confirmed HIV positive. Among the 91, 13 were subsequently excluded from the TDR survey: 5 reported previous ARV exposure, 2 had insufficient blood drawn for HIVDR testing, 4 declined to participate, and 7 were not approached for consent. Therefore, 73 clients were included in the survey. Seventy-two matched plasma and DBS specimens and 1 plasma-only specimen were available for genotyping.

Genotyping of Plasma Specimens and Presence of TDR Mutations

All 73 plasma specimens were successfully genotyped as described above. Following the WHO survey method, the first 47 consecutive specimens were analyzed for the presence of TDR mutations. No specimen had nucleoside reverse-transcriptase inhibitor (NRTI) mutations, specimen 23 had non-NRTI (NNRTI) mutations (Y181C and G190A), and specimen 24 had a protease inhibitor (PI) mutation (F53Y). Using the WHO TDR survey method, TDR to NRTIs, NNRTIs, and PIs was classified as low (<5%) (Table 1).

When all 73 plasma specimens were analyzed, 5 were found to have TDR mutations: 1 had a PI mutation (F53Y), 1 had an NRTI mutation (M184MV), 2 had NNRTI mutations (1 Y181C and G190A, and 1 K103KN), and 1 specimen had both NRTI (D67N, K70R, M184V, T215F, K219EQ) and NNRTI (K101E, G190CS) mutations. Point prevalence estimates with 95% confidence intervals are reported in Table 1.

Amplification and Genotyping of DBS Specimens

Of the 72 plasma-matched DBS specimens, 69 (96.0%) were successfully genotyped. Among the first 47 consecutively collected specimens, specimen 23 had the NNRTI mutations Y181C and G190A and specimen 24 had the PI mutation F53Y, as seen in matched plasma specimens; however, specimen 47 had the NNRTI TDR mutation P225H/P, which was not identified in the matched plasma specimen. Overall, this
also translated to a low prevalence of TDR to NRTI and PI but moderate prevalence of TDR to NNRTI. Specimens with HIVDR were cross checked with epidemiological data to verify no previous pregnancy if the participant was female. Point prevalence estimates with 95% confidence intervals are reported in Table 1. The genotyping concordance rate for the first 47 matched plasma and DBS specimens was 97.9%.

Notably, when the 69 matched plasma and DBS specimens were analyzed, 5 TDR mutations were identified in both plasma and DBS specimens; however, the distribution of TDR mutations differed between plasma and DBS specimens: 1 plasma sample had the M184MV mutation, which was not detected in the DBS specimen, and another DBS specimen had the P225HP mutation, which was absent in plasma.

**DISCUSSION**

**Overall Results and Interpretation of Drug Mutations**

A previous TDR survey conducted in Hanoi, Vietnam among VCT clients in 2006 demonstrated TDR <5% for all drug classes [13], and studies of ART-naive persons in HCMC [8, 14] and the north of Vietnam [15, 16] using different methods have documented low rates of TDR, although some studies report multiclass-resistant virus. Using plasma specimens, this survey in VCT attendees in HCMC documented low prevalence of TDR (<5%) to all drug classes. However, when DBS specimens were used, the prevalence of NNRTI TDR was classified as moderate (5%–15%) and multidrug-resistant virus was detected. In HCMC, the transmission of drug-resistant HIV may be due to several factors, including the widespread use of ARVs in the private sector (especially before ART program scale-up in 2005), where suboptimal regimens or ART adherence can lead to treatment failure and acquired drug resistance, and, to a lesser extent, injection drug use, which may be associated with suboptimal adherence and acquired drug resistance.

The Vietnam Ministry of Health’s Guidelines for Diagnosis and Treatment of HIV/AIDS recommend NNRTIs be part of the first-line regimen, and up to 97% of patients receiving ART currently receive an NNRTI-based regimen [17]. The moderate NNRTI TDR observed in this survey is therefore consistent with the common use of NNRTIs (ie, efavirenz, nevirapine) in Vietnam. The observed case of dual-class TDR may have been due to inclusion of a client who had not disclosed previous ARV exposure or may in fact have been true TDR; however, inclusion of this specimen does not alter the low prevalence classification for plasma specimens but does change the NNRTI TDR prevalence from low to moderate (5.8%) when all 69 DBS specimens are analyzed. The moderate TDR prevalence classification observed in this survey using DBS specimens necessitates action to raise awareness among treatment sites to support and optimize patient adherence and identify early treatment failure among patients who have a history of prior suboptimal ARV use.

Additionally, results should raise concern among ART program managers in HCMC and prompt investigations into programmatic indicators associated with acquired drug resistance and TDR, such as population-level ART adherence, rates of loss to follow-up and retention at treatment sites, and ARV drug supply quality and continuity. Finally, because of these findings, a follow-up TDR survey in HCMC began in October 2011; results should help program managers better track trends in TDR.

**Genotyping From DBS**

The high amplification success rate and high concordance in genotyping results between plasma and DBS specimens support the use of DBS specimens as a sample source for future
surveys and studies. The collection of DBS specimens is technically simpler and less time-consuming, and it requires less blood. Moreover, specimens can be transported at ambient temperature. Although genotyping results from matched plasma and DBS are highly concordant in the current survey and other reports, studies demonstrate that concordance between plasma and DBS is not always 100% [18, 19]. In this survey, we were able to genotype 96% of the plasma-matched DBS specimens. Two factors that may have contributed to failure to amplify from DBS in this survey include low viral load and poor quality of DBS due to the high ambient temperature and humidity in HCMC.

When the WHO TDR prevalence classification method was applied, the prevalence of NNRTI TDR differed between plasma and DBS specimens because an NNRTI mutation was detected in 1 DBS specimen and not in the corresponding plasma specimen. Mutation differences could arise from archived proviral DNA in genotypes obtained from DBS [19]. Nonetheless, a correlation was observed between the prevalence classification using the WHO survey method and point prevalence estimates using all 73 specimens genotyped. Drug classes with low prevalence by the WHO survey method had point prevalences of <5%, and classes with moderate prevalence by the WHO method had point prevalences of 5%–15% (Table 1). Because of the small sample size (total number of specimens collected, N = 73), our confidence intervals were wide, suggesting little added value in the collection of large numbers of specimens to classify TDR beyond the sample size of ≤47 recommended by WHO. The WHO method also consumes less time and resources, an advantage in resource-constrained contexts such as Vietnam.

Limitations of This Survey
Survey results are subject to potential limitations, including the relatively high rate (21%) of refusal to provide informed consent among those eligible to be included in the survey. If these clients had TDR, this could have affected the classification. In this survey, where additional blood was required, client refusal may have been unavoidable; therefore use of remnant diagnostic specimens in future surveys would be expected to increase the participation rate. In addition, this survey was performed at only 6 of a total of 20 VCT sites in HCMC; therefore, participants may not represent the entire population receiving HIV testing in HCMC. Inclusion of more sites in different districts may have provided better representativeness and shortened the specimen collection phase. Finally, results from this survey should not be generalized to all of Vietnam, given the different levels of access to and scale-up of ART throughout Vietnam’s 63 provinces.

CONCLUSIONS
The prevalence of TDR to NRTI and PI in HCMC, Vietnam was found to be low (≤5%), whereas the prevalence of TDR to NNRTI was moderate (5%–15%). Follow-up surveys in the same geographic area and expansion to other areas should be considered and implemented to provide ART program planners with a more complete picture of current and future TDR trends in Vietnam.

Notes

Acknowledgments. The authors acknowledge Dr Nguyen Thi Hoang Lan from Ho Chi Minh City Preventive Medicine Center (PMC), Mr Tran van Anh Phuong and Ms Vo Thi Mong Hien, VCT coordinators at the Ho Chi Minh City AIDS Committee (PAC), and other PAC and PMC staff for their efforts in survey implementation. We are greatly indebted to all staff at 6 VCT sites (District 1, District 2, District 4, District 8, District 10, and Binh Thanh). We thank all the VCT clients whose participation made this survey successful. We thank Drs Lisa Cosimi and Ritu Shrivastava for their contribution to the development of the protocol and Dr Michael Jordan (WHO Geneva) for his assistance in data analysis, interpretation of results, and finalization of the manuscript. We acknowledge our colleagues at CDC/GAP Vietnam, Drs Bruce Struminger and Nicholas Medland and Mr Kyle Bond, for their support.

Disclaimer. The conclusions and opinions expressed in this article are those of the authors and do not reflect those of their respective organizations, including the Centers for Disease Control and Prevention.

Financial support. This work was supported by the US President’s Emergency Program for AIDS Relief (PEPFAR) through a cooperative agreement between the US Centers for Disease Control and Prevention and the National Institute for Hygiene and Epidemiology (Hanoi, Vietnam). Dr Nguyen Bui Duc received training support from the Emory AIDS International Training and Research Program (NH/IFC D43 TW01042). 

Supplement sponsorship. This article was published as part of a supplement entitled “The World Health Organization HIV Drug Resistance Prevention and Assessment Strategy: Global, Regional, and Country Progress,” sponsored by The Bill & Melinda Gates Foundation (38180).

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

7. Giang LT, Thuy HT, Nhung VT, et al. ARV resistance in patients with treatment failure to first-line regimens in Ho Chi Minh City,


