

HIV drug resistance threshold survey using specimens from voluntary counselling and testing sites in Hanoi, Vietnam

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Background: In countries where antiretroviral therapy has been available or is being rapidly expanded, the World Health Organization (WHO) recommends surveillance for transmitted HIV drug resistance (HIVDR) by threshold surveillance methods using specimens from antenatal clinics or voluntary counselling and testing (VCT) sites. The aim of this study was to implement the HIVDR threshold survey in VCT sites in Vietnam, where HIV prevalence is high. Estimating transmitted resistance in the infected population will enable the appropriateness of current antiretroviral drug regimens to be assessed and will inform plans for future HIVDR surveillance.

Methods: Consecutive blood specimens were collected from 70 newly diagnosed HIV-positive clients 18–24 years of age at two sites in Hanoi, Vietnam. Informed consent and serum specimens were obtained from each eligible client, with serum frozen at -70°C until

shipping to Thailand for resistance testing using the TruGene system.

Results: From February until August 2006, 559 clients were eligible to participate in this survey. Of the 535 clients (95.7%) who agreed to participate, 70 (13%) were HIV-positive and were included in the survey. Of the 70 specimens sent for genotyping, 52 consecutive samples were amplified, 49 of which could be genotyped. Only 1 of 49 genotyped specimens had mutations associated with drug resistance (L74V and Y181C) in the reverse transcriptase gene, indicating that the prevalence of transmitted HIVDR to all drugs and drug classes evaluated was $<5\%$.

Conclusion: The prevalence of transmitted HIVDR was low in Hanoi as determined using threshold surveillance methods. The Ministry of Health plans to repeat this survey methodology in one more province and to confirm these findings by expanded HIVDR surveillance.

Introduction

The first HIV case in Vietnam, a developing country with a population of 83.1 million [1], was reported in 1990. Since then, the number of HIV cases has dramatically increased. Through 2006, ~20,000 AIDS cases and 11,000 AIDS-related deaths were reported to the Ministry of Health (MOH) [2]. Vietnam has a concentrated epidemic; the majority of cases are among intravenous drug users (IDU) and commercial sex workers (CSW). The Joint United Nations Programme on HIV/AIDS (UNAIDS) estimated that

the overall prevalence of HIV among adults (15–49 years old) was 0.5% [3]; however, the prevalence among IDUs, CSWs and pregnant women was 26%, 3.5% and 0.37%, respectively [4].

Antiretroviral therapy (ART) was first introduced to Vietnam in the mid-1990s. Initially, most regimens included only two nucleoside reverse transcriptase inhibitors (NRTIs) selected from zidovudine, lamivudine, stavudine and didanosine, which were available through the national programme. The Vietnam

National Guidelines for Diagnosis and Treatment of HIV/AIDS published in 2000 recommended dual therapy [5]. The number of patients who had access to free ART was initially limited to a few patients in the national programme. However, some individuals were able to purchase locally produced and black market antiretrovirals (ARVs) without a prescription in local pharmacies. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs) became available more recently (around 2004), but were not consistently available causing many patients to switch between dual and triple therapy during the course of their treatment. In 2005, the updated National Guidelines for Diagnosis and Treatment of HIV/AIDS published by the MOH recommended triple therapy with two NRTIs (stavudine plus lamivudine or zidovudine plus lamivudine) plus one NNRTI (nevirapine or efavirenz) in the first-line regimen [6]. Starting in 2005, Vietnam underwent a rapid ART scale-up supported by the US President's Emergency Plan for AIDS Relief (PEPFAR), the Global Fund for AIDS, Tuberculosis and Malaria (GFATM) and the national programme. Free standardized ART supported by these programmes has been available mostly in large cities hardest hit by the HIV epidemic. ARVs in the first-line regimen (stavudine, zidovudine, lamivudine, nevirapine and efavirenz) and a limited amount of ARVs for the second-line regimen (abacavir, didanosine, nelfinavir and lopinavir/ritonavir) are officially provided in treatment sites. The MOH/Vietnam Administration for AIDS Control (VAAC) reported that, as of September 2007, 15,000 patients had received free ART. Vietnam's target by September 2009 is 22,000 patients receiving ART.

It is important for Vietnam to understand the extent of HIV drug resistance (HIVDR) in the country; in the context of rapid ART scale-up, and given the history of provision of suboptimal drug regimens, a risk for transmitted drug resistance exists. In 2006, PEPFAR Vietnam funded a collaboration between the MOH's National Institute for Hygiene and Epidemiology (NIHE), the World Health Organization (WHO), and the US Centers for Disease Control and Prevention (CDC) to conduct an HIV drug resistance threshold survey (HIVDR-TS) to inform ART programme scale-up and plan for future HIVDR surveillance activities.

Methods

The Vietnam HIVDR-TS protocol was adapted from the WHO HIVDR-TS protocol for evaluation of transmitted HIV drug resistance using specimens from unlinked HIV serosurveys in resource-limited settings [7].

Geographical area

Hanoi, the capital city of Vietnam, is ranked second among provinces in the number of reported HIV infections (11,600 by December 2006) and sixth in HIV prevalence (362/100,000 persons). The number of patients on ART in the public sector in Hanoi as of December 2006 was 672, but the number of patients on ART through private sectors was unknown. Hanoi was chosen for this survey because the city had ART available since the mid-1990s, had access to ART through the national programme and other donors, and also had national referral centres for HIV patients. Suboptimal regimens and treatment failures have been seen among HIV-infected patients in Hanoi. Therefore, HIV drug resistance is likely to have emerged among some treated patients in Hanoi and could potentially be transmitted.

Population

The WHO protocol recommends choosing treatment-naïve pregnant women <25 years old in their first pregnancy attending antenatal clinics, or clients at voluntary counselling and testing (VCT) sites who are <25 years old ('eligible clients'). These populations would be more likely to be infected relatively recently; that is, since the start of ART availability during which transmission of resistant strains was possible. Exclusion of older individuals also minimizes the risk that previously diagnosed drug-experienced individuals will be included. In Vietnam, the prevalence of HIV among pregnant women was very low (0.37% as of 2005 [4]), and prevalence among VCT clients was much higher (~17%); therefore, VCT clients from 18 to 24 years old were chosen for this survey in order to ensure sufficient numbers for the analysis.

Sites

Two VCT sites in Hanoi were selected as sites for specimen collection. These sites were within 1 h travel to the referral HIV laboratory at NIHE. Each site served ~150–200 clients per month with 15–20% of clients testing positive for HIV. Of these clients, 20% were under 25 years old.

Specimen collection procedures

At the two VCT sites, routine procedures included performing pre-test counselling and consent for an HIV test. If verbal consent was given by the client, 3 ml of blood were drawn for HIV serology testing. All testing was anonymous, but linked through a unique client code.

An amendment to the existing verbal consent was developed to explain the survey and ask the client to participate in the HIVDR-TS. If the client agreed, 5 ml of blood were drawn instead of 3 ml. Serum was then

separated on-site and aliquoted into three vials. Two of these aliquots (0.5 ml each) were reserved for HIVDR testing, the remainder was used for routine HIV serology testing. The serum specimens for HIVDR testing were kept at refrigeration temperature (4°C) until they were shipped to the HIV referral laboratory at NIHE to be frozen. Specimens from clients subsequently determined to be HIV-negative were discarded.

Sample size and statistical approaches

The sample size and statistical approaches of this survey were adapted from the WHO protocol [7]. A binomial sequential sampling strategy was used. According to this strategy, a maximum of 47 consecutive HIV-infected specimens should be genotyped to categorize the HIVDR prevalence into three categories: low (<5%), moderate (5–15%) and high (>15%). However, to ensure successful amplification of 47 specimens, it is recommended that 60–70 HIV-infected specimens be collected. Results from resistance tests (that is, the presence of mutations) were used to complete a simple sampling and classification plan [7], and the classification was made on the basis of that plan. In this survey, specimens from 70 consecutive HIV-infected clients were collected.

Serological testing

HIV serology testing was performed per the standard MOH protocol [6], which followed WHO strategy III [8].

Questionnaire

At the VCT sites, a questionnaire ('VCT client intake form') is used to collect client information. In this survey, the existing VCT questionnaire was used without modification. Questions regarding previous ARV drug exposure were not added to the questionnaire, because it would change the routine VCT protocol and create additional work load for staff.

HIVDR testing

Of the two sets of 70 frozen serum aliquots received at NIHE, one set was shipped to the Thai Ministry of Public Health – US CDC Collaboration (TUC) HIV/Sexually Transmitted Infections Research laboratory in Bangkok, Thailand for HIVDR testing. The other set remains at NIHE and will be analysed at NIHE as part of a laboratory capacity building effort. The date and time of blood draw of each specimen was recorded. HIV-positive specimens were sorted in order of date and time of blood draw. According to the HIVDR-TS protocol, specimens were amplified and sequenced consecutively in that order. HIVDR testing was performed using the TruGene HIV-1 genotyping assay (HIV-1 TruGene™ Genotyping system, Bayer HealthCare, Tarrytown, NY, USA). Mutations

were classified as either resistance mutations, silent mutations, polymorphisms or unexpected mutations at resistant sites, based on the Bayer Guidelines 10.0 library and aligned with a clade B HIV-1 reference sequence (HIV-1_{LAV}). Mutations detected were compared with the WHO recommended drug resistance mutations for surveillance ('WHO list') [9] and International AIDS Society (IAS) HIVDR mutations list (autumn 2006) [10].

HIV subtyping

To determine the genotypes of newly generated sequences, preliminary phylogenetic analyses were performed using the National Centre for Biotechnology Information (NCBI) genotyping tool (<http://www.ncbi.nlm.nih.gov/projects/genotyping/>).

Ethical considerations

The confidentiality of clients participating in this survey was maintained using the existing anonymous testing protocol at VCT sites. Information on the VCT client intake form was not available to study staff. The survey received a non-research determination by the Associate Director of Science, National Centre for HIV, Hepatitis, STD and TB Prevention, CDC. The survey was approved by the MOH of Vietnam.

Results

Survey population

From 10 February to 11 August 2006, a total of 1,872 clients received services at the two VCT sites. Of these, 559 (30%) clients were eligible to participate in this survey by age criteria. However, 12 of the 559 were not tested for HIV because they refused HIV testing or for unknown reasons. Of the remaining 547, 11 were not approached for consent and one refused consent. Thus, among those who were approached for consent, the consent rate was high (535/536 or 99.9%). All 11 clients who were not approached for HIVDR-TS consent were HIV-negative, whereas the one non-consenting client was HIV-positive. Of the 535 clients who agreed to participate, 70 (13%) were HIV-positive and were included in the survey. The number of clients surveyed by age, and the HIV status of those in each age group, is shown in Table 1.

Amplification of serum specimens

The first 52 consecutive HIV-positive serum specimens were used for genotyping. Three (5.8%) of these 52 specimens were not amplifiable for genotyping; therefore, 49 were amplified and sequenced to detect mutations. This met the requirement of 47 amplifiable samples in the WHO HIVDR-TS protocol.

Presence of drug resistance mutations

Reverse transcriptase mutations

Of 49 specimens tested, six (12%) specimens had mutations associated with HIVDR in the reverse transcriptase (RT) according to the TruGene criteria: V118I was found in two, T69N in three, and both L74V and Y181C were found in one specimen. Of these four RT mutations, V118I and T69N are not in the WHO list of recommended mutations for use in HIVDR surveillance nor in the IAS HIVDR list of mutations; therefore, they were not considered transmitted drug-resistant mutations and were likely to be polymorphisms. The L74V (an NRTI mutation) and Y181C (an NNRTI mutation) are in both the WHO and IAS lists [9,10] and might represent transmitted mutations. Although 49 specimens were genotyped, according to the HIVDR-TS methodology, genotyping can be stopped when the lower limit is reached; in this case, because only one specimen with drug-resistant mutations was detected, genotyping was not stopped until specimen number 44 when the running total was less than the lower limit (Table 2). This translated to a low (<5%) prevalence of transmitted resistance to all relevant ARV drugs and drug classes.

Protease mutations

Amino acid changes in the HIV protease were found in all 49 amplified specimens; however, none of the changes found in the protease region were in the WHO list for surveillance of transmitted mutations [9]. Therefore, they were all considered polymorphisms. In this case, as no specimen with drug-resistant mutations was detected, sampling was stopped at specimen number 34 when the running total was less than the lower limit, demonstrating a low (<5%) prevalence of transmitted protease inhibitor resistance.

HIV subtypes

All HIV strains found were circulating recombinant forms (CRF). Of 49 specimens sequenced and analysed, 17 were CRF01 (or CRF01_AE), 10 were CRF15 (or CRF15_01B) and 22 appeared to be closely related to both CRF01 and CRF15.

Table 1. Number of clients by age and HIV status

Age, years	Clients, <i>n</i>	HIV-positive clients, <i>n</i> (%)
18	15	0 (0)
19	20	2 (10.0)
20	52	4 (7.7)
21	52	3 (5.8)
22	66	7 (10.6)
23	127	16 (12.6)
24	203	38 (18.7)
Total	535	70 (13.0)

Discussion

Overall results and interpretation of drug mutations

This survey found a low threshold prevalence of transmitted HIV drug resistance (<5%) in Hanoi, suggesting that the first-line regimens used in the national ART programme should be effective. The finding of a low prevalence may reflect the fact that, although ART has been scaled-up in the city, the number of patients receiving ART in Hanoi may not be high enough to generate and transmit resistant mutations. Moreover, most HIV infections are in IDUs, who tend to have poor access to healthcare, including ARVs; therefore, drug-resistant mutations might be less likely to emerge, and be transmitted within this group [11,12].

A recent study in Ho Chi Minh City, Vietnam [13] found 13 sequences carrying drug-resistant mutations in 200 newly diagnosed HIV patients with unknown duration of infection. These mutations included one M41L, four K219Q and four M184I mutations in the RT gene, and one D30N and three L90M mutations in the protease gene. These mutations are in the WHO list of mutations; the prevalence of HIVDR of 6.5% (95% confidence interval 3.1–9.9) found in this study reflects some degree of transmitted resistance in Ho Chi Minh City. However, the confidence interval indicates that the prevalence of transmitted HIVDR could not be confirmed to be >5%. If prevalence of transmitted resistance is subsequently seen to be higher, it could be associated with the fact that the number of patients on ART is higher and that ART began earlier in Ho Chi Minh City compared with Hanoi. Unlike the present survey, the Ho Chi Minh City study included HIV-infected patients with a larger age range (15–52), which might not represent a recently infected population. Only seven persons with mutations were questioned again and confirmed to have no previous ART exposure; therefore, if some of the patients were in fact not treatment-naïve, the prevalence of transmitted resistance could be overestimated. Expansion of the threshold survey to Ho Chi Minh City would help to address this question.

In this survey, the L74V mutation, which confers resistance to abacavir and didanosine, was unexpected as abacavir and didanosine have not been used commonly in Vietnam. Although some didanosine was available early in the mid-1990s, abacavir and didanosine are now restricted to a limited number of patients who need second-line regimens. This mutation was not found in the Ho Chi Minh City study cited above [13] nor in any of the 635 CRF01 strains reported in the article by Shafer *et al.* [9] from which the WHO list was derived. The Y181C mutation, which confers cross-resistance to all Food and Drug Administration (FDA)-approved NNRTIs, was found in this survey

Table 2. Sampling and classification plan for reverse transcriptase mutations*

Sample number genotyped	Lower limit	Running total of specimens with HIVDR	Upper limit
1	ND	0	ND
2	ND	1	ND
3	ND	1	ND
4	ND	1	ND
5	ND	1	ND
6	ND	1	ND
7	ND	1	ND
8	ND	1	ND
9	ND	1	ND
10	ND	1	ND
11	ND	1	ND
12	ND	1	ND
13	ND	1	ND
14	ND	1	5
15	ND	1	5
16	ND	1	5
17	ND	1	5
18	ND	1	5
19	ND	1	5
20	ND	1	5
21	ND	1	5
22	ND	1	5
23	ND	1	5
24	ND	1	5
25	ND	1	6
26	ND	1	6
27	ND	1	6
28	ND	1	6
29	ND	1	6
30	ND	1	6
31	ND	1	6
32	ND	1	6
33	ND	1	6
34	1	1	6
35	1	1	7
36	1	1	7
37	1	1	7
38	1	1	7
39	1	1	7
40	1	1	7
41	1	1	7
42	1	1	7
43	1	1	7
44	2	1	7
45	2		7
46	2		8
47	2		8
Stop	Stop	Stop	Stop

*Each line in the classification table included a lower and an upper limit. The lower and upper limits were calculated using binomial sequential sampling methods and were constant [7]. After each specimen was genotyped, the total number of specimens that had mutations (third column) was compared with the limits in the same line. If the number was less than the lower limit, sampling could be stopped and prevalence was classified as low (<5%). If the number was greater than the upper limit, sampling could be stopped and prevalence was classified as high (>15%). If after 47 consecutive specimens were genotyped, the total number was still within the limits (that is, the number was from 2 to 8), the prevalence was classified as moderate (5–15%). In this survey, only one specimen had reverse transcriptase mutations and after analysis of 44 specimens the total number was 1, less than the lower limit, so the prevalence of resistance was classified as low. HIVDR, HIV drug resistance; ND, no decision.

and in 0.2% of the 635 CRF01 strains reported by Shafer and colleagues [9], but not in the Ho Chi Minh City study [13]. Therefore, the result of this survey, especially the specific drug-resistant mutations found, suggest that additional surveys might be useful to better understand the HIVDR patterns in Vietnam.

The study population and specimen processing

After 6 months, 70 consecutive HIV-positive specimens were obtained from eligible VCT clients. The consent rate was very high. The few clients who were not approached for consent were HIV-negative; therefore, this survey was able to follow the protocol for obtaining consecutive HIV-positive clients. Only one HIV-positive client did not agree to participate. However, if this client had drug-resistant mutations in the RT, it could affect the interpretation of the threshold classification towards moderate prevalence (the running total after 47 specimens would be two and be within the two limits, indicating moderate prevalence).

All specimens had adequate volume, high quality and were carefully shipped and stored. This was reflected in the high rate of successful amplification. In the three samples that failed to produce an amplification signal, a reason could not be determined; according to records, these specimens were processed according to protocol and shipped in good condition. Viral loads were not established to see if these samples had undetectable or low viral loads, thereby effecting ability to amplify virus. The success of this sample collection phase was the result of a concerted effort to train VCT staff and coordinate closely with the HIV referral laboratory at NIHE.

HIV subtypes

HIV types detected in this survey were all recombinant forms. Previous studies in Vietnam also found a predominance of CRF01 subtype [13]. However, the proportion of HIV strains of the CRF15 subtype (or intersubtype recombinant of CRF01 and subtype B) was found to be higher in this survey than in other studies from the South of Vietnam [13]. The 22 sequences with close relationships to both CRF01 and CRF15 might also indicate a recombination of those CRFs. Determining whether the subtype distribution differs between the North (for example, Hanoi) and the South of Vietnam will need further investigation with a larger sample size.

Limitations of the survey

This survey should be interpreted in the context of several limitations. First, the survey could not exclude clients who had been exposed to ARVs; instead a proxy measure (age range) was used. An additional questionnaire would help resolve this problem, but would

require extensive training, more work for staff, and would change the routine VCT data collection. As this survey is intended to be incorporated into routine programmatic activities, temporary changes in the VCT procedures for survey purposes should be avoided if possible. Second, the survey was performed at only two VCT sites in Hanoi, which might not represent the entire testing population of the city. Inclusion of more sites in different districts may provide better coverage, shorten the specimen collection phase and allow for the selection of clients from a lower age range, thus improving the proxy measure of 'recent infections'. 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 the 64 provinces.

Future directions and recommendations

To further assess the picture of HIVDR in Vietnam, MOH/NIHE is planning to expand the survey to other provinces where drug-resistant mutations are likely to develop. One threshold survey in Ho Chi Minh City started in October 2007 and finished specimen collection. Results from such surveys will provide a more complete picture of transmitted HIVDR in Vietnam. Additional surveys to assess whether individuals in different HIV risk categories have differing risks of HIVDR transmission might also be helpful. Finally, given the rapid ART scale-up, population-based monitoring of HIVDR in clients on ART should also be considered.

Although a high prevalence of transmitted HIVDR mutations has not been found in Hanoi, Vietnam, prevention of HIVDR emergence is important. The national ART programme should continue to develop monitoring systems for drug quality and delivery to maximize adherence to ART and ensure that the national ART protocol is being followed.

Summary

A newly developed threshold surveillance method for HIVDR recommended by the WHO was implemented at two VCT sites in Hanoi, Vietnam. The prevalence of transmitted HIV drug-resistant mutations was found to be <5%, suggesting that the national ART programme's first-line regimens can be used with confidence in the area. Programmes providing ART should maintain vigilance to ensure adherence and continued supply of high-quality ARV drugs. The implementation of this survey has also demonstrated initial success that will strengthen the national ART programme in Vietnam. HIVDR surveillance capability is being built at the national level in Vietnam, laboratory expertise is being developed, and regional laboratory expertise has been used to strengthen national laboratory capacity.

However, these results may not be representative of the HIVDR transmission in all of Vietnam and expansion of HIVDR surveillance is warranted.

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Disclosure statement

The authors declare that they have no competing interests.

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