Transmitted HIV Drug Resistance Among Drug-Naive Subjects Recently Infected With HIV in Mexico City: A World Health Organization Survey to Classify Resistance and to Field Test Two Alternative Patient Enrollment Methods

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In 2004, the World Health Organization performed a survey to assess transmitted drug resistance in Mexico City among drug-naive persons with newly diagnosed human immunodeficiency virus (HIV) infection and likely to be recently infected who were attending 3 voluntary counseling and testing sites. A parallel study comparing 2 alternative methods of enrolling survey participant was conducted in 9 voluntary counseling and testing sites in central Mexico. In study arm 1, subject information, consent and blood specimens were obtained during the HIV diagnostic testing visit. In study arm 2, consent and blood specimens were obtained at the return visit, only from those who were HIV infected. This survey classified nonnucleoside reverse-transcriptase inhibitor and nucleoside reverse-transcriptase inhibitor transmitted drug resistance as <5% and 5%–15%, respectively. Arm 2 yielded major advantages in cost and workload, with no evidence of increased sampling bias.

In Mexico, an estimated 220 000 persons had human immunodeficiency virus (HIV) infection in 2009 [1]; 8800–21 000 persons were newly infected with HIV, and between 9800–15 000 persons died of AIDS. The prevalence of HIV infection in the adult population in Mexico is among the lowest in the region and has stabilized over the past decade at 0.3%. However, higher estimated rates have been found in certain subpopulations: 9.9% among men who have sex with men, 5.5% among female sex workers, and 2.8% among injection drug users (IDUs) [2, 3]. The epidemic has had a varied impact across the country, with the highest numbers of cumulative cases reported in Mexico City and the states of Mexico, Veracruz, and Jalisco [2].

Since 2003, the Mexican government has had a policy of universal access for antiretroviral therapy (ART), and nearly all HIV medications on the market in the United States are available in Mexico through both public and private programs [4]. However, not everyone who is ART eligible has access to treatment. On the basis of the new World Health Organization (WHO) treatment guidelines released in 2010, Mexico has delivered ART to 54% of eligible individuals [1, 3].

A recent large, national, prospective study reported 7.2% prevalence of transmitted drug resistance (TDR) to any drug class among 1655 chronically infected and antiretroviral (ARV) drug-naive patients from 12 Mexican states who were enrolled from 2003 through 2010 [5]. However, data on prevalence of TDR in Mexico among persons with newly diagnosed HIV infection who are
ARV naive are limited. Most studies have methodological problems that limit interpretation—above all, the use of convenience samples from chronically infected individuals and use of mutation lists that include polymorphisms [6, 7].

In 2004, the WHO developed methods to assess TDR in individuals with newly diagnosed HIV infection who were likely to be recently infected and who were ARV naive, to generate comparable TDR prevalence data across regions and time. The methodology consists of classification of TDR to 3 drug classes as above or below 2 thresholds, 5% and 15%, using a maximum of 47 specimens [8, 9]. The method includes a defined list of resistance mutations that are not polymorphic in any HIV subtype and are associated with ARV in ≥1 subtypes [8]. Results from WHO surveys apply to the population and to the geographic area where the survey is conducted. This survey used WHO methods to assess TDR in a population with newly diagnosed HIV infection, likely to be recently infected and ARV naive, who were attending voluntary counseling and testing (VCT) sites in Mexico City.

In addition, we conducted a parallel study comparing 2 different approaches to TDR surveillance at VCT sites, the first enrolling participants at the HIV diagnostic testing visit and the second at the follow-up visit. We evaluated each approach from a cost and work standpoint and in terms of its adequacy for producing reliable estimates of HIV drug resistance prevalence.

METHODS

HIV Drug Resistance Transmission Survey

From January through July 2004, ARV-naive individuals with newly diagnosed HIV infection who were seeking HIV diagnostic testing in 3 large VCT sites in Mexico City were evaluated for inclusion in the survey. In Mexico City, these 3 sites (Centro de Atención Profesional para Personas con VIH/SIDA, Centro Nacional para la Prevención y el Control del VIH/SIDA, and Fundación Mexicana para la Lucha Contra el SIDA) account for 45% of new HIV diagnoses from VCT and 28% of all new HIV diagnoses and, therefore, provide reasonable representation of the population in this city who have received new diagnoses. To minimize the risk of including individuals with long-term infection and ARV exposure, all persons meeting the following criteria were consecutively enrolled: new HIV infection diagnosis or first HIV-positive test result <6 months before the survey start date, self-reported ARV naive, resident of Mexico, age 14–25 years, no signs or symptoms of AIDS assessed by medical staff of the site, and signed informed consent. Persons refusing participation were replaced by the next eligible person.

Demographic information was recorded from all persons, including those who refused to participate. Questionnaires were transferred to Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ) for quality control and coding and were entered into a central local database. Data were finally transferred to the International Data Center, Frontier Science and Technology Research Foundation (FSTRF), for the analysis. For each participant, 2 tubes of ethylenediaminetetraacetic acid anticoagulated whole blood (8 mL total) were collected. Blood was centrifuged, and plasma was sent to the INCMNSZ within 24 hours of collection, where it was stored at −70°C and then shipped frozen to the WHO-accredited laboratory for genotyping (National HIV and Retrovirology Laboratories, Public Health Agency of Canada). Specimens were genotyped as described elsewhere [10]. TDR was classified using standard WHO methods [8, 9]. In addition, HIV subtype was determined by phylogenetic analysis of amplified sequences.

Nested Study

Two enrollment methods were compared. For study arm 1, the questionnaire and consent were administered during the initial visit of every person seeking an HIV test, and if consent was obtained, 2 specimens were collected: 1 for HIV testing and 1 for resistance testing. If the participant tested positive for HIV, the questionnaire was sent to INCMNSZ and the specimen was processed for resistance testing; otherwise, the form was retained for auditing use only, and the extra specimen was discarded. For study arm 2, the questionnaire was administered during the initial visit; however, consent was not requested, and an extra specimen was not obtained. Approximately 2–4 weeks later, when the participants returned to receive the Western blot confirmatory test results, those who tested positive for HIV were asked for consent to participate in the study and for an extra specimen for resistance testing.

The nested study was fielded at the same time as the TDR survey and used identical specimen handling procedures. It was conducted in 9 VCT sites in 7 states in central Mexico. The procedures worked quite differently for the 2 arms. Administering both processes simultaneously would have been burdensome for VCT sites, and therefore, the arms were implemented sequentially in participating sites: arm 1 for the first 3 months (January–April) and arm 2 for the latter 3 months (May–July).

Information on all the VCT sites in the states of central Mexico was collected. The following eligibility criteria were used to identify representative sites for each state: (1) >50 new HIV diagnoses per year; (2) capacity to accurately determine eligibility and enroll the eligible, administer informed consent, complete subject questionnaires, answer queries about data mismatches, and preserve confidentiality; and (3) ability to collect blood samples, centrifuge and freeze specimens at least to −20°C, or ability to transport specimens to INCMNSZ on a same-day basis. All sites in central Mexico that met these criteria were recruited, except in Veracruz where the state’s largest testing site did not participate and the next largest one was recruited so that the state would be represented. One site in the state of Jalisco was unable to follow survey procedures and
was omitted from the analysis, reducing the original sample of sites from 10 to 9. This sample included 3 VCT sites in Mexico City and 1 each in Toluca (state of Mexico), Morelia (Michoacán), Cuernavaca (Morelos), Oaxaca (Oaxaca), Puebla (Puebla), and Tlaxcala (Tlaxcala). Except for the states of Veracruz and Mexico, the centers participating in the study were those responsible for a good proportion (22%–41%) of all new diagnoses in their respective states.

The 2 arms were compared for burden for both the site and the processing laboratory and, likely, data quality and representativeness. Burden was measured by cost, time, and resources required to conduct the survey in each arm and was calculated using the following criteria: (1) site workload, defined as time spent by the VCT staff to collect specimens, informed consent, and questionnaires; (2) processing laboratory workload, defined as time spent by laboratory staff at the processing laboratory to handle specimens (including receiving, checking, logging, and storing specimens) and quality assurance participant data (including receiving, checking, and data entry); and (3) direct cost, defined as cost for shipment of specimens from VCT sites to the processing laboratory and cost of laboratory equipment and supplies. Representativeness was assessed by comparing characteristics (sex, age, risk factors for HIV, and reported HIV-negative test result in the past year) of the resulting arm 1 and 2 samples.

RESULTS

HIV Drug Resistance Survey
The first consecutive 47 specimens from the 54 eligible participants enrolled in the 3 VCT sites in Mexico City during the survey period were genotyped. The mean age of participants was 22 years (range, 18–25 years); 90% were male. All sequences were HIV subtype B. TDR to nucleoside analog reverse transcriptase inhibitors (NRTIs) was classified as moderate (5%–15%), with mutations detected in 3 samples (L210W + T215S, T69ADNT, and M184V + T215ST). TDR to nonnucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs) was <5%, with no mutations detected for these classes. Specimens with HIVDR were cross checked with epidemiological data to verify no previous pregnancy if the participant was female.

Nested Study
Figure 1 shows study flow in a Consort-like diagram. During the 6-month study period, 7746 persons were screened for HIV infection at the 9 VCT sites. As expected, approximately one-tenth (782 participants) were HIV positive. Of these, 516 (66%) were included in the analysis dataset. The various losses (ineligibility, nonreturn in arm 2, nonconsent, and amplification failures) are shown in the figure. Table 1 shows the comparison in labor and costs between arms 1 and 2. The differences are substantial, with major savings in arm 2 on both dimensions: 5.75 hours of labor and $25.70 in other direct costs per study subject in the analysis dataset. Given these results, the key question is what loss of quality, if any, the savings of arm 2 entail and whether one will get different results if the more economical arm 2 approach is used. Supplementary Table 1 shows characteristics of clients enrolled in the 2 arms. None of the differences are significant; in fact, arm 1– and arm 2–enrolled participants appear to be similar. Specifically, the 2 arms showed no difference for any of the following variables: age, sex, risk factor for HIV infection, and having a negative HIV test result within the past year. Although these findings do not entirely remove the possibility of different sequencing results (which unfortunately are not available), they make the likelihood of such differences low.

Supplementary Table 2 examines one possible source of bias—nonreturn—by comparing returning and nonreturning patients. Supplementary Table 3 is a parallel analysis of nonconsenting patients. Overall, the results in these 2 tables suggest that neither nonreturn nor nonconsent marked a distinctive population, one from which different results would be expected. The only significant difference found was that nonconsenters were considerably less likely to report a heterosexual risk factor in arm 1 and in the 2 arms combined.

DISCUSSION

HIV Drug Resistance Survey
Newly infected persons are an optimal population in which to assess TDR, because HIV tends to revert to wild type over time, with resistant subspecies remaining but not present in sufficient density to be detected by standard sequencing methods [11]. However, because no country has routine, universal HIV testing, it is challenging to construct a valid sample of new infections. Therefore, adapting the WHO recommendations, we used age <25 years and absence of signs and/or symptoms of AIDS as a surrogate for recent infection, and excluded individuals who reported previous ARV use or a diagnosis of HIV infection >6 months before the survey start date. However, we acknowledge that these criteria may not have completely eliminated chronically infected individuals or ARV-exposed individuals from our sample.

Diagnostics of recent infections occur in various settings, including private medical practices, antenatal clinics, sexually transmitted diseases clinics, and institutions that require HIV testing. In Mexico, however, the largest number of new HIV diagnoses comes from VCT sites. VCT sites were therefore used as the primary sampling units for this survey. Ideally, all eligible VCT sites in Mexico City that meet eligibility criteria would have been included. However, for practical reasons, the survey was restricted to the 3 largest ones.

Despite results showing low TDR to NNRTI and PI, the detection of a moderate (5%–15%) level of TDR to NRTI merits...
attention and indicates the need to repeat the survey in the same region and population. Possibly, the survey should also be expanded to additional areas in the country where ARVs have been widely available. Avila et al [5] recently presented data on a large prospective study conducted in 12 Mexican states from 2005 through 2010 that assessed TDR among 1655 chronically HIV-infected and ARV-naive patients. The results are similar to those observed in this survey, supporting the findings of highest rate of TDR for NRTI (4.2%), followed by NNRTI (2.5%) and PI (1.7%). A high frequency of TDR to NRTIs was observed in other Latin American countries [12], likely related to the widespread use of mono- or dual-NRTI therapy. In Mexico,
zidovudine and dual combinations with lamivudine and didanosine were commonly used, beginning in the early 1990s and continuing even after 2003 when the public health approach for HIV treatment was launched, giving the opportunity to transmit resistant strains from virologically nonsuppressed individuals.

**Nested Study**

Arm 1 had the advantage of low or no selection bias, because all consenting VCT attendees were enrolled at their first VCT visit. However, this approach can be labor intensive and costly, because all resistance specimens and participant information are collected and stored from all persons seeking HIV testing regardless of their HIV status. In settings such as Mexico, where only 10% of those tested in VCT sites are HIV positive, this approach is particularly burdensome, multiplying many costs by a factor of 10. In addition, because in arm 1, informed consent for resistance testing was asked of all persons undergoing HIV testing, a level of discomfort could have been generated in those who have not yet received a diagnosis, potentially resulting in a high number of persons refusing to participate in the HIV drug resistance survey. In settings with high rates of HIV positive results, on the other hand, the savings from arm 2 would be smaller.

Given the results about cost, the key question is what loss of quality, if any, the savings of arm 2 entail and whether one will get different results if the more economical arm 2 approach is used. The results in Supplementary Table 1 are reassuring, showing that the 2 methodologies, in terms of the characteristics of the participants enrolled, are indistinguishable.

Probing more deeply, one might want to examine the possibility of sampling bias in arm 2, from 2 potential sources. First, because nonreturning patients are not sampled under arm 2, results could be skewed if HIV drug resistance patterns differed significantly between returning patients and those which failed to return. For instance, if HIV drug resistance patterns among IDUs differed significantly from other risk groups, a hypothetical disproportionate loss of IDUs because of fear of legal consequences if the testing center did not observe its confidentiality promises may significantly impact on the TDR levels reported by this type of survey. However, our comparison of characteristics between returners and nonreturners identified no significant differences.

Second, arm 2 had a higher rate of nonconsent, possibly because consent was requested at a very difficult moment in the patient’s life. To the extent that nonconsenters are different from consenters, the higher nonconsent rate in arm 2 could be a source of additional bias in addition to that inherently resulting from the need for consent. However, the differences were unremarkable, with the only exception that consenters reported heterosexual risk at a significantly higher rate than those who refused consent. The reasons for this difference, which could be either a reporting difference or a difference in actual behavior, are unclear. In any case, it did not have an effect large enough to create bias in the arm 2 sample.

Overall, the lack of differences based on nonreturn and/or nonconsent explains the lack of differences in patient characteristics between the 2 arms, because return and consent are the leading possible sources of bias in arm 2.

Study results support the use of the simplified and cost-effective sampling of patients at the return diagnostic visit as an approach for resistance surveillance. However, these findings cannot be applied to any area, but only in contexts that are epidemiologically very similar to those presented here. In addition, because we were not able to measure resistance prevalence in the 2 arms, there is a need for caution and further research before adopting the methodology tested in arm 2 on a wide scale.

One methodological issue not directly explored in this study was the requirement for informed consent. Gains in both cost and representativeness might be obtained through use of procedures and ethics committee waivers obviating a consent requirement.

Another issue was the use of 2 periods to define arms 1 and 2. Considering the differences in the work process for the 2 arms and sites receiving no financial compensation, we concluded that administering the 2 processes simultaneously would be burdensome and that poor fieldwork would result. As such, the design unfortunately risked confounding results, with changes
because of time. However, no event (such as a change in HIV policy or a new campaign) that could affect the study was observed. Seasonal weather differences between the periods of arm 1 and arm 2 also appeared to be unlikely to confound results.

CONCLUSIONS

Despite results showing low (<5%) TDR to NNRTI and PI, the detection of a moderate (5%–15%) level of TDR to NARTI merits attention and indicates the need to repeat the survey in the same area and population. The 2 methods assessing 2 alternative approaches to recruit survey participants (arms 1 and 2) were practical for the staff at the VCT sites involved in the study; however, arm 2 provided major savings in cost and labor, with no signs of increased bias or loss of quality.

Supplementary Data

Supplementary materials are available at Clinical Infectious Diseases online (http://www.oxfordjournals.org/our_journals/cid/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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