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First Molecular Surveillance Report of HIV Type 1 in Injecting Drug Users and Female Sex Workers along the U.S.–Mexico Border

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Abstract

HIV prevalence is increasing among high-risk populations in the Mexican–U.S. border cities of Tijuana and Ciudad Juarez. In 2005, the molecular epidemiology of HIV-1 was studied among injecting drug users (IDU) and female sex workers (FSW) in these cities, which are corridors for over two-thirds of the migrant flow between Mexico and the United States. Eleven samples (eight IDU and three FSW) were successfully amplified, sequenced, and analyzed. The results revealed that all 11 samples were subtype B. There was no phylogenetic clustering or separation of the strains between IDU and FSW or between Tijuana and Ciudad Juarez nor was the Mexican strain phylogenetically distinct from other subtype B strains. Two of three drug naive FSWs had low-level HIV-1 resistance mutations. This community-based study demonstrated that HIV-positive IDUs and FSWs in Ciudad Juarez and Tijuana were predominantly infected with subtype B. Further prevalence studies on HIV-1 resistance mutations among FSWs in these border cities are warranted.

Mexico shares a 2100-mile border with the United States and the human immunodeficiency virus (HIV) epidemics on both sides of the border are consequently interrelated.^{1,2} HIV prevalence is rising among high-risk populations in Mexico–U.S. border cities, especially among female sex workers (FSW). In Tijuana, the prevalence of HIV infection among 15-to 49-year-old adults was recently estimated to be between 0.3 and 0.8%,¹ suggesting that up to 1 in 125 adults within this age group may be HIV infected. Tijuana and Ciudad Juarez, which are located adjacent to San Diego, CA and El Paso, TX, respectively, are situated on major drug trafficking routes, and have a thriving sex trade industry.^{2,3} Baja California (the state where Tijuana is located) ranks second among Mexico's 32 states in terms of the cumulative rate of new AIDS cases, after the Distrito Federal (i.e., Mexico City), whereas the state of Chihuahua (where Ciudad Juarez is located) ranks 18th.⁴

Tijuana and Ciudad Juarez account for over two-thirds of the migrant flow between Mexico and the United States.⁵ According to Mexican census data, approximately 60% of Tijuana residents originate from other cities, most commonly other cities in central and southern Mexico, and in Ciudad Juarez, 40% of residents are migrants.⁶

There have been a limited number of studies performed on the genetic diversity of the HIV-1 strains in Mexico.^{7,8} Such studies are needed due to the increasing prevalence of HIV-1 subtype

C isolates that has been reported elsewhere in Latin America and the rapidly changing nature of HIV epidemics.⁹ In Mexico, as in the United States and Canada, the predominant subtype has been reported to be subtype B.⁷ The populations of Tijuana and Ciudad Juarez are highly mobile, and so molecular surveillance of HIV-1 subtypes in the Mexico–U.S. border region is of interest. Additionally, since highly active antiretroviral therapy is provided free in Mexico but barriers to care persist, studies investigating the prevalence of HIV-1 resistance mutations are also needed for HIV-1 therapeutic guidelines.

The present study examined the molecular epidemiology of HIV-1 among two high-risk populations, injecting drug users (IDU) and FSWs, in Tijuana and Ciudad Juarez.

A total of 35 HIV-positive samples were collected from two independent studies of IDU and FSW in Tijuana and Ciudad Juarez, which have been described previously.^{10,11} Briefly, the IDU samples were obtained from a cross-sectional study of 222 IDUs in Tijuana and 206 IDUs in Ciudad Juarez who were recruited through respondent-driven sampling between February and April 2005.⁸ The FSW samples were obtained from a prevalence study performed both in Tijuana and Ciudad Juarez.¹⁰

A total of 21 HIV-positive samples came from Ciudad Juarez (6 IDUs and 15 FSWs) and 14 from Tijuana (6 IDUs and 8 CSWs). The serum collected from each study was obtained and viral RNA was extracted using a QIAmp RNA extraction Kit (QIAagen, Valencia, CA). The extracted RNA was used for reverse transcription using Superscript III (Invitrogen) priming from the 3' end using RT3474R (below). Synthesized cDNA was then polymerase chain reaction (PCR) amplified at the protease and reverse transcriptase region (ProRT) using a nested strategy. The first PCR round was performed using the amplification primers Pro5F (5'-AGAAATTGCAGGGCCCCCTAGGAA) and RT3474R (5'-GAATCTCTCTGTTTCTGCCAG) in combination with Amplitaq Gold (Applied Biosystems, Foster City, CA). The second round was completed using 1 µl of product from the first round and the following two primers: Pro3F (5'-AGANCAGAGCCAACAGCCCCACCA) and ProRT (5'-TTTCCCCACTAACTTCTGTATGTCATTGACA). The amplicon produced was 1.1 kb in length corresponding to nts 2166 and 3307 on HXB-2 (GenBank accession no. K03455).

The amplified product was prepared for automated sequencing by running it on a purification column (Microcon, Amicon YM-50 column) and was sequenced using fluorescent dye terminators on a 3130 ABI automated sequencer (Applied Biosystems, Foster City, CA). The amplified and sequenced region included all of the protease and the amino-terminus of the reverse transcriptase (RT) protein. All sequences obtained were assembled using Sequencher 4.2.2 (Gene Codes Inc., Ann Arbor, MI), and aligned with representative standard subtype references from the United States and Latin America using MacGDE. Phylogenetic analysis was conducted using neighbor-joining with Kimura's two-parameter model of distance calculation and a bootstrap computed using maximum parsimony.^{12,13}

Eleven of the 35 samples were successfully amplified, sequenced, and analyzed. Eight of the samples were from IDU and three were from FSW. The low amplification rate was due to problems with sample processing and shipping, which directly affected the success of cDNA synthesis and PCR amplification. Phylogenetic analysis showed that all 11 samples were subtype B (Fig. 1A). There was no phylogenetic clustering of IDU strains separate from FSW strains or Tijuana strains separate from Ciudad Juarez strains (Fig. 1B). It is also evident that there is no distinct difference between Mexican Bs and the subtype B that is circulating in the rest of the world.

Two of the three FSWs, both from Tijuana, had low-level antiretroviral resistance mutations in spite of the fact that they were drug naive. Female sex worker CJ157 had the protease

inhibitor resistance mutation G48E and female sex worker TJ108 had the nucleoside reverse transcriptase mutation T69N. Although the source for this resistance cannot be determined, further analysis of the subjects' backgrounds revealed that the clients of subject CJ157 were mostly from Mexico and a few from the United States. In addition, subject TJ135 reported exposure to injected drugs and had shared needles and/or injecting equipment.

In the IDU risk population two subjects reported having worked outside of Mexico in the last 10 years (JSPR10M, RSM011), one of whom reported having lived and worked in San Diego, California (RSM011). Six out of the eight subjects who were asked about sharing syringes reported having used a needle or syringe that they knew or suspected had been used before by someone else. Subjects also reported allowing other people to use their needle or syringe after them: five with friends, two with people that they did not know very well, and one (JSPR10M) with someone who lived in the United States. Furthermore, one reported having a casual sexual partner that lives in the United States.

This community-based study demonstrated that HIV-positive IDUs and FSWs in Ciudad Juarez and Tijuana are predominantly infected with subtype B. This subtype is the most prevalent in the United States as it is in the rest of North America, Western Europe, and Australia.⁷ These results are consistent with the results of a previous study done of the C2–V5 region of envelope in several regions of southern Mexico.⁸

The common gateway of entrance and exit along the border between the United States and Mexico is reflected in the molecular epidemiology of HIV in the border communities. Because the HIV prevalence rate in the United States (0.6%) is double that of Mexico (0.3%), contact with the United States is more of a threat to Mexicans than the other way around.^{14,15} As is visible phylogenetically, there are close interactions between viruses from Mexico and the United States. In a recent study it was found that out of 116 HIV-positive residents of San Diego, 64% crossed the Mexican border at least once a month in the past year.¹⁶ A recent study of men having sex with men (MSM) living in Tijuana and San Diego found high rates of HIV positivity in both groups (19% and 35%, respectively) and nearly half of the Tijuana group and three-quarters of the San Diego sample reported sex with partners from across the border.¹⁷ Multiple individuals in this study reported behavioral risks with people from the United States.

Although the sample size is small, the antiretroviral drug resistance found in two drug-naïve FSWs is of concern; the potential for primary drug resistance poses a potential public health threat to both sides of the border. There is a long documented history of cross-border utilization of services by HIV-positive clients. Both Tijuana and Ciudad Juarez have been involved in efforts to ensure continuity of care because of this long documented trend. The north–south interactions underscore the importance of further studies in Mexican high-risk groups to determine the rate of primary drug resistance not only in individuals in the Mexican border but throughout the country. A study of this magnitude would be beneficial because although antiretroviral drugs are available as part of the Mexican universal health care system, coverage and adherence are sporadic.¹⁸ The documentation mentioned above also strengthens the argument that the Mexican health care system has a long history of providing erratic services. Testing for HIV-1 drug resistance will aid clinicians in their route of treatment for patients who have shown virological failure; this will not only increase the patients' chance of survival but it will also result in savings in treatment cost.¹⁹

Nucleotide accession number: GenBank accession numbers for the sequences of this study are DQ631415–Q631425.

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FIG. 1.
(A) Neighbor-joining phylogenetic analysis of 11 partial protease/reverse transcriptase (ProRT) sequences of HIV-1 from IDU and FSW in Mexico along with reference strains. **(B)** Neighbor-joining phylogenetic distribution of (ProRT) sequences of HIV-1 from IDU and FSW in Mexico with representative world subtype B strains.