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HIV, HBV, and HCV Molecular Epidemiology Among Trans (Transvestites, Transsexuals, and Transgender) Sex Workers in Argentina

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Commercial sex work is frequent among male-to-female transvestites, transsexuals and transgenders in Argentina, leading to high susceptibility to HIV, HBV, and HCV among other sexually transmitted infections. In a global context of scarce data on the trans sex workers population, this study was aimed to study the genomic characterization of these viruses. Plasma presence of HIV, HBV, and HCV genomic material was evaluated in samples from 273 trans sex workers. Genomic sequences of HIV-*gag*, *pol*, and *vif-vpu* genes, HBV-S gene, and HCV-5′UT and NS5B genes were obtained. Molecular characterization involved phylogenetic analysis and several in silico tools. Resistance-associated mutations in HIV and HBV *pol* genes were also analyzed. The HIV genomic characterization in 62 trans sex workers samples showed that 54.8% of the isolates corresponded to BF intersubtype recombinants, and 38.7% to subtype B. The remaining were classified as subtypes C (4.8%) and A (1.6%). HBV and HCV co-infection prevalence among HIV positive trans sex workers yielded rates of 3.2% and 6.5% respectively. Drug resistance-associated mutations were found in 12/62 (19%) HIV *pol* sequences, but none among HBV. Based on phylogenetic relationships, HIV isolates characterized as subtypes BF and B appeared intermingled with those from other high-risk groups. Despite trans sex workers declared not to have received antiviral treatment, complex drug resistance-associated mutation patterns were found in several HIV isolates. Planned prevention, screening, and treatment are needed to reduce further transmission and morbidity. ***J. Med. Virol. 86:64–70, 2014.***

KEY WORDS: HIV; trans sex workers; viral hepatitis

INTRODUCTION

Commercial sex work is a high-risk activity that renders individuals at an increased risk of infection with HIV, HCV, HBV, and several other sexually transmitted infections [McKeganey, 1994; Talbott, 2007; Giami and Le Bail, 2011]. Thus, sex workers require special attention from epidemiological surveillance systems and public health agencies worldwide.

In Argentina, male-to-female transvestites, transsexuals and transgenders (collectively referred to as trans) sex workers are highly vulnerable and marginalized populations. As shown in our previous study focused on trans sex workers in Argentina, a high prevalence of sexually transmitted infections was found in this study population [Dos Ramos Farias et al., 2011]. Of note, the prevalence of HIV (34.1%), HBV (40.2%) and *Treponema pallidum* (50.4%) infections was alarmingly high, as was the incidence of HIV (10.7 per 100 person-year) infection, while the prevalence of HCV was 4.5%. The HIV prevalence observed was associated with a variety of factors such as commercial sex work, low level of education and strong social stigma and discrimination [Barreda, 2006; Dos Ramos Farias et al., 2011].

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The extensive diversity of HIV, HBV, and HCV represents a challenge for vaccine development and effectiveness [Taylor et al., 2008; Klenerman and Gupta, 2012; Lai et al., 2012]. It has been shown that besides prevention and diagnosis, performing genetic characterization studies is also relevant in the clinical context, since genotypes/subtypes can have an impact on treatment efficacy and severity of liver disease [Asselah et al., 2010; Wainberg and Brenner, 2010; Kim et al., 2011], as well as transmission and disease progression rates [Scott and Gretch, 2007; Tebit et al., 2007; Pujol et al., 2009].

As a result of the limited inclusion of trans sex workers in surveillance systems [Tsakris et al., 1997; Spizzichino et al., 2001; Bauer et al., 2012], and because of scarce data on HIV, HBV, and HCV genomic characterization among this population in the global context [Gutierrez et al., 2004], the present study was aimed to fully characterize HIV, HBV, and HCV variants circulating in trans sex workers from Argentina at the molecular level.

MATERIALS AND METHODS

Study Subjects and Plasma Samples

Frozen plasma samples from 273 trans sex workers obtained during a cross-sectional study conducted from October 2006 to December 2009 in five densely populated cities of Argentina (Buenos Aires, La Plata, Córdoba, Rosario, and Santiago del Estero) were studied [Dos Ramos Farias et al., 2011]. Most participants were young (mean age 29 years old, IQR 24–35), and nearly half of them had not completed high school (123 out of 273). The study population included individuals born in Argentina (n = 259), Peru (n = 8), Paraguay (n = 5), and Ecuador (n = 1). One third of the participants reported irregular use of condom mainly when engaged in commercial sex work with clients who offer them more money, evidencing higher rates of unsafe sex practices, even though they had a good general knowledge of HIV transmission routes. Regarding condom use with stable partners, as reported in female sex workers, this was generally irregular.

HIV infection diagnosis involved two screening techniques: ELISA (Enzygnost Anti-HIV 1/2 Plus ELISA, Dade Behring, Marburg, Germany) and particle agglutination (Fujirebio Diagnostics, Tokyo, Japan). Positive samples were confirmed subsequently by Western blot (New LAV Blot I, Bio-Rad Laboratories, WA). HBV infection markers such as surface antigen (HBsAg) and anti-core antibody (anti-HBc) were determined by ELISA (HBsAg (V2) Abbott AxSYM System, Core AxSYM System Abbott, Wiesbaden, Germany). For epidemiological purposes, a sample was considered HBV-positive if at least one of these markers was reactive. In order to determine HCV infection, anti-HCV antibodies were tested by ELISA (HCV version V3.0, Abbott AxSYM System, Wiesbaden, Germany). Those plasma samples positive for HIV by Western blot, reactive for HBsAg and/or anti-HBc, or with anti-HCV results, were selected for HIV-RNA, HBV-DNA, and/or HCV-RNA detection, respectively.

This study was performed in compliance with international and national ethical guidelines for biomedical research involving human subjects [CIOMS, 2002]. The research project was approved by Nexo AC Institutional Biomedical Review Board (IRB 5349), and carried out under all federal regulations governing the protection of human subjects in research.

HIV-RNA, HBV-DNA, and HCV RNA Isolation, PCR, and Sequencing

HBV DNA was obtained from 200 μl of plasma using a commercial kit (QIAamp DNA mini kit, Qiagen, Hilden, Germany) according to the manufacturer's instructions, and used as a template for a nested PCR amplification of the HBV S genomic region (585 nt, from nucleotide 203–787), as described previously [Quarleri et al., 2007].

HIV and HCV viral genomes were obtained from plasma samples using a commercial kit (QIAamp Viral RNA kit, Qiagen), and used as template for reverse transcription and PCR amplifications.

For HIV, amplicons from partial *pol* (1,496 bp, positions 2253–3749), *gag* (1,097 bp, positions 1231–2328) and *vif-vpr-tat* (314 bp, positions 5545–5859, HXB2 numbering, GenBank accession number K03455) coding sequences were obtained. PCR products were used as a template for double strand automated sequencing (ABI PRISM 3100 automated sequencer, Applied Biosystems, Foster City, CA and Big Dye Terminator sequencing kit, Amersham, Stockholm, Sweden).

Presence of HCV-RNA was first evaluated using reverse transcription (RT)-nested PCR amplification of the 5′untranslated region (5′UTR), as described elsewhere [Quarleri et al., 2000]. Positive HCV-RNA samples were further studied performing a PCR amplification of the NS5B gene as described previously [Laperche et al., 2005]. Briefly, cDNA was amplified by a heminested PCR, obtaining a 401-bp product that encompassed nucleotide positions 8245 and 8645 based on the standard numbering system [Choo et al., 1991]. DNA from purified PCR products (QIAquick PCR Purification Kit, Qiagen) was used as a template for double strand automated sequencing (ABI PRISM 3100 automated sequencer, Applied Biosystems and Big Dye Terminator sequencing kit, Amersham) as described elsewhere [Quarleri et al., 2004].

Phylogenetic Analysis

HIV *pol*, HBV-S, and HCV-NS5B nucleotide sequences were edited and assembled with Sequencer v.4.10.1 (Gene Codes).

For the HIV phylogenetic inference, 132 HIV *pol* reference sequences were retrieved from GenBank.

Among such references, 63 were previously characterized in Argentina from infected individuals with a well-defined risk of infection, including injecting drug users (n = 22), men who have sex with men (n = 3), women who have sex with men (n = 10), heterosexual men (n = 5), mother-to-child transmission (n = 7), female sex workers (n = 8), commercial sex workers (n = 3), male sex workers (n = 3) and unknown transmission route (n = 2). Likewise, reference sequences for the various HBV and HCV genotypes/subtypes were obtained from GenBank and the European Hepatitis C database [Kuiken et al., 2005; Combet et al., 2007].

Multiple sequence alignment was achieved by the Mafft program [Katoh et al., 2002, 2009]. Each viral nucleotide sequence dataset was input into phylogenetic studies and analyzed by Maximum Likelihood technique. For probabilistic analyses, the sequence evolution model was inferred by MrAIC [Nylander et al., 2004]. The model which best fitted the data was GTR + I + G. Maximum likelihood trees were inferred with PhyML version 3.0 [Guindon and Gascuel, 2003], using a BIONJ starting tree reordered by SPR in search of the tree space. Tree searches were performed as described elsewhere [Dilernia et al., 2008]. Shortly, cycles of random addition sequences plus tree-bisection-reconnection were followed by ratcheting, tree-drifting and tree fusing until no further improvement of tree lengths were observed. Phylogenetic grouping robustness was evaluated by bootstrap analysis using ML (1,000 replicates) with the PhyML software.

HIV and HBV Genotypic Drug Resistance Testing

In addition, HIV and HBV *pol* sequences were analyzed in search of resistance mutations using the Stanford University Calibrated Population Resistance Tool, HIV Drug Resistance Database (http://sierra2.stanford.edu/sierra/servlet/JSierra), and the HepSEQ-Research Database System website (http://www.hepseq.org/Public/Web_Front/main.php), respectively.

HIV, HBV, and HCV Genotyping Analysis

HIV *gag*, *pol* and *vif-vpr-tat* gene nucleotide sequences were used for subtype assignment and recombination analysis using SimPlot (version 2.5; Stuart Ray, http://www.med.jhu.edu), and the following tools available online: jumping profile Hidden Markov Model (jpHMM), and NCBI genotyping tool (http://www.ncbi.nlm.nih.gov/projects/genotyping/formpage.cgi).

HBV *pol* gene nucleotide sequences and HBV genotypes were defined using the Oxford HBV subtyping tool (http://www.bioafrica.net/rega-genotype/html/citetoolhbv.html) and the STAR genotyping tool (http://www.vgb.ucl.ac.uk/starn.shtml).

Similarly, HCV NS5B gene nucleotide sequences (400 bp, positions 8245–8645) were used for subtyping using tools available online: Oxford HCV subtyping (http://www.bioafrica.net/regagenotype/html/subtypinghcv.html) and NCBI viral genotyping (http://www.ncbi.nlm.nih.gov/projects/genotyping/formpage.cgi).

Statistical Analysis

Comparisons among proportions were analyzed by parametric (Student's *t* test) and non-parametric methods (Mann–Whitney *U* test). Univariate analyses were performed by Chi-square test or Fisher's exact test, according to the sample size. Statistical analyses were carried out using SPSS 15.0 (SPSS, Inc., Chicago, IL).

RESULTS

Prevalence of HIV, HBV, and HCV Infections Among Trans-Sex Workers

HIV infection was confirmed in 93/273 samples. HBV and HCV serology tests were performed in 264/273 individuals, and 106/264 and 12/264 samples exhibited serological markers associated with infection, respectively. It was not possible to determine the hepatitis serological status in nine patients due to the scarce sample material obtained. Seventy-five out of 106 HBV positive samples (HBsAg and/or anti-HBc reactive) exhibited anti-HBs antibodies and were assumed as samples from individuals who recovered from natural infection (resolved infection). Among the remaining 31samples, only 3 (12%) were positive for HBV-DNA, and were subjected to genotypic characterization. In this group of samples, 25 and 6 samples corresponded to HBV-HIV coinfected and HBV-monoinfected, respectively, which represents a coinfection prevalence of 3.2%.

Samples in which only anti-HBc antibodies were detected could correspond to patients on the time gap between the disappearance of HBsAg and the appearance of anti-HBs antibodies, or be false positive.

HIV viral load (VL) was determined in the 93 HIV-infected trans sex workers, and 62 exhibited detectable viremia ($\geq$50 copies/ml).

Regarding HCV, 6 out of 12 individuals exhibiting detectable anti-HCV antibodies and HCV-RNA were coinfected with HIV, thus representing a coinfection prevalence of 6.5%. Only one HCV-monoinfected individual had detectable HCV-RNA.

HIV, HBV, and HCV Genotyping and Phylogenetic Analysis

HIV genotyping analysis was performed by examining 3 genomic regions, as described in M&M, to minimize erroneous subtype assignment (Table I). Together with the phylogenetic analysis performed on *pol* sequences, this showed that 34 and 24 out of 62 samples corresponded to BF intersubtype recombinants (54.8%) and subtype B (38.7%), respectively. Three samples (1 from Ecuador and 2 from

TABLE I. HIV Subtype Assignment Based on Each Partial Gene Analyzed by Online Genotyping Tools and Simplot

gag	pol	vif-vpr-tat	Number of isolates (%)
B	B	B	24 (38.7)
F	BF	BF	5 (8.1)
F	BF	F	19 (30.1)
B	BF	BF	1 (1.6)
F	BF	B	2 (3.2)
F	F	F	1 (1.6)
BF	BF	BF	2 (3.2)
B	B	BF	2 (3.2)
BF	B	BF	1 (1.6)
B	B	F	1 (1.6)

Argentina) were ascribed to subtype C (4.8%) and one sample (from Peru) to subtype A (1.6%). HIV *pol* sequences did not form any statistically significant cluster during the phylogenetic analysis, appearing intermingled with other HIV isolates identified previously in individuals with other risks associated with infection (Fig. 1) (GenBank accession numbers: JX847141–JX847202). A complete coincidence for HIV-subtype assignment was found between the phylogenetic inference method and the analysis carried out with genotyping tools. The frequencies of subtype B and BF intersubtype recombinant variants found in Argentina among trans sex workers exhibit statistically significant differences when compared with those reported previously among men who have sex with men and heterosexuals ($P < 0.05$), but this significance was not observed when compared with female sex workers ($P > 0.05$).

Primary drug resistance-associated mutations (to NRTIs, NNRTIs, and PIs) were found in 12 out of 62 (19%) HIV *pol* sequences (Table II).

The HBV genomic characterization based on S gene nucleotide sequences of the samples with detectable HBV-DNA showed coincident results using both phylogenetic and online genotyping tools. Two were ascribed to genotype A2 and one to genotype C (Fig. 1). No drug resistance-associated mutations were identified when the analysis of HBV *pol* gene was carried out.

HCV genotyping results based on NS5B gene sequences were identical using phylogenetic and online typing analyses. Viral variants in HCV RNA-positive samples (n = 7) were ascribed to genotype 1a (n = 2), 1b (n = 1), 3a (n = 2), 4a (n = 1), and 4d (n = 1).

DISCUSSION

Trans sex worker individuals have become one of the fastest growing populations within the HIV pandemic [Sebastian, 1999; Lombardi and van Servellen, 2000]. This marginalized population is difficult to study, mainly due to their behavior and frequent migrations [Spizzichino et al., 2001; Perrin et al., 2003; Wright, 2003; The United Nations Population Fund, 2011]. Transsexualism is a worldwide phenomenon, not limited to western societies, and this is mainly related to surgical techniques and accessibility to surgical interventions which improved progressively.

In Latin America, commercial sex work frequently represents a way of procuring financial resources for survival among trans people. Consequently, epidemiological surveillance of sexually transmitted infections in this population is a topic of high relevance in the context of public health.

To our knowledge, the present study is the first to address the HIV, HBV, and HCV genomic characterization in trans sex workers from Argentina, and to a lesser extent, from other Latin American countries (i.e., people born in Peru, Paraguay and Ecuador that live and work in Argentina).

Despite the high prevalence of sexually transmitted infections found among trans sex workers, the genomic characterization of HIV reveals the presence of viral variants that prevail in other high-risk groups, and in the general population. The HIV subtypes B and BF intersubtype recombinants characterized in Argentinean trans sex workers have been found in other at-risk populations. Regarding frequencies, a dichotomy was reported about viruses found in the heterosexual community vs. the homosexual community, depicting two independent HIV epidemics in the same geographic region [Avila et al., 2002; Dilernia et al., 2007a]. Here, subtype B and BF intersubtype recombinant frequencies found among trans sex workers appear distinguishable from these two, but similar to the frequencies found in female sex workers. These two at-risk groups predominantly engage in commercial sex with male customers Nevertheless, the phylogenetic relationship established among HIV isolates showed no evidence of a clustering phenomenon according to the related infection risk. Taking this into account, we hypothesize that trans sex workers could play a role in bridging the gap between men who have sex with men and heterosexual populations. In order to draw a more definitive conclusion in this matter, further studies on well-defined trans sex worker populations, their clients and related individuals are needed.

This study shows that BF intersubtype recombinant variants were most frequently found among trans sex workers followed by subtype B variants. Subtypes C and A, detected previously in Argentina [Gomez-Carrillo et al., 2004; Pando et al., 2006, 2007; Segura et al., 2007; Dilernia et al., 2007a] were also present but in a low frequency. As shown in the results section, subtype C was found in samples from 2 Argentinean patients and 1 from a patient born in Ecuador, while the sample harboring the subtype A variant was obtained from a patient born in Peru. This result highlights the role of migration in the HIV epidemic dynamics.

All participants reported neither being aware of their serological status nor having received antiviral

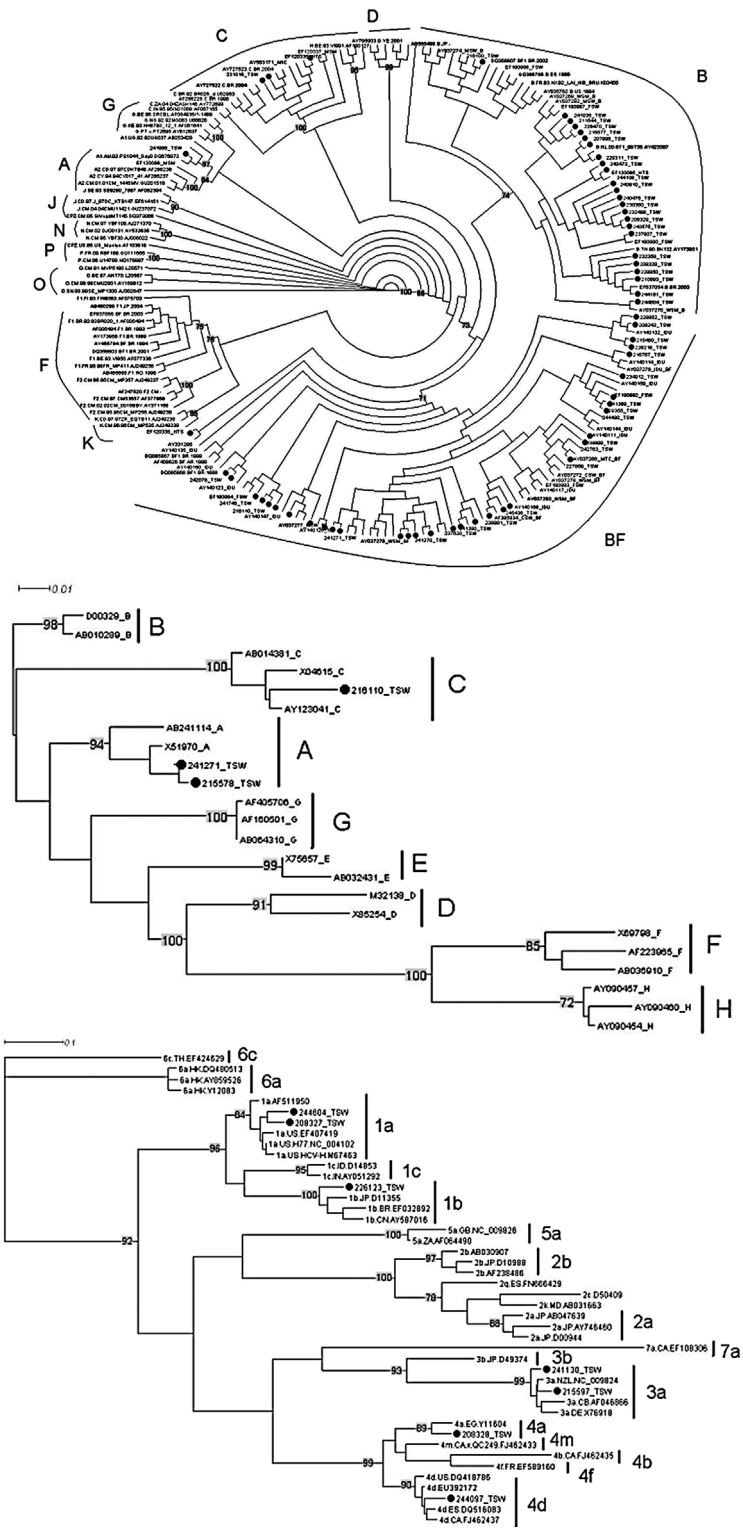

Fig. 1. Maximum-likelihood analysis of HIV pol gene (upper tree), HBV S gene (middle tree), and HCV NS5B gene (lower tree) nucleotide sequences from transsexual workers (black dots) obtained by direct sequencing. The HIV, HBV, and HCV reference sequences were retrieved from GenBank and Hepatitis C database and are indicated by their accession number. Among the HIV reference sequences, those previously obtained in Argentinean individuals are also indentified with the infection associated-risk (see M&M). The phylogenetic trees topology were evaluated by bootstrap analysis (1,000 replicates) and significant values are shown at each node. The subtypes/genotypes for each virus are indicated in bold capital letters and lines. The ruler shows the branch length for a pairwise distance equal to 0.1.

TABLE II. Drug Resistance-Associated Mutations Found in TSW Samples

	Resistance mutations associated to		
Sample subtype	NRTI	NNRTI	PI
B	—	K103N	—
B		Y181C	
BF	M184V, T215Y	K103N	V82A
BF	M184V	K103N	M46L, I54V, V82A
BF	M184V	K103N	—
BF	—	—	M46I, I54V, V82A
BF	D67N, K70R, T215E, K219Q	K101E, G190S	—
BF	—	G190A	—
BF	—	—	V82F, L90M
BF	M41L, L74V, M184V, T215Y	K101E, Y181C, G190A	I54V, V82A, L90M
BF	D67N	—	—
C	D67N, T215S, K219E	—	—

Corresponding subtypes and drug class are shown.

treatment. Nevertheless, our results on viral load determinations raised the question if some of them could have been taking medication without reporting it. In addition, some drug resistance mutation patterns observed indicate that they could have provided false information or that infection with high resistant HIV variants may have occurred. The fact that primary resistance mutations were found in 20% of the samples may hamper future treatment options and prognosis, while increasing the possibility of transmission of drug resistant variants to the uninfected population. It is important to highlight that drug-resistance surveillance among newly HIV-1 diagnosed individuals in Argentina has been shown previously to be between 4% and 9%, and up to 33% depending on the study population [Vignoles et al., 2007; Dilernia et al., 2007b; Pando et al., 2011].

Regarding the HBV and HCV genomic characterization, our study allowed us to determine the presence of different genotypes in this population. Nevertheless, the sample size is too small to draw a conclusion.

In summary, the trans sex worker population from Argentina exhibits the highest HIV prevalence among at risk groups, where HBV and HCV coinfection is also detected. The HIV molecular characterization reflects a similar scene to that observed before in other at risk groups. Thus, the trans sex worker group may play a central role as a niche for different sexually transmitted infections, especially HIV. All these conditions distinguish this group from other populations studied previously in Argentina, and highlight the need for well-designed periodic surveillance studies of sexually transmitted diseases in such populations, which will allow the early recognition of the increase in the rate of some infections and future changes in viral diversity, as well as the design of specific early interventions. Fully understanding the genetic diversity of these human pathogens and its implications for prevention, vaccine development, and chemotherapy will allow the control of the epidemic.

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