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# Incidence of HIV Type 1 Infection, Antiretroviral Drug Resistance, and Molecular Characterization in Newly Diagnosed Individuals in Argentina: A Global Fund Project

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## Abstract

An HIV incidence estimation was performed among men who have sex with men (MSM), drug users (DUs), sex workers (SWs), and pregnant women (PW) from Argentina. Volunteers older than 18 years old without a previous HIV-positive diagnosis were included. HIV-positive samples were analyzed by the Serological Testing Algorithm for Recent HIV Seroconversion (STARHS) to estimate incidence. By partial RT-PCR and sequencing of the HIV *pol* gene, an HIV subtype and resistance profile were determined. A total of 12,192 volunteers were recruited from October 2006 to September 2008. A higher HIV prevalence was detected among trans SWs (33.9%, 38/112), male SWs (10.8%, 12/111), and MSM 10.4% (161/1549). HIV incidence estimates by STARHS was also higher on trans SWs (11.31 per 100 person-years), male SWs (6.06 per 100 person-years), and MSM (6.36 per 100 person-years). Antiretroviral primary resistant mutations were detected in 8.4% of the study group, with a higher frequency in female DUs (33.3%). Phylogenetic analysis showed that 124 (57.9%) samples were subtype B, 84 (39.3%) intersubtype BF recombinants, 5 (2.3%) subtype C, and 1 (0.5%) subtype F in the *pol* region. Subtype B was most commonly found in MSM and male SWs whereas the intersubtype BF recombinant was more prevalent in female DUs, female SWs, and PW. Given the high HIV prevalence and incidence found in most of these groups, monitoring the continuing spread of the HIV epidemic is essential for determining public health priorities, assessing the impact of interventions, and estimating current and future health care needs.

## Introduction

**M**ONITORING THE CONTINUING SPREAD OF THE HIV EPIDEMIC is essential for determining public health priorities, assessing the impact of interventions, and estimating current and future health care needs. Although HIV-1 prevalence estimates are the most commonly available surveillance data, HIV-1 incidence provides a more useful tool to estimate epidemiological trends, a more sensitive indicator for evaluating the impact of interventions, and a more accurate prediction of the number of infected people in a population requiring treatment. However, HIV incidence data are rarely available since collection requires difficult, prolonged, and expensive follow-up. As an alternative, approximately 10 years ago, a laboratory assay that can distinguish recent from long-term HIV-1 infections based on changes in antibody quantity was developed. Thus, it is now possible to estimate HIV incidence in cross-sectional HIV prevalence surveys with the use of a Serologic Testing Algorithm for Recent HIV Seroconversion (STARHS).[1]

Cross-sectional HIV prevalence studies recently conducted among several vulnerable groups in Argentina have showed high HIV-1 prevalence among men who have sex with men (MSM) (13.8%),[2] injecting drug users (IDUs) (44.3%),[3] noninjecting cocaine users (NICUs) (6.3%),[4] patients with tuberculosis (TB) (17.1%),[5] patients attending clinics for sexually transmitted infection assessment (7.4%),[6] and female sex workers (3.4%).[7] Nevertheless, no HIV incidence cohort

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studies in populations vulnerable to HIV infection had been carried out in Argentina until 2003 when our group began the first incidence study following a cohort of MSM for 1 year.[8] This study showed a high HIV incidence, 3.9% person-years. Although a high retention rate (91.5%) was observed in this MSM group, follow-up of other hidden populations is not usually easy, mainly due to the fact that many participants are missed and also because of the high cost of these studies. For these reasons, the STARHS was used in these previously tested populations showing an annual incidence of 6.7, 3.1, 2.4, 2.0, 1.3, and 0% person-years for MSM, NICUs, TB, STIs, female sex workers (SWs), and IDUs, respectively.[9]

Transmission of drug-resistant HIV has been documented and associated with suboptimal response to antiretroviral treatment.[10] In Argentina, Dilernia *et al.* recently reported a primary drug resistance prevalence of 4.2% over a sample of 284 newly HIV diagnosed individuals,[11] whereas Vignoles *et al.* reported 9% over a sample of children born to HIV-infected mothers.[12]

It has been previously indicated that HIV molecular epidemiology has a particular trend in Argentina, with the co-existence of subtype B and intersubtype BF recombinants, with different proportions related to the transmission route.[13]

The aim of our study was to estimate HIV incidence, a drug resistance profile, and viral subtype characterization in recently diagnosed drug-naive individuals from different vulnerable populations in Argentina in order to update epidemiological and molecular HIV data.

## Materials and Methods

### Study design

From October 2006 to November 2008, a cross-sectional HIV seroprevalence survey was conducted at three different nongovernmental organizations (Nexo AC, AMMAR, and Intercambios AC) and one hospital (Hospital Materno Infantil Ramón Sardá), in the context of a Global Fund Project (Subproject no. 112). SWs, MSM, drug users (DUs), and pregnant women (PW) were included in the study. The project was coordinated by the National Reference Center for AIDS in conjunction with the social groups represented through the nongovernmental organizations.

This research was reviewed by the institutional review boards and the scientific ethical committee of the School of Medicine, University of Buenos Aires and was conducted in compliance with all federal regulations governing the protection of human subjects.

### Inclusion criteria

Volunteers were considered eligible for these surveys when they were older than 18 years and declared no previous HIV-positive diagnosis. Attendees who provided written informed consent were invited to receive HIV testing, offered pretest and posttest counseling, and interviewed on sociodemographic characteristics and their sexual preferences. Specific inclusion criteria were taken into account for each group. MSM were individuals from Buenos Aires who reported having had sex with men at least once in the past 6 months. SWs were men, women, and male-to-female trans (transvestites, transgenders, and transsexuals) who declared that they had exchanged sex for money in the past six months.

Recruitment was carried out in the cities of Buenos Aires, Santiago del Estero, Viedma, Rosario, Paraná, Córdoba, La Plata, and Mendoza. DUs were men, women, and male-to-female trans who declared having used drugs once a week on average in the past 6 months. Recruitment was carried out in Buenos Aires, Rosario, La Plata, Córdoba, and Tucumán. PW were individuals from Buenos Aires with confirmed pregnancy.

### Blood sample collection and HIV diagnosis

Approximately 10 ml of anticoagulated blood was collected from each volunteer. Plasma was separated by centrifugation and conserved at $-80^{\circ}$C for HIV diagnosis. HIV diagnosis was assessed by using two screening tests (GENSCREEN Ultra HIV Ag-Ab, Bio-Rad; Marnes-la-Coquette, France; SFD HIV 1/2 PA, Bio-Rad FUJIREBIO INC., Tokyo, Japan), and further confirmation of reactive samples by a Western blot assay for HIV-1 (New Lav Blot I, Bio-Rad, Marnes-la-Coquette, France).

Patients from Buenos Aires whose result were positive were offered HIV-1 viral load and $CD4^{+}$ T lymphocytes count testing for free at the National Reference Center for AIDS; HIV-positive patients from other cities outside Buenos Aires were referred to local hospitals.

### "Detuned assay" to identify recent infections

HIV-positive plasma samples were tested using a modified or "detuned" version of an HIV-1 enzyme immunoassay (Vironostika HIV-1 Microelisa System; bioMerieux Inc., North Carolina) to classify samples as potential recent infections (time of infection less than 4–6 months prior to sample collection) or longstanding infections. For this, the STARHS strategy[1] was performed, as previously described.[9]

### HIV-1 RNA isolation and sequencing

RNA was isolated from HIV-positive plasma samples by the QIAamp viral extraction kit (QIAGEN GmbH, Hilden, Germany). The *pol* gene was amplified from positions 2143 to 3798 (HXB2 numbering) by reverse transcriptase-polymerase chain reaction (RT-PCR), and sequenced in an ABI Prism 3100/3100-*Avant* sequencer (Applied Biosystems, Foster City, CA), as previously described.[14]

### Drug resistance analysis

Sequence edition was performed (Sequencher 4.9, Gene Codes Corporation, USA) and primary resistance mutations (PRMs) associated with reduced susceptibility to protease (PR) and RT inhibitors were detected by means of the Stanford University calibrated population resistance tool, using the 2009 surveillance drug resistance mutations list.[15] When used to assess resistance in a population-sampled set of HIV-1 sequences obtained from untreated individuals, the list provides an estimate of transmitted drug resistance in agreement with WHO guidelines.

### Phylogenetic analysis

Sequence alignment was performed by CLUSTAL W (BioEdit 7.0.4.1 sequence alignment editor).[16] Neighbor-joining trees were constructed under the Kimura two-parameter

model with the MEGA3 program.[17] Sequences were individually analyzed by SimPlot 2.5 and recombination analysis was later performed by bootscanning analysis.[18]

### Viral load and CD4 testing

Plasma viral load was assessed by branched DNA technology, with a detection limit of 50 HIV-1 RNA copies/ml (Versant HIV-1 RNA 3.0 assay; Siemens, Tarrytown, NY). $CD4^+$ T lymphocytes from peripheral blood were counted by flow cytometry (Coulter XL; Coulter Co., Hialeah, FL).

### Statistical analysis

Chi-square test and Fisher's exact test were used to compare proportions and trend analysis was performed by the Chi-square test. A Mann–Whitney test was performed for nonparametric analysis.

### Results

### Study population

T1 ▶ A total of 12,192 volunteers were included in the cross-sectional HIV prevalence survey.[19] Table 1 describes the number of volunteers in each group stratified by city. Overall, most of the volunteers (89.9%) were mainly recruited in Buenos Aires city, involving individuals from the city itself and the surrounding areas. However, SW samples were recruited mainly in La Plata city as well as in Buenos Aires (Table 1). Among drug users, 8.5% of the volunteers declared having used injecting drugs. Only eight MSM and two SWs declared having used injecting drugs at any time in their lives. No data of illegal drug use were available from pregnant women.

### Cross-sectional HIV prevalence survey

HIV diagnosis was performed on all the volunteers. Among the 12,192 self-reported HIV-negative or unknown status volunteers enrolled in the surveys, 273 individuals
T2 ▶ were HIV positive. Table 2a shows the HIV prevalence of the different groups stratified by gender.

HIV prevalence in DUs is higher in men than in women, though not statistically significant ($p > 0.05$). When IDUs and non-IDUs were compared no statistical difference was observed (7.9% vs. 3.9%, $p > 0.05$).

In SWs, significant differences were observed. Trans SWs had a higher prevalence as compared to men ($p < 0.0001$) and women ($p < 0.0001$), and men also had a significantly higher prevalence as compared to women ($p < 0.0001$).

HIV seroprevalence was analyzed according to stratification among cities only on SWs, since only a few MSM were recruited outside Buenos Aires, no HIV-positive cases were detected on DUs outside Buenos Aires, and all PW were from Buenos Aires city. Table 2b shows HIV prevalence in SWs from the different cities. Some significant differences were observed among female SWs: HIV prevalence was higher in Buenos Aires, Paraná, and Córdoba as compared to La Plata ($p < 0.001$, $p < 0.05$, and $p < 0.05$, respectively). There was a significantly lower HIV prevalence on trans sex workers from Santiago del Estero as compared to the same population from Buenos Aires, Rosario, and Córdoba ($p < 0.05$).

HIV prevalence in pregnant women is lower, but without statistical significance, than the epidemiological data reported from several testing centers from Buenos Aires city (Centros de Prevención, Asesoramiento y Diagnóstico) (0.1% vs. 0.36%, $p > 0.05$).

A total of 139 patients who were diagnosed as HIV positive (50.9%) on the prevalence surveys attended the National Reference Center for AIDS for viral load and $CD4^+$ T cell count testing. Most of them belonged to the MSM group (123), 14 were DUs, and 2 were SWs. The time between the first sample (for HIV diagnosis) and the second sample (for viral load and CD4 counts) oscillates between 1 week and 6 months, with 78% of the individuals coming back before 3 weeks. The median viral load for these patients was 4.29 log of viral RNA/ml (19,603 copies/ml) and the median number of $CD4^+$ T cells was 470 cells/ml.

### HIV incidence

Annual HIV incidence was estimated by testing 262 HIV-positive plasma samples from the cross-sectional studies.

TABLE 1. VOLUNTEERS INCLUDED IN THE HIV PREVALENCE STUDY STRATIFIED BY GROUP AND CITY, ARGENTINA, 2006–2008[a]

| City | MSM n (%) | SWs n (%) | DUs n (%) | PW n (%) | Total n (%) |
|---|---|---|---|---|---|
| Buenos Aires | 1,519 (98.1) | 295 (22.1) | 580 (78.6) | 8,570 (100) | 10,964 (89.9) |
| Santiago del Estero | 22 (1.4) | 148 (11.1) | — | — | 170 (1.4) |
| Viedma | — | 27 (2.0) | — | — | 27 (0.2) |
| Rosario | — | 46 (3.4) | 106 (14.4) | — | 152 (1.2) |
| Salta | 6 (0.4) | — | — | — | 6 (0.1) |
| Paraná | 2 (0.1) | 30 (2.2) | — | — | 32 (0.3) |
| Córdoba | — | 205 (15.4) | 7 (0.9) | — | 212 (1.7) |
| La Plata | — | 425 (31.8) | 38 (5.1) | — | 463 (3.9) |
| Tucumán | — | — | 7 (0.9) | — | 7 (0.1) |
| Mendoza | — | 159 (11.9) | — | — | 159 (1.3) |
| Total | 1,549 | 1,335 | 738 | 8,570 | 12,192 |
| Mean age (SD) | | | | | |
| Men | 30.7 (9.9) | 29.2 (9.8) | 29.0 (8.3) | — | 30.3 (9.6) |
| Women | — | 33.4 (11.2) | 29.6 (9.3) | 26.3 (7.4) | 32.6 (11.0) |
| trans | — | 30.2 (8.4) | 25.6 (6.5) | — | 29.6 (8.3) |

[a]MSM, men who have sex with men; SWs, sex workers; DUs, drug users; PW, pregnant women.

Table 2a. HIV Prevalence among Self-Reported HIV-Negative or Unknown Status Volunteers Enrolled in the Survey Stratified by Group and Gender, Argentina, 2006–2008[a]

| | Men | | Women | | Trans | |
|---|---|---|---|---|---|---|
| Group | HIV positive/total | HIV prevalence % (95% CI) | HIV positive/total | HIV prevalence % (95% CI) | HIV positive/total | HIV prevalence % (95% CI) |
| MSM | 156/1,518 | 10.3 (8.7–11.8) | — | — | 5/31 | 16.1 (5.4–33.7) |
| DUs | 24/489 | 4.9 (2.9–6.9) | 7/241 | 2.9 (0.6–5.2) | 0/8 | — |
| SWs | 12/111 | 10.8 (4.6–17.0) | 21/1112 | 1.9 (1.0–2.7) | 38/112 | 33.9 (24.7–43.1) |
| PW | — | — | 10/8570 | 0.1 (0.0–0.2) | — | — |

[a]MSM, men who have sex with men; SWs, sex workers; DUs, drug users; PW, pregnant women; HIV, human immunodeficiency virus; CI, confidence interval.

Table 2b. HIV Prevalence among Self-Reported HIV-Negative or Unknown Status SWs Enrolled in the Survey Stratified by City and Gender, Argentina, 2006–2008[a]

| | MSWs | | FSWs | | TSWs | |
|---|---|---|---|---|---|---|
| City | HIV positive/total | HIV prevalence % (95% CI) | HIV positive/total | HIV prevalence % (95% CI) | HIV positive/total | HIV prevalence % (95% CI) |
| Buenos Aires | 4/32 | 12.5 (3.5–29.0) | 10/241 | 4.1 (1.4–6.9) | 10/22 | 45.5 (22.4–68.5) |
| Santiago del Estero | 1/12 | 8.3 (0.2–38.5) | 1/108 | 0.9 (0.0–5.0) | 4/28 | 14.3 (4.0–32.7) |
| Viedma | 0/1 | — | 0/26 | — | — | — |
| Rosario | — | — | 0/41 | — | 4/5 | 80.0 (28.3–99.5) |
| Paraná | — | — | 2/30 | 6.7 (0.8–22.1) | — | — |
| Córdoba | 0/2 | — | 6/155 | 3.9 (0.5–7.2) | 19/48 | 39.6 (24.7–54.4) |
| La Plata | 7/51 | 13.7 (3.3–24.1) | 1/365 | 0.3 (0.0–1.5) | 1/9 | 11.1 (0.3–48.2) |
| Mendoza | 0/13 | — | 1/146 | 0.7 (0.0–3.7) | — | — |
| Total | 12/111 | 10.8 (4.6–17.0) | 21/1112 | 1.9 (1.0–2.7) | 38/112 | 33.9 (24.7–43.1) |

[a]MSWs, male sex workers; FSWs, female sex workers; TSWs, trans sex workers.

STARHS showed that, overall, 57 (21.8%) samples belonged to individuals with recent HIV infection. HIV incidence was 6.33 (95% CI 4.40–8.26) in MSM, 1.65 (95% CI 0–3.36) in male DUs, 2.72 (95% CI 0–5.80) in female DUs, 2.15 (95% CI 0–6.36) in male SWs, 0.62 (95% CI 0–1.30) in female SWs, 11.31 (95% CI 0.38–22.2) in trans SWs, and 0.05 (95% CI 0–0.12) per 100 person-years in PW.

No difference in viral load values was observed between HIV recent or established infections (4.1 vs. 4.3 log of viral RNA/ml, $p > 0.05$). However, patients with recent infections had significantly higher $CD4^+$ T cell counts (613 vs. 423 cells/ml, $p < 0.05$, Mann–Whitney test).

### Prevalence of transmitted drug resistance

A total of 214 plasma samples from newly HIV diagnosed individuals were sequenced. HIV-positive samples that could not be successfully PCR amplified had significantly lower viral load levels ($p < 0.05$). Of the study group, 18 individuals (8.4%) had PRM; however, results differed by group and gender. Higher frequencies of resistance mutations were found in women DUs (33.3%, 95% CI 0.8–90.6), male DUs (15.4%, 1.9–45.4), and trans SWs (13.3%, 95 CI 3.7–70.7), followed by male SWs (9.1%, 95% CI 0.2–41.3) and MSM (7.7%, 95% CI 2.7–12.6). No resistance mutations were detected in pregnant women and female sex workers.

A higher percentage of primary resistance mutations was detected in those individuals with established infection as compared with those with recent infection, though without statistical significance (8.3% vs. 7.5%, $p > 0.05$). Also, a higher but still not significant prevalence of PRM was observed in those individuals infected with subtype B variants vs. those infected with intersubtype BF recombinants (9.7% vs. 6.7%, $p > 0.05$).

Most patients had primary resistance mutations associated with only one group of antiretroviral drugs (61.1%), mainly with nonnucleoside RT inhibitors (NNRTIs, 45.4%) and, to a lesser extent, with nucleoside RT inhibitors (NRTIs, 27.3%) and PR inhibitors (PIs, 27.3%). The most prevalent primary resistance mutations was K103N (44.5%), followed by M184V (33.3%) and M41L (22.2%) (Table 3).

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### Subtype characterization

Phylogenetic analysis showed that 124 (57.9%) samples were subtype B, 84 (39.3%) intersubtype BF recombinants, 5 (2.3%) subtype C, and 1 (0.5%) subtype F in the *pol* region. Figure 1 shows the frequency of different HIV subtypes in each group.

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Subtype B was most commonly found in MSM and male SWs whereas the intersubtype BF recombinant was more prevalent in female DUs, female SWs, and PW. Moreover, intersubtype BF recombinants were also more frequently found in male DUs and in trans SWs, but with a lower frequency. In addition, subtype B prevalence in MSM was significantly higher when compared to male DUs and female DUs (69.6% vs. 43.7%, $p < 0.05$), female SWs (69.6% vs. 25%, $p < 0.05$), and trans SWs (69.6% vs. 40.0%, $p < 0.05$). No

TABLE 3. PRIMARY DRUG RESISTANCE MUTATIONS AMONG NEWLY HIV DIAGNOSED INDIVIDUALS, ARGENTINA, 2006–2008[a]

| Group | ID | Subtype | PIs | NRTIs | NNRTIs |
|-------|----|---------|-----|-------|--------|
| MSM | M1 | B | L90M | | |
| | M2 | BF | M46I I54V L76V V82S | | K103N |
| | M3 | B | | M41L | |
| | M4 | B | | M41L T215E | |
| | M5 | C | | D67N T215S K219E | |
| | M6 | B | | M184V T215Y | K103N |
| | M7&8 | B | | | G190A |
| | M9&10 | B | | | K103N |
| Dus | D1 | B | M46I | | |
| | D2 | B | V82A | M41L D67N K70R M184V T215Y K219E | K103N |
| | D3 | B | | M184V | G190A |
| SWs | S1 | BF | L90M | | |
| | S2 | BF | V82A | M184V T215Y | K103N P225H |
| | S3 | BF | | M184V | K103N |
| | S4 | B | | | K103N |
| | S5 | BF | I54V V82A L90M | M41L L74V M184V T215Y | K101E Y181C G190A |

[a]PIs, protease inhibitors; NRTIs, nucleoside reverse transcriptase inhibitors; NNRTIs, nonnucleoside reverse transcriptase inhibitors; MSM, men who have sex with men; SWs, sex workers; DUs, drug users; HIV, human immunodeficiency virus.

subtype prevalence differences were observed between recent or established infections and no significant differences in viral load were observed among the different subtypes.

## Discussion

This study shows the high impact of HIV in male-to-female trans workers, with approximately 34% of the individuals being HIV positive, in agreement with the results previously reported by Toibaro et al.[20] In Argentina, male-to-female trans individuals make up a population that is especially hidden, difficult to reach, and marginalized from the general population.[21] Other researchers have asserted that many trans people lack employment, live below the poverty line, and are engaged in high-risk sex work because of the discrimination and stigma they experience for being trans.[22,23] All these results emphasize the need to focus efforts on this group in order to achieve HIV risk reduction.

For the first time, HIV incidence has been estimated in trans SWs in Argentina, showing the highest incidence ever described in our country (11.3 per 100 person-years). This result, together with HIV prevalence, clearly shows that the male-to-female trans group is at very high risk for HIV infection. This trans group also has a high prevalence of coinfections with other sexually transmitted infections, as reported by our group. Preliminary analysis of socioepidemiological data showed several risk factors that could be associated with both high HIV prevalence and incidence. Among them are the fact that this group has a very low formal educational level, highly irregular use of condoms either with partners (60%) or clients (40%), and a high percentage of illegal drugs use (35%).

Another important issue of this prevalence study on self-reported HIV-negative individuals is the sustained high prevalence in MSM. Since the first prevalence studies were performed in 2000, the HIV prevalence in those individuals who were diagnosed for the first time was around 11%.[24] Epidemiological data on socioeconomical characteristics of this group show that they are men with high formal educational levels, mostly with stable employment and access to condoms (data not shown). Other studies on sexual risk behavior predictors are in progress in Buenos Aires with the aim of better understanding the sustained HIV prevalence and incidence in this group.[25] As previously reported by the CNRS in a preceding incidence study during 2000–2001[9] and a follow-up study during 2003–2004,[8] a high annual HIV seroincidence rate was found in MSM (6.33 per 100 person-years). The highly sustained HIV prevalence and incidence values reported since 2000 in this population[2] are consistent evidence of the impact of HIV on MSM that should be taken into account by public health officials in order to implement specific strategies for HIV prevention in this group.

Based on our results, in the overall vulnerable population, excluding pregnant women (as they are not considered an at-risk group), 7.3% of the individuals who self-reported as HIV negative were actually HIV positive. However, the studies of incidence estimation showed that not all of these diagnoses were recent infections. When the individuals were stratified by group, this percentage was considerably higher in some of them. This cross-sectional incidence study on people who self-reported as HIV negative showed that only approximately 20% of the HIV-positive individuals had been recently infected. This highlights the striking fact that most of the HIV-positive individuals had been infected for more than 6 months prior to recruitment and that they were unaware of their HIV status. These results, together with the fact reported by the National Health Ministry that 29.0% of men and 16.9% of women receive their HIV diagnosis during a symptomatic period, show the necessity of encouraging HIV diagnoses in vulnerable groups.[26,27]

Our study shows that 8.4% of newly diagnosed individuals belonging to different vulnerable groups harbor resistant mutations associated with reduced susceptibility to antiretroviral drugs. These results are in agreement with previous studies in adults, where resistance prevalence was 7.7%[28] and in children, with 9% of children harboring antiretroviral-resistant mutations.[12] When analyzed by group, the PRM prevalence was 7.7% in MSM, similar to previous results reported by our laboratory in MSM studied between 2003 and 2005 (8.2%).[11]
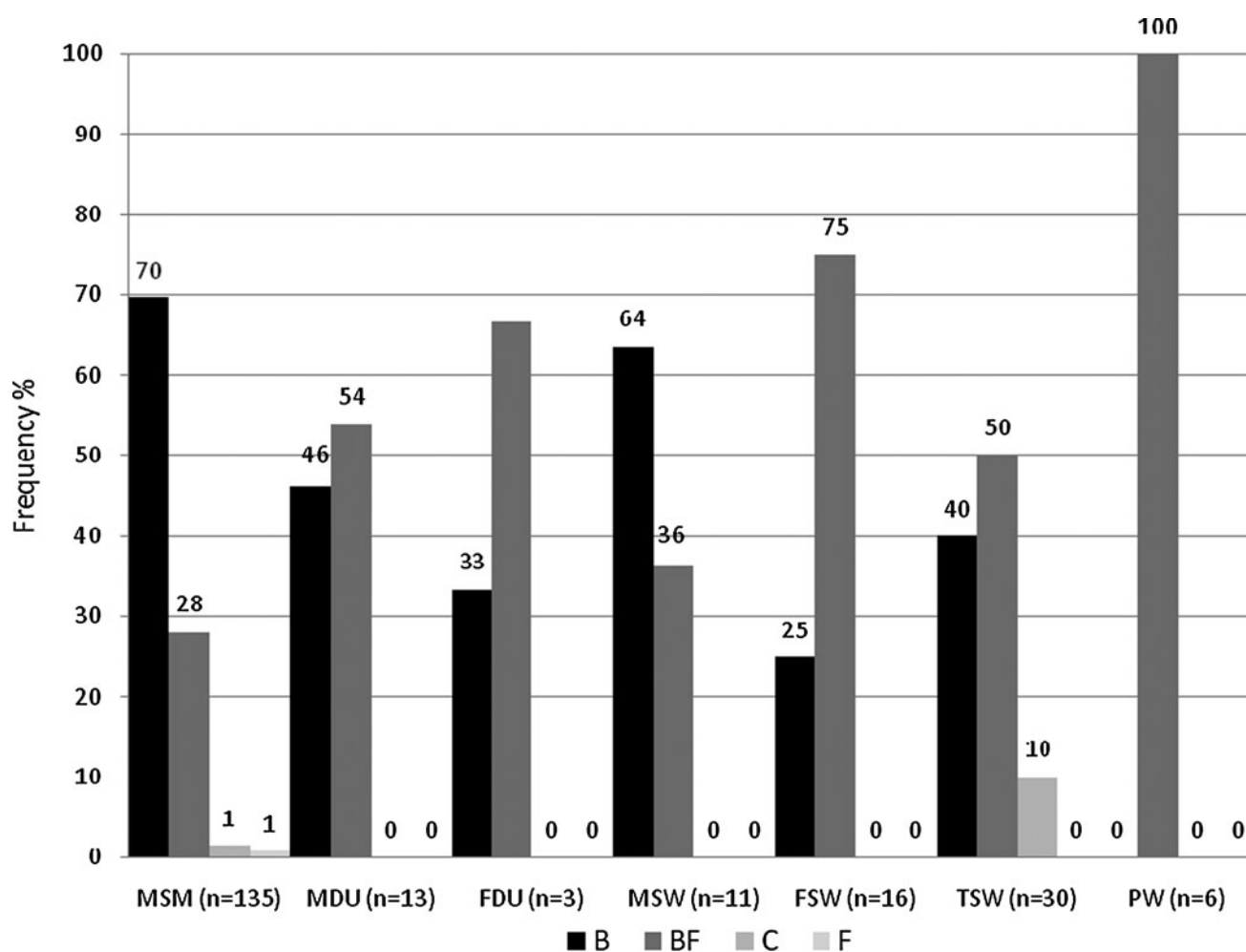
**FIG. 1.** HIV subtype frequency among volunteers enrolled in the surveys stratified by group, Argentina, 2006–2008. MSM, men who have sex with men; MDUs, male drug users; FDUs, female drug users; MSW, male sex workers; FSWs, female sex workers; TSWs, transsexual workers; PW, pregnant women.

Transmission of drug-resistant HIV-1 is well documented and is associated with suboptimal virologic response to a first antiretroviral treatment, limiting the options of active drugs.[10] Therefore, recent U.S. guidelines[29] recommend resistance testing for all subjects beginning treatment, regardless of evidence of recent infection. In addition, the World Health Organization recommends the establishment of HIV drug resistance sentinel surveillance systems in developing countries where treatment access is being expanded. In agreement with this, the results of the present study may help facilitate the tracking of trends on the transmission of resistance locally.

The phylogenetic analysis showed, as previously described by our group, that subtype B is the most common subtype found in MSM and BF recombinants are more frequently found in heterosexuals and drug users.[13,30] A higher frequency of non-B subtypes was found in MSM in our study as compared with our previous study (30.4% vs.10.5%, $p < 0.001$). This significantly higher frequency may have occurred because of the mixture of groups over time. Continued efforts to monitor the genetic makeup of HIV strains are critical in order to better assess the ongoing nature of the HIV epidemic in this region.

One of the main objectives of this project was to access people from different vulnerable populations from different cities other than Buenos Aires, where most of the previous studies had been performed. In this sense, this aim could be partially achieved in the SW group and, to a less extent, in the DU group. Regarding SWs, we can hypothesize that all the previous work conducted through many years since the first HIV prevalence study was performed[7] by the NGO recruiting SWs all around the country has probably led to the success of having access to individuals from different parts of the country. In the drug users group, this was the first time this kind of collaborative study between governmental and nongovernmental treatment centers was performed. Unfortunately, this was not the case for the MSM group, in which around 98% of the samples belonged to individuals recruited in Buenos Aires city. More field work is needed to reach this hidden population outside the city of Buenos Aires.

In conclusion, our results suggest that the HIV epidemic in Argentina is concentrated, with HIV prevalence higher than 5% in more than one defined subpopulation and lower than 1% in pregnant women. This study also shows the value of the interaction between the academic and the social groups

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represented through the nongovernmental organizations. The complexity we found in recruitments outside Buenos Aires points out the need to implement new strategies to obtain more access to vulnerable groups throughout the country as well as to encourage NGOs already working with these populations on HIV prevention and diagnosis.

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## Author Disclosure Statement

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AU1: Disclosure statement accurate? If not, amend as needed.