The Prevalence of Drug Resistance Mutations Among Treatment-Naive HIV-Infected Individuals in Beijing, China

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Abstract

To investigate the prevalence of HIV-1 genotypic mutations for drug resistance among patients in Beijing, blood samples from 145 newly confirmed (2006–2007), treatment-naive HIV-1-infected individuals were analyzed. Seven subtypes or CRF were subsequently determined and scored by the Stanford HIV Drug Resistance algorithm: CRF01_AE HIV-1 (27.6%), subtype B (24.1%), CRF07_BC (21.4%), subtype B (20.7%), CRF08_BC (3.4%), subtype C (2.1%), and CRF06_cpx (0.7%). Eleven of the 145 subjects studied were found to harbor the strains resistant to either protease inhibitors (PIs) (3.4%), or nucleoside reverse transcriptase inhibitors (NRTIs) (2.1%), or nonnucleoside reverse transcriptase inhibitors (NNRTIs) (3.4%). Although the prevalence of drug resistance was relatively low among the treatment-naive HIV-1 patients in Beijing in comparison to those in industrialized countries, we will continue monitoring newly infected subjects for any potential alteration of the prevalence pattern to ensure the success of the ongoing scale-up of antiretroviral treatment.

HIV type 1 (HIV-1) is the most basic type of HIV virus. Despite efforts by researchers and clinical professionals to control the HIV epidemic and improve the quality of the life of HIV-1-infected individuals, an estimated 740,000 people have been living with HIV and 26,000 died of AIDS in China in 2009 as reported by UNAIDS.1 Beijing, the capital of China and one of the world’s most populated cities, has over 12 million inhabitants and receives over 9 million migrants annually. Since the first case of AIDS was detected in 1985,2 the development of HIV-1 in Beijing has been on the rise and can be classified into three stages: the initial stage (1985–1991), the spread stage (1992–1997), and the rapid growth stage (1998 to now). By the end of May 2009, 6383 cumulative cases of HIV-1 infection had been reported in Beijing. Of these, 21.04% were permanent residents of the city, 73.98% were migrants, and 3.87% were foreigners. Beijing is nationally ranked twelfth in terms of HIV-1 prevalence. Based on previously published data, the modes of HIV-1 transmission in this city were sexual activity-related transmission (38.99%), intravenous drug use (IDU) (31.74%), contaminated blood transfusion (10.94%), and mother-to-child transmission (1.0%). An increase in the proportion of sexual transmission was observed to harbor the strains resistant to either protease inhibitors (PIs) (3.4%), or nucleoside reverse transcriptase inhibitors (NRTIs) (2.1%), or nonnucleoside reverse transcriptase inhibitors (NNRTIs) (3.4%). Although the prevalence of drug resistance was relatively low among the treatment-naive HIV-1 patients in Beijing in comparison to those in industrialized countries, we will continue monitoring newly infected subjects for any potential alteration of the prevalence pattern to ensure the success of the ongoing scale-up of antiretroviral treatment.

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A total of 200 drug-naive HIV-1 patients diagnosed during 2006 and 2007 in the Beijing Center for Disease Prevention and Control (BJCDC) participated in this study (50 in 2006 and 150 in 2007). Enrollment in this study was preapproved by participants with their informed consent after ensuring anonymity as well as by the Committee on Human Research at BJ CDC. The plasma from these patients’ blood samples were separated and stored at ~80°C. Demographic data and possible HIV-1 exposure risk factors were requested by a designed questionnaire during the interview. CD4+ lymphocyte counts were determined by flow cytometry (FC500, Beckman Company, USA) at the time of blood sample collection (within 24 h).

RNA was isolated from the plasma samples using the QIAamp viral extraction kit (QIAGEN, Germany). The HIV-1 pol gene (protease 1–99 amino acids and part of reverse transcriptase 1–250 amino acids) was amplified by reverse transcriptase polymerase chain reaction (RT-PCR) (Takara One Step RNA PCR kit) with outer primer pair MAW 26 (5'-GGGAATATGGAAAGGAAGAC-3', 2028–2050 nt, HXB2) and RT21 (5'-CTGATTATTTGCTTAAAGCTTITTGATGGG-3', 3509–3539 nt, HXB2), followed by nested PCR (Takara Ex Taq PCR kit) with inner primer pair PRO-1 (5'-ACGGGGTGCGGAGCCTCARTATTAA-3', 2147–2166 nt, HXB2) and RT20 (5'-CTGGCAGTTCATGGCTGC-3', 3441–3462 nt, HXB2). The amplifications were carried out in a thermal cycler (170–9703, Bio-Rad) for first-round RT-PCR (50°C for 30 min and 94°C for 2 min), followed by 35 cycles (94°C for 30 s, 55°C for 30 s, 72°C for 2 min), and a final extension for 7 min at 72°C.

From the first-round PCR products, 5 μl was used as a template for the nested PCR with inner primers and the same cycling condition as the first-round PCR except the cycles were at 50°C for 30 min. The second-round PCR products were purified using spin columns (QIAGEN, Germany) and were sequenced with primers PRO-1, RTAS (5'-CTCATGGCGTGTTGCAC-3', 2524–2539 nt, HXB2), and RTB (5'-CTCCAGTTCATGGCTGC-3', 2946–2967 nt, HXB2) using an ABI 3730 sequencer. For analysis of HIV-1 drug resistance mutations, each sample sequence was compared with the subtype B consensus sequence and was interpreted by the HIVdb program (http://hivdb.stanford.edu/).

For HIV-1 subtyping, the edited sequences were aligned against reference sequences available at the Los Alamos database, http://www.hiv.lanl.gov/content/index. Phylogenetic trees were constructed by the neighbor-joining method with 1000 bootstrap replicates using Mega 4.0.7

The GenBank accession numbers of nucleotide sequences reported in this article are JF906562-JP906700. The Chi-square test or Fisher exact test was performed to detect significant differences in prevalence of drug resistance of populations with different risk factors and in the frequency of amino acids at specific positions between different subtypes. Statistical significance was defined as p < 0.05. All the statistical analyses were performed with Microsoft Excel software.

Of the 200 treatment-naive HIV-1-infected subjects, 145 (72.5%) were successfully sequenced and were subjected to drug resistance analysis including protease inhibitor (PI), nucleoside reverse transcriptase inhibitor (NRTI), and non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance mutations. The remaining 55 samples that could not be successfully sequenced were excluded from the analysis to ensure the accuracy of this study. Of the 145 patients whose samples were included, 130 were males, aged 13–68 years (average: 31.9 years) and 15 were females, aged 23–43 years (average: 32.3 years). They were from 22 provinces including Beijing, Hebei, Shanxi, Inner Mongolia, Liaoning, Jilin, Heilongjiang, Jiangsu, Zhejiang, Anhui, Fujian, Jiangxi, Shan- dong, Henan, Hubei, Hainan, Chongqing, Sichuan, Gansu, Qinghai, Ningxia, and Xinjiang. The patients from Beijing, Xinjiang, Henan, Sichuan, and Hebei constituted 32.4% (47 of 145), 10.3% (15 of 145), 9.0% (13 of 145), 9.0% (13 of 145), and 6.2% (9 of 145) of all the patients investigated, respectively.

In addition to the Han ethnic group that accounted for the majority of our study participants (120 of 145; 82.8%), HIV-1 was also detected in five other minority populations including Uygur (16 of 145; 11.0%), Yi (0.7%), Dai (0.7%), Hui (0.7%), and Manchu (0.7%). With regard to transmission routes, five patients (3.4%) were former plasma/blood donors (FPDs), 28 (19.3%) through heterosexual contact, 69 (47.6%) through homosexual contact, and 22 (15.2%) were IDUs. The transmission routes for the remaining 21 subjects were unknown. The median CD4 cells count of the samples analyzed was 374 (range: 9–1133) cells/μl.

Phylogenetic tree analysis of pol sequences revealed that the majority of the HIV-1 isolates (40, 27.6%) studied belonged to CRF01_AE, and the second most prevalent strain was subtype B’ (35, 24.1%), followed by CRF07_BC (31, 21.4%), subtype B (30, 20.7%), CRF08_BC (5, 3.4%), subtype C (3, 2.1%), and CRF06_cpx (1, 0.7%). The distribution of HIV-1 strains was uneven among different risk groups. CRF01_AE and subtype B were two strains more frequently found in homosexual individuals (29, 42.0% and 23, 33.3%, respectively) than in other HIV-1-infected populations, and the CRF07_BC strain had the highest frequency among IDUs (20, 90.9%). There were more diversified HIV-1 subtypes in heterosexual infectors, in which subtype B’, CRF07_BC, and CRF01_AE were among the most prevalent, while B, CRF08_BC, C, and CRF06_cpx could also be detected.

Eleven out of 145 isolates (7.6%) were identified as resistant to one or more antiretroviral drugs. Of these, one of them (0.7%) was resistant to both PIs and NNRTIs, and one (0.7%) to NRTIs and NNRTIs (Table 1).

Primary drug resistance mutations to PIs were found in five samples (3.4%) with one single major mutation at M46L, which confers low resistance to ATV and NFV. All of the five subject were infected through homosexual activities, with three from Beijing, one from Shanxi, and one from Heilongjiang province. Four of the five subjects were infected with the CRF01_AE strain. Various minor mutations were also detected on the PR gene (Table 2). Minor mutations may not result in a significant decrease in sensitivity to PIs, though T74S could confer potent level resistance to NFV.

Mutations associated with resistance to reverse transcriptase inhibitors (RTIs) were found in seven (4.8%) subjects. Six of them were resistant to either NRTIs (n = 3, 2.1%) or NNRTIs (n = 5, 3.4%) and one (0.7%) was resistant to both NRTIs and NNRTIs. The mutation sites in the respective subtypes are listed in Tables 3 and 4. Mutations T69D and V118I caused low level resistance to DDI; D67DN and V118I caused low level resistance to AZT; and A62V, K65R, T69D, V75LV, and K219R caused intermediate level resistance to 3TC, ABC, AZT, D4T, DDI, FTC, and TDF. Each of the three types of mutation-induced drug resistance was found in each respective patient.
The K101E and G190A mutations conferred intermediate level resistance to DLV, EFV, and ETR and high level resistance to NVP; G190AG conferred intermediate level resistance to EFV, low level resistance to ETR, and high level resistance to NVP; K103N conferred high level resistance to DLV, EFV, and NVP; V108I and Y181C conferred intermediate level resistance to EFV and ETR and high level resistance to DLV and NVP; and F227FL conferred low level resistance to NVP. Each type of mutation-induced drug resistance was also found in its corresponding patient. The most frequent NRTI-related and NNRTI-related mutations were V118I and V106I, which were found in 23 (15.9%) and 11 (7.6%) samples, respectively. Other mutations, which by itself did not confer any drug resistance to NRTI and NNRTI, were also identified (Tables 3 and 4).

Major mutation M46L was observed in 10.0% of the CRF01_AE strains and in 1.0% of the non-AE strains (Fisher exact test, \( p < 0.05 \)). A71V and A71T, the two most predominant minor mutations in the PR gene, were both observed in 10.8% of the B/B¢ strains with A71V in 3.8% and A71T in 1.3% of the non-B strains (Fisher exact test, \( p > 0.05 \) and \( p < 0.05 \)). In the RT gene, V118I, V106I, and V179E, the three predominant

### Table 1. Genotypic Resistance Mutations Detected and Phenotypic Resistance Mutations Predicted of HIV-1 Circulating in Beijing

<table>
<thead>
<tr>
<th>ID</th>
<th>Gender</th>
<th>Age</th>
<th>Risk group</th>
<th>Subtype</th>
<th>Province</th>
<th>Resistance mutation</th>
<th>Predicted phenotypic resistance</th>
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<tbody>
<tr>
<td>06117</td>
<td>M</td>
<td>41</td>
<td>Homo</td>
<td>01AE</td>
<td>Beijing</td>
<td>M46L</td>
<td>ATV(L), N FV(L)</td>
</tr>
<tr>
<td>06590</td>
<td>M</td>
<td>31</td>
<td>Homo</td>
<td>B'</td>
<td>Henan</td>
<td>A71V M62V, K65R, T69d, V75LV, K219R V108L, Y181C</td>
<td>3TC(L), ABC(L), AZT(L), D4T(L), ddI(L), FTC(L), TDF(L)</td>
</tr>
<tr>
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<td>01AE</td>
<td>Heilongjiang</td>
<td>M46L</td>
<td>ATV(L), N FV(L)</td>
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<td>B'</td>
<td>Shandong</td>
<td>A71V D67DN, V118I</td>
<td>AZT(L)</td>
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<tr>
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<td>41</td>
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<td>B'</td>
<td>Henan</td>
<td>G190AG</td>
<td>EFV(I), ETR(L), NVP(H)</td>
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<td>ATV(L), N FV(L)</td>
</tr>
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<td>ddI(L)</td>
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<td>B'</td>
<td>Hebei</td>
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<tr>
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<td>B</td>
<td>Shanxi</td>
<td>M46L V118I</td>
<td>NVP(L), DLV(L), EFV(I), ETR(I), NVP(H)</td>
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<td>C</td>
<td>Beijing</td>
<td>T74S</td>
<td>K103N</td>
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</table>

Pls, protease inhibitors; NRTIs, nucleoside reverse transcriptase inhibitors; NNRTIs, nonnucleoside reverse transcriptase inhibitors; AZT, zidovudine; NFV, nelfinavir; ABC, abacavir; ATV, atazanavir; ddI, didanosine; d4T, stavudine; DLV, delavirdine; EFV, efavirenz; FTC, emtricitabine; NVP, nevirapine; TDF, tenofovir; 3TC, lamivudine; ETR, etravirine; L, low-level resistance; I, intermediate level resistance; H, high-level resistance; homo, homosexual; heter, heterosexual.

The K101E and G190A mutations conferred intermediate level resistance to DLV, EFV, and ETR and high level resistance to NVP; G190AG conferred intermediate level resistance to EFV, low level resistance to ETR, and high level resistance to NVP; K103N conferred high level resistance to DLV, EFV, and NVP; V108I and Y181C conferred intermediate level resistance to EFV and ETR and high level resistance to DLV and NVP; and F227FL conferred low level resistance to NVP. Each type of mutation-induced drug resistance was also found in its corresponding patient. The most frequent NRTI-related and NNRTI-related mutations were V118I and V106I, which were found in 23 (15.9%) and 11 (7.6%) samples, respectively. Other mutations, which by itself did not confer any drug resistance to NRTI and NNRTI, were also identified (Tables 3 and 4).

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### Table 2. Amino Acid Substitutions in the HIV Protease Gene of 145 Treatment-Naive Patients at Positions Associated with Resistance to Protease Inhibitors

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Number</th>
<th>M46L</th>
<th>A71AT</th>
<th>A71T</th>
<th>A71V</th>
<th>L10I</th>
<th>L10IL</th>
<th>L10V</th>
<th>M46MT</th>
<th>Q58E</th>
<th>T74S</th>
<th>V11I</th>
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</tr>
<tr>
<td>Total</td>
<td>145</td>
<td>5</td>
<td>2</td>
<td>8</td>
<td>10</td>
<td>6.9%</td>
<td>3</td>
<td>2.1%</td>
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<td>0.7%</td>
<td>3</td>
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</table>
mutations, were detected in 35.4%, 16.9%, and 6.2% of B/B’ strains, respectively, while none of them was found in non-B/B’ strains (Fisher exact test, \( p < 0.05 \)). K103R was observed in 7.5% of the CRF01_AE strains and 1.0% of the non-AE strains (Fisher exact test, \( p > 0.05 \)).

The comparison of the HIV drug resistance rates in three major risk groups revealed that the difference among heterosexual individuals (2 of 28, 7.1%), homosexual individuals (8 of 69, 11.6%), and IDUs (0 of 22, 0%) (Fisher exact test, \( p > 0.05 \)) was not statistically significant. There was also no significant difference found in drug resistance rate between female (2 of 15, 13.3%) and male (9 of 130, 6.9%) subjects. Although there was an increase in drug resistance in 2007 (8.1%, 9 of 111), it was not statistically significant (\( p > 0.05 \)) compared with that in 2006 (5.9%, 2 of 34). The rate of high-level resistance to one or more antiretroviral drugs was also not significant (\( p > 0.05 \)) between 2007 (2.7%, 3 of 111) and 2006 (2.9%, 1 of 34). The drug resistance rates for the main subtypes B, B’, CRF01_AE, and 07BC are 3.3% (1 of 30), 14.3% (5 of 35), 10.0% (4 of 40), and 0% (0 of 31), respectively, with no statistical significance of the differences found.

Our study was the first large-scale cross-sectional survey on HIV drug resistance in treatment-naive HIV-infected individuals in Beijing. It revealed that the overall prevalence of drug resistance was 7.6% (11 of 145), with 3.4% for PIs, 2.1% for NRTIs, and 3.4% for NNRTIs. The drug resistance rate in this study was higher than the 3.8% HIV drug resistance rate of 676 treatment-naive HIV patients during 2004 and 2005 in a nationwide investigation,\(^9\) the 4.4% among 91 HIV patients in Liaoning province,\(^9\) the 2.0% in Thailand during 2003 to 2006,\(^10\) and the 4.3% in South Korea from 1999 to 2005.\(^11\) However, the rate in our study was lower than the rates in North America and in Western Europe where the varying prevalence of drug resistance was reported to be from 9.1% to 14.6%.\(^12\),\(^13\)

As far as transmission routes were concerned, the prevalence rates of drug resistance in this study were 7.1% (2 of 28), 11.6% (8 of 69), and 0% (0 of 22) among heterosexual, homosexual, and IDU individuals, respectively. The unexpected higher percentage of resistance was likely due to the high percentage (47.6%, 69 of 145) of homosexual patients, of which the prevalence of drug resistance was 11.6% (8 of 69), similar to that reported before.\(^14\) If the homosexual group was not counted, the prevalence would be 3.9% (3 of 76), which was similar to other previous reports in China.

Our result are consistent with the high prevalence (15%) of drug resistance found in homosexual people in a previously reported study in Beijing.\(^14\) In this study, 11.6% of treatment-naive homosexual participants carried drug-resistant mutations, a much higher proportion than that from earlier studies on nonhomosexual populations conducted in China. It is possible that homosexual patients have been primarily infected with drug-resistant strains derived from other countries with more diverse and prolonged ART experience. The other possibility could be that some patients misreported their ART status as naive rather than “not-currently-taking.” Since ART use was self-reported in this study. Based on the preliminary information we obtained, at least a few patients had started ART by themselves in the acute phase of their HIV infection to have their viral load decreased to the “set point.” If this were true, it could lead to an overestimation of the prevalence of HIV drug resistance.

The amino acid substitutions associated with resistance to PIs and RTIs had been extensively characterized on subtype B, which is predominant in developed countries. Information on the drug resistance-related mutation in non-B subtypes, however, is still lacking in China.

In this study, CRF01_AE constituted the most prevalent HIV-1 strains, 27.6%, followed by subtype B’, CRF07_BC, B, CRF08_BC, C, and CRF06_cpx, which account for 21.4%, 20.7%, 3.4%, 2.1%, and 0.7%, respectively. Given the complexity of HIV subtypes circulating in Beijing, we collected the information on different profiles of drug resistance-related mutations from each subtype. Our studies identified HIV-1 drug resistance-associated mutations in subtype B (one), B’ (five), C (one), and CRF01_AE (four), revealing a substantial difference in the profile of drug resistance mutations between the subtypes studied. We observed a high drug resistance of 10.0% (4 of 40) and 14.3% (5 of 35) in CRF01_AE and B’ and a low resistance of 3.3% (1 of 30) and 0% (0 of 31) in B and CRF07_BC strains, respectively. CRF01_AE mainly conferred resistance to PIs; of the five PIs resistance mutations that were identified, CRF01_AE constituted 80% (4 of 5). Meanwhile, subtype B’ mainly conferred resistance to RTIs, constituting 71.4% (5 of 7) of the RTIs resistant isolate.

Of the 11 subjects resistant to antiretroviral drugs, the profiles of mutations were identical for PIs, but diversified for RTIs, with every RTI drug-resistant strain carrying a unique mutation pattern. These results revealed the presence of a particular pattern of the drug resistance-associated mutations occurring in patients in Beijing and also suggested that these PI-resistant strains may have a common ancestor. We also found that the rate of high-level resistance to NNRTIs was high, with five samples that conferred resistance to NNRTIs and four (80%) showing high-level resistance, which represents a challenging situation for the use of NNRTIs.
The first-line ARV drugs widely used in Beijing included efavirenz (EFV), lamivudine (3TC), indinavir (IDV), didanosine (ddI), zidovudine (AZT), stavudine (d4T), and nevirapine (NVP) and the second-line drugs included tenofovir (TDF), lopinavir (LPV), and ritonavir (RTV). In this study, 11 subjects were identified as conferring resistance to 13 drugs, some of which had not been introduced into Beijing such as atazanavir (ATV), nelfinavir (NFV), abacavir (ABC), emtricitabine (FTC), delavirdine (DLV), and etravirine (ETR). These data indicate that HIV drug-resistant isolates from other countries may have disseminated into Beijing, which will no doubt compromise the success of future ART in these patients.

We determined several subtype-specific drug resistance mutations in the PR and RT regions. Major mutations M46L (10%) and K103R (7.5%) were more frequent in CRF01_AE strains than in non-CRF01_AE strains with mutations M46L (1.0%) and K103R (1.0%). The B/B¢ subtype was characterized by a higher frequency of substitutions at mutations A71V (10.8%) and A71T (10.8%), compared with the non-B/B¢ subtype, in which the frequencies of substitution at these codons were 3.8% and 1.3%, respectively. Mutations V118I (35.4%), V106I (16.9%), and V179E (6.2%) were restricted only to B/B¢ strains. The V118I mutation, which does not confer any drug resistance to NRTIs by itself, appeared in 23 subjects. However, when V118I occurs with T69D and D67DN, it confers a low-level resistance to DDI and AZT. K103N occurs in about 0.7% of untreated persons, though by itself, it confers a high level of resistance to three NNRTIs including DLV, EFV, and NVP.

Some of these subtype-specific drug resistance mutations in drug-naive individuals with respect to B and non-B subtypes are similar to those reported in other studies, while the major mutation M46L associated with HIV-1 CRF01_AE was first reported in this study. Because of the characteristics of the corresponding subtype, these specific mutations may provide insight into the emergence of resistant phenotypes.

In this study, 7.6% of patients have mutations conferring resistance to PIs and RTIs. The baseline prevalence of drug resistance mutations was rather high when compared with those in other studies conducted in China on treatment-naive HIV-1 patients. It is our belief that most patients will be sensitive to the ART provided free by the Chinese government, though we observed that an emerging number of patients exhibited resistance to antiretroviral drugs that had not been introduced to the Chinese market. The information on drug resistance and related HIV strain mutations obtained in this study is believed to be of importance in guiding the recommendations on selective usage of different antiretroviral drugs for the optimal treatment of HIV patients in China.

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Author Disclosure Statement

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References

3. Community Prevents the AIDS Propaganda Activity Organization Committee Beijing, China: The epidemic situation in Beijing was released by June 30, 2009. CAPITAL MEDICINE 2009;16(15) [in Chinese].

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