BACKGROUND: The global human immunodeficiency virus (HIV)-1 epidemic is becoming increasingly diverse and complex. Molecular epidemiologic characteristics were studied for HIV-1–infected blood donors from five Chinese regions to determine genotype diversity and drug resistance mutations (DRMs) profile.

STUDY DESIGN AND METHODS: HIV-1 confirmed-reactive serum samples were collected from 172 blood donors from five blood centers during 2007 to 2010. HIV-1 Pol including whole protease and partial reverse transcriptase genes was amplified, sequenced, and analyzed for the subtype determination and drug resistance profile description.

RESULT: A total of 113 amplified sequences including 82 from Kunming blood center and 31 from four other blood centers had the following genotype characteristics: G (0.9%), B (2.7%), circulating recombinant form (CRF) 01_AE (32.7%), CRF07_BC (22.1%), and CRF08_BC (41.6%). Female donors represent 45.1% of all cases and 63.9% cases with DRMs. The prevalence of samples with potential low or higher resistance among Chinese blood donors is 4.4%.

CONCLUSION: HIV-1 infection in Chinese blood donors is genetically diverse and the subtype distribution reflects that from the high-risk populations. Our results support continuous molecular epidemiologic surveillance for HIV-1 in blood donors as a part of a comprehensive HIV control program.

In 2009, there were an estimated 740,000 people living with human immunodeficiency virus Type 1 (HIV-1) in China with AIDS becoming China’s leading cause of death among infectious diseases. Genetic diversity has been a feature of HIV-1 infection in humans. The currently detected distinct phylogenetic groups or subtypes of HIV-1 include Subtypes A1, A2, B, C, D, F1, F2, G, H, J, and K as well as many recombinants named as circulating recombinant forms (CRFs). Both viral and host factors contribute to this high genetic diversity. These factors include the rapid viral duplication rate.
the error-prone reverse transcription process, recombination between different viral strains, and the selective pressure from the host immune response and antiviral treatment. Increasing HIV-1 genetic diversity has been reported in China as the AIDS pandemic progresses.5,6

Unique distribution patterns of HIV-1 subtypes in different Chinese regions correlate with the route of the spread of the virus.7-10 For example, while the prevalent HIV-1 subtypes in the Yunnan Province are CRF-BC and CRF01_AE, which are primarily transmitted through heterosexual sex and injecting drug use (IDU),11 Subtype B is primarily found in inland regions such as Henan with the major route of transmission being related to the unsafe blood collection practices of the 1980s and 1990s.12,13

Monitoring drug resistance mutation (DRM) profiles in HIV-1–infected individuals is another important element of an HIV molecular epidemiologic surveillance program. Mortality rates have been greatly reduced with the introduction of highly active antiretroviral therapy (HAART) treatment.14 Unfortunately, drug resistance from antiretroviral therapy treatment (ART) has become a major obstacle leading to treatment failures.15,16 Moreover, transmitted drug resistance emerges frequently among treatment-naïve individuals and therefore compromises the effect of ART.17-19 Although there have been several studies examining the genetic diversity and DRMs in HIV-1–infected individuals from high-risk populations in China,20-27 there are very limited data from HIV-1–infected volunteer blood donors.

We present results from a study that used phylogenetic tools to determine the HIV-1 subtypes and detect DRMs in infected blood donors who attempted to donate between 2007 and 2010 at five blood centers located in geographically diverse Chinese regions (Kunming Blood Center in Yunnan Province, Liuzhou Blood Center in Guangxi Province, Urumqi Blood Center in Xinjiang Province, Luoyang Blood Center in Henan Province, and Mianyang Blood Center in Sichuan Province; see map in Fig. 1).

Surveillance of HIV-1 genetic diversity is an integral part of a comprehensive program to develop better prevention and treatment strategies. Studies in blood donors contribute to our knowledge about the current molecular epidemiologic features of HIV-1 in the general population. Insight into the trend and makeup of HIV-1 genetic variety is imperative for effective vaccine development. Knowledge about the HIV-1 subtype distribution in infected donors is critical for developing sensitive and specific donor screening test for HIV-1. Most HIV-1–infected blood donors have been previously untreated with HAART. Blood donors should therefore be part of a molecular epidemiologic surveillance program because information from this population is valuable in monitoring the molecular diversity trend of the epidemic and will help to design more effective treatment approaches for treatment-naïve individuals.28 In addition to benefiting the development of effective HIV-1 treatment strategies, baseline information on DRMs of HIV-1–infected donors will also help the blood centers and local public health offices improve their service to infected donors by providing this information during donor counseling.

**MATERIALS AND METHODS**

**Samples**

From October 2007 to August 2010, a total of 172 HIV confirmed-reactive serum samples of 717,390 blood donors were collected from five blood centers participating in the NHLBI-sponsored Retrovirus Epidemiology Donor Study-II (REDS-II) International-China Program: Kunming (n = 111), Urumqi (n = 35), Liuzhou (n = 14), Mianyang (n = 6), and Luoyang (n = 6). Routine screening tests were performed at each blood center by two of the following four enzyme immunoassays for anti-HIV-1/2 (bioMérieux, Marcy l’Etoile, France; Bio-Rad, Inc., Hercules, CA; KeHua, Inc., Shanghai, China; and WanTai, Inc.,
Screening test HIV-reactive serum samples with either kit then underwent confirmatory testing at the Institute of Blood Transfusion, Chinese Academy of Medical Sciences by Western blot (Ausia, Inc., Hangzhou, China).

**Extraction, amplification, and sequencing of HIV-1 RNA**

HIV-1 RNA was extracted from 350 μL of serum using a viral RNA isolation kit (MagMAX, Ambion, Inc., Austin, TX) according to the manufacturer’s specifications, and eluted to a 75-μL suspension. Reverse transcription nested polymerase chain reaction (RT-PCR) was applied using reverse transcriptase (ReverTra Ace, Toyobo [Shanghai], Inc., Shanghai, China) and high-fidelity PCR polymerase (KOD FX, Toyobo, Inc.) to amplify approximately 1036 bp of the pol region including the entire protease gene (297 nucleotides encoding 99 amino acids) and partial polymerase gene (the first 244 amino acids) for genotyping and DRM analysis. Ten microliters of RNA suspension was used in RT reaction with primer (RT21, 5ʹ-CTGCTGCTATTAAAG TCTTTTGATGGG-3ʹ, HXB2, 3509-3539) placed at room temperature for 10 minutes and then 42°C for 30 minutes, followed by 95°C for 5 minutes, and put on ice. The amplification of first-round PCR using 10 μL of RT product as template was amplified with inner primers (Pro-1, 5ʹ-CCTCTGCTATTTAAG TCTTTTGATGGG-3ʹ, HXB2, 3509-3539) placed at room temperature for 10 minutes and then 72°C 1 minute, followed by 72°C 10 minutes. The second-round PCR taking 5 μL of the first-round PCR product as template was amplified with inner primers (Pro-1, 5ʹ-CCTCTGCTATTTAAG TCTTTTGATGGG-3ʹ, HXB2, 3509-3539) was run under the following conditions: one cycle of 94°C for 5 minutes, 55°C for 1 minute, and 72°C for 2 minutes 30 seconds and then 30 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute 30 seconds followed by 72°C for 10 minutes. The second-round PCR taking 5 μL of the first-round PCR product as template was amplified with inner primers (Pro-1, 5ʹ-CCTCTGCTATTTAAG TCTTTTGATGGG-3ʹ, HXB2, 3509-3539) under the following conditions: one cycle of 94°C for 2 minutes, 63°C for 50 seconds, and 72°C for 1 minute 30 seconds and then 35 cycles of 94°C for 30 seconds, 63°C for 30 seconds, and 72°C 1 minute, followed by 72°C 10 minutes. The PCR products were purified and both DNA strands were sequenced directly with inner primers by a DNA sequencing company (BGI, Inc., Beijing, China).

**Genotypic drug resistance analysis**

The profile of genotypic drug resistance was determined by submitting the sequences to the Stanford HIVdb Program Genotypic Resistance Interpretation Algorithm (http://hivdb.stanford.edu). HIVdb is an expert system that accepts user-submitted HIV-1 pol sequences and returns inferred levels of resistance to 20 FDA-approved ARV drugs including eight protease inhibitors (PIs), seven nucleoside and nucleotide reverse transcriptase inhibitors (NRTIs), four nonnucleoside reverse transcriptase inhibitors (NNRTIs), and one integrase inhibitor. The HIVdb system assigns each HIV-1 DRM a drug penalty score and a comment; the total score for a drug is derived by adding the scores of each mutation associated with resistance to that drug. Using the total drug score, the program reports one of the following levels of inferred drug resistance: susceptible, potential low-level resistance, low-level resistance, intermediate resistance, and high-level resistance.

**Statistical analysis**

Demographic information was extracted from the donor database founded by the REDS-II program,30 and the statistical analysis was processed with computer software (SPSS, Version 13.0, SPSS, Inc., Chicago, IL).

**Gene accession numbers**

The sequences in our study can be retrieved in GenBank with accession numbers from JF505510 to JF505622.

**RESULTS**

Of 717,390 donors analyzed in this study, the HIV-1 screening-positive rates of five blood centers were Kunming (605/274,720, 0.22%), Urumqi (331/143,150, 0.23%), Liuzhou (94/57,720, 0.16%), Mianyang (47/78,000, 0.06%), and Luoyang (55/163,800, 0.03%), and the confirmatory rates were Kunming (113/605, 18.68%), Urumqi (35/331, 10.57%), Liuzhou (14/94, 14.89%), Mianyang (6/47, 12.77%), and Luoyang (6/55, 10.91%). Each blood sample underwent two round screening tests resulting in a relative high false-positive rate at the confirmatory stage.

**Demographic characteristics**

Of the 172 confirmed HIV-1–reactive samples, DNA from 113 donors was successfully amplified and analyzed for partial HIV-1 pol region: Kunming (n = 82/111), Urumqi

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(n = 14/35), Liuzhou (n = 10/14), Mianyang (n = 5/6), and Luoyang (n = 2/6). Demographic information of these 113 donors is shown in Table 1. As a group, there are more males than females and 20.4% of these donors were from non-Han ethnic background while the overall percentage of non-Han donors from these five blood centers for the study period is 13.3% (unpublished REDS-II China data). Most donors (89.4%) were first-time donors. A total of 84.9% of donors in this study were between 20 and 40 years of age and the majority of them had high school or lower education.

### HIV-1 subtype distribution

For the whole group, the HIV-1 subtype distribution by phylogenetic analysis was as follows: G = 1 (0.9%), B = 3 (2.7%), CRF01_AE = 37 (32.7%), CRF07_BC = 25 (22.1%), and CRF08_BC = 47 (41.6%; Fig. 2). CRF_BC and AE were dominant subtypes in this study. The subtype distribution of samples from Kunming was B = 1 (1.2%), CRF01_AE = 21 (25.6%), CRF07_BC = 13 (15.9%), and CRF08_BC = 47 (57.3%). All CRF08_BC strain samples in this study were from Kunming. All 10 samples from Liuzhou were CRF01_AE. Two samples from Luoyang were both of B subtype. The only subtype G was from Urumqi.

### Drug resistance–associated mutation analysis

Of 113 samples analyzed, 36 (31.9%) were found to have DRMs for the whole group. All samples with DRMs were first-time donors. Thirty-one samples from Kunming displayed 36 DRMs, and the rate of DRM within samples from Kunming was 37.8% (31/82). There were six (15.0%) PI minor DRMs, 18 (45.0%) NRTI DRMs, and 16 (40.0%) NNRTI DRMs. Samples from Kunming had two (5.6%) PI minor DRMs, 18 (50.0%) NRTI DRMs, and 16 (44.4%) NNRTI DRMs. Characteristics of samples with DRMs are shown in Table 2. A large proportion of donors with DRMs was female (23/36 = 63.9%).

No major PI DRMs were found. Half of the six PI minor DRMs were L10I/V. The 18 NRTI DRMs from 16 samples (all from Kunming) include T69S/D, L210M/F, and A62V with T69S as the dominant mutation. Of the 20 NNRTI DRMs from 19 samples, nine were E138A/K. The DRMs and their subtype distribution are shown in Fig. 3. No significant subtype difference was found in PI minor DRMs, while CRF08_BC was dominant in both NRTI and NNRTI DRMs, especially in T69S/D and E138A/K.

Five (4.4%) samples displayed potential low-level resistance or above (Table 3). K7 and K25 showed potential low-level resistance to NNRTI due to V179D mutation. K54 displayed potential low-level resistance to stavudine (D4T) and low-level resistance to didanosine (DDI) because of the A69D mutation. L1 had a V108I mutation, which is associated with potential low-level resistance to efavirenz and nevirapine. G1 harbored T74S and E138K mutations causing potential low-level resistance to nelfinavir, efavirenz, etravirine, and low-level resistance to nevirapine.
DISCUSSION

As in many countries, China has seen the spread of HIV-1 infection from high-risk groups into the general population over the past 25 years. The HIV-1 epidemic in China has been complex both in the routes of viral spread and in the genetic variability of the HIV-1 strains. HIV-1 genotype diversity contributes to monitor the mode of viral transmission. To develop effective prevention and treatment strategies, it is critical to collect ongoing molecular epidemiologic information, not only from high-risk groups, but also from infected individuals from the general population such as volunteer blood donors.

To investigate the genetic complexity of transmitted variants of HIV-1 in blood donors, serum samples were collected from five Chinese blood centers located in regions with the highest HIV prevalence in China. Four blood centers are from the vast Western part of China except Luoyang blood center (Henan Province), which represents the central Chinese regions. Overall, CRF_BC (63.7%) and CRF01_AE (32.7%) are the dominant subtypes among blood donors from Western China. Kunming (Yunnan Province) has the largest number of HIV-infected people in China. Eighty-two samples from this study were from Kunming. The subtype distribution in Kunming blood donors from this study is similar to findings from an earlier study during 2005 to 2006. The subtype distribution showed differences between Kunming and the other blood centers. CRF08_BC was only identified in Kunming from this study. This finding is consistent with previous reports that although CRF08_BC is distributed nationwide, it is a rare subtype in other regions. CRF01_AE is the dominant subtype in the other four regions (51.6%), except for Kunming (25.6%). Samples from Liuzhou (Guangxi Province) were all subtype CRF01_AE. This subtype was first introduced from Southeast Asia into mainland China via the Guangxi Province, the region bordering with Southeast Asia. CRF01_AE is the dominant strain among high-risk groups from this region. CRF07_BC has been the main subtype in Urumqi (Xinjiang Province), a subtype of HIV found commonly in the IDU population. Not surprisingly, 11 of 13 strains from Urumqi blood donors in this study are CRF07_BC.

While the majority of Chinese HIV-1–infected individuals acquired the infection through IDU or sexual transmission, the Henan Province, which is located in central China, is unique in that most of its HIV-1–infected individuals were infected by unsafe blood collection (mostly commercial plasma collection) practice before the mid-1990s. Previous studies have shown that most infected individuals from Henan display Subtype B as a result of the Founder’s effect. Unsafe blood collection practice was banned by the Chinese government in the late 1990s. There have also been several large-scale government-funded campaigns to provide HIV-1 testing for people with a history of donating or selling blood products previously and their family members. Both of the two young donors in this study from Luoyang in the Henan Province have subtype B infection. Our results suggest that the distribution of HIV-1 subtype distribution

Fig. 2. Phylogenetic tree of the HIV-1 sequences. (○) Kunming (K), (■) Urumqi (U), (△) Luoyang (L), (◇) Liuzhou (G), (●) Mianyang (M), and (●) reference sequences.
in infected blood donors matches that from local high-risk populations. This finding indicates the appropriateness of using blood donor screening as one of the tools in monitoring HIV epidemic characteristics in the general population. Although it cannot be ruled out that some of the infected donors may have been test seekers, our findings support the theory that HIV-1 continues to spread from isolated high-risk groups into the general population. Additional studies that are under way will help to determine risk factors associated with these infections. Because all blood donors underwent screening with a health history questionnaire including history of treatment before giving a blood sample for testing for HIV-1, most HIV-1–infected blood donors had no previous treatment with ART. Both US and Chinese governments have national programs to monitor DRMs in treatment-naïve populations because such information is valuable for rational design of ART treatment regimen.

The Stanford HIVdb Program Genotypic Resistance Interpretation Algorithm is mainly based on studies of Subtype B HIV-1 and is not suitable for all non-B subtypes on DRM determination. Some non-B subtype–dependent DRMs are described to be polymorphic, but whose relations to drug effect are still unclear. In this study, T69S/D and E138A/K were the most commonly observed NRTI and NNRTI–associated mutations. Both of those mutations were predominantly found in CRF08_BC subtype, supporting earlier research that T69S is a polymorphism with CRF08_BC subtype specificity; however, we also observed the E138A/K mutation’s association with CRF08_BC subtype. Although the E138A/K mutation is described as a polymorphism associated with a decreased etravirine response in the drug resistance database, Codon 138 of four CRF08_BC reference sequences are all E, which suggests that the E138A/K mutation does relate with the drug effect. Further in-depth research is needed because CRF08_BC is a common subtype among the HIV-1–positive population in China.

Compared with the other four regions, samples from Kunming exhibited a higher DRMs prevalence rate. This result may be due to two major factors. First, the earliest outbreak of the HIV-1 epidemic in China was at the border of the Yunnan Province adjacent to the “Golden Triangle” of Southeast Asia as a result of rampant drug trafficking and use during the 1990s. The government enforced the drug ban act, and consequently, the spread of HIV-1 via IDU declined. However, early infected persons who received antiretroviral treatment are still a large transmission source. Second, the Yunnan Province is an underdeveloped area in China, where unsafe sex behaviors are probably more common.

Only five samples (4.4%) from this study harbored DRMs that reach potential low-level resistance or higher

<table>
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<th>TABLE 2. Characteristics of donors with DRMs*</th>
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* Data are reported as number (%).

Fig. 3. DRMs and their subtype distribution.
resistance, indicating a relatively low prevalence of drug resistance among Chinese blood donors. This rate is lower than that from infected donors in the United States (9.9% between 2005 and 2007),46 in Brazil (6.3%),47 and in Europe (9.1%)48 and is likely because of the comparably shorter ART history in China. This rate is similar to that from other Chinese treatment-naïve individuals from high-risk groups including IDUs, heterosexual, and paid plasma donors (3.842 and 4.4%23). The reported rate from the group of men who have sex with men in China was higher (15%, p < 0.05).49 The ART regimens used in China’s national Free Care Program include four NRTIs and two NNRTIs.50 NRTI and NNRTI-associated mutations are the major DRMs (38/44) in this study, likely reflecting the selective pressure by HAART on circulating strains among infected patients.

A study in US blood donors found that among donors with drug-resistant strains, 90% of them were male and the median age was 42 years.46 Our study found that all five donors with potential low-level or higher level resistance were female with a median age of 26. In addition, 63.9% (23/36) of all donors in our study with any DRMs were also female. In fact, the percentage of females among all infected blood donors in Kunming has increased from 30.6% (2005 to 2006)27 to 50% in our study (p < 0.05). Although our study does not directly address the route of transmission in infected donors, the observed female predominance and younger age distribution likely indicate a more prominent role of heterosexual transmission for infected blood donors in China. The reported occupation for donor K25 was “health care personnel.” Therefore, it is possible that this donor may have acquired her infection from an occupational exposure, although this possibility cannot be confirmed by our study.

The relatively low successful amplification rate (113/172) is likely caused by low viral load of some samples and by sample storage and shipment problems during long-distance specimen transportation especially during the earlier stage of this project from remote blood centers. It is not surprising that 82 of the 113 successfully amplified samples were from Kunming Blood Center. Kunming (Yunnan Province) has the highest prevalence of HIV-1 infection among the general population and blood donors. Our future efforts will strive to include more samples from other blood centers to obtain an even more comprehensive and representative understanding of HIV-1 subtype and the DRMs profile of diverse Chinese regions, and we will trace risk factors of the HIV-1–infected donors to clarify the relation between transmission routes and genotypes diversity. Furthermore, full-length sequencing on certain clade of HIV-1 will be carried out to provide more precise information on virus spread and evolution among Chinese blood donors.

Our findings are consistent with the increasing complexity of HIV-1 genetic variation and wide geographic distribution of DRMs among blood donors in China. Our results support continuous HIV-1 molecular epidemiologic surveillance in blood donors as part of a comprehensive HIV control program.

**CONFLICT OF INTEREST**

There are no conflicts of interest.

**REFERENCES**


29. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular


