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ORIGINAL RESEARCH

Pretreatment drug resistance in people living with HIV: A large retrospective cohort study in Chongqing, China

Min Liu[1] | **Xiao-Qing He**[1] | **Ren-Ni Deng**[2] | **Sheng-Quan Tang**[1] | **Vijay Harypursat**[1] | **Yan-Qiu Lu**[1] | **Kun He**[1] | **Qin Huo**[1] | **Hong-Hong Yang**[1] | **Qian Liu**[1] | **Yao-Kai Chen**[1,2]

[1]Division of Infectious Diseases, Chongqing Public Health Medical Center, Chongqing, China

[2]Department of Clinical Laboratory, Chongqing Public Health Medical Center, Chongqing, China

Correspondence
Yao-Kai Chen, Division of Infectious Diseases, Chongqing Public Health Medical Center, 109 Baoyu Road, Shapingba District, Chongqing, 400036, China.
Email: yaokaichen@hotmail.com

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Abstract

Objectives: The emergence of pretreatment drug resistance (PDR) caused by increased usage of antiretroviral therapy (ART) represents a significant challenge to HIV management. In this study, we evaluated the prevalence of PDR in people living with HIV (PLWH) in Chongqing, China.

Methods: We retrospectively collected the data of 1110 ART-naïve PLWH in Chongqing from January 1, 2018 to June 30, 2021. HIV-1 genotypes and drug resistance were analyzed using the HIV-1 *pol* sequence. Risk factors associated with PDR were evaluated via the logistic regression model.

Results: Nine genotypes were detected among 1110 participants, with CRF07_BC (55.68%) being the dominant genotype, followed by CRF01_AE (21.44%), CRF08_BC (14.14%), and other genotypes (8.74%). Of all the participants, 24.14% exhibited drug resistance mutations (DRMs). The predominant DRMs for non-nucleoside reverse transcriptase inhibitors (NNRTIs) and nucleoside reverse transcriptase inhibitors (NRTIs) were V179D/E/A/DIN (13.60%) and M184V/I (1.44%), respectively, whereas only two major DRMs (M46L and I54L) were identified for protease inhibitors (PIs). The total prevalence of PDR was 10.54%, with 2.43%, 7.66%, and 1.71% participants exhibiting PDR to NRTIs, NNRTIs, and PIs, respectively. Furthermore, female PLWH, delays in ART initiation, and the CRF08_BC genotype were associated with a higher risk of PDR.

Conclusions: Our study provides the first large cohort data on the prevalence of PDR in Chongqing, China. HIV-1 genotypes are diverse and complex, with a moderate level of PDR, which does not reach the threshold for the initiation of a public health response. Nevertheless, continuous surveillance of PDR is both useful and advisable.

KEYWORDS
genotype, HIV-1, mutation, pretreatment drug resistance, risk factors

Min Liu, Xiao-Qing He and Ren-Li Deng equally contributed to this manuscript.

INTRODUCTION

The advent of effective antiretroviral therapy (ART) has led to a substantial decrease in HIV-related morbidity and mortality. However, the emergence of HIV drug resistance (HIVDR), which can result in ART failure, poses a significant challenge for ART in the treatment of HIV infection [1]. Pretreatment drug resistance (PDR), which is usually detected among individuals initiating ART, regardless of prior antiretroviral (ARV) drug exposure, has become increasingly recognized in recent years [2]. According to the WHO 2019 HIV Drug Resistance Report [2], most low- and middle-income countries have a high prevalence (above 10%) of PDR to efavirenz (EFV) and nevirapine (NVP), which has prompted a number of measures in response. First, guidelines recommend baseline genotypic drug resistance testing during initial visits for all patients in developed countries to guide the selection of appropriate and optimal ART regimens [3, 4]. Additionally, the WHO recommends that first-line ART regimens should be changed from non-nucleoside reverse transcriptase inhibitor- (NNRTI-) based to non-NNRTI-based regimens in countries where the prevalence of PDR to NNRTIs equals or exceeds a threshold of 10% [5]

The National Free Antiretroviral Treatment Program of China, initiated in 2003, currently recommends two nucleoside reverse transcriptase inhibitors (NRTIs) combined with one NNRTI as the preferred, standardized, first-line ART regimen [6]. However, as the widespread use of ART has increased in China, so has the prevalence of PDR [1]. In Shanghai, Tianjin, and Liangshan Prefecture of Sichuan Province, the prevalence of PDR already exceeds 10% (17.4%, 11.5%, and 12.2%, respectively) [7–9]. Nevertheless, due to limited resources and a lack of widely available drug resistance testing, Chinese guidelines do not specifically recommend routine PDR testing. Thus, it is important to perform region-specific studies of HIV-1 PDR.

Chongqing, located in southwest China, has more than 30 million inhabitants and is one of the four centrally administered municipalities in China. By the end of October 2020, Chongqing ranked sixth in China, in the number of people living with HIV (PLWH; 53 994), with 45 732 individuals (82.5%) receiving ART, and 97.4% of those on ART having achieved virological suppression [10]. In addition, there were 6525 newly reported cases of HIV infection in Chongqing in 2020, with the majority being older males. More than 95% of the newly reported cases acquired HIV through sexual transmission, with heterosexual transmission accounting for 82.4% of cases, and homosexual transmission accounting for 14.4% of cases [10]. However, there is limited knowledge of PDR rates in Chongqing. Therefore, the aim of this study is to investigate the prevalence of PDR and the risk factors associated with PDR development in Chongqing, China.

METHODS

Study design and participants

A total of 1110 patients with HIV-1 who met the following inclusion criteria were enrolled in our study: (1) visited Chongqing Public Health Medical Center between January 1, 2018 to June 30, 2021; (2) ART-naïve, which refers to PLWH who had no previous evidence of initiating ART; (3) successfully completed testing for $CD4^+$ T-cell counts, HIV-1 RNA viral load, HIV-1 genotype, and PDR, and all testing reports were traceable. The baseline data of these participants, including demographic information (gender, age, level of education, marital status) and epidemiological information (route of infection, duration between HIV diagnosis and ART initiation) were collected and analyzed anonymously; thus, the usual requirement for written or oral informed consent was waived. The study was approved by the ethics committee of Chongqing Public Health Medical Center.

RNA extraction, nested PCR, and sequencing of viral DNA

We amplified the nucleotide sequence of protease and reverse transcriptase present in the '*pol*' region of HIV-1 (approximately 1500 bp), using a method developed in our laboratory. RNA was extracted using a viral nucleic acid extraction kit (Jiangsu Shuoshi Company, China). Then, the target fragment was amplified using a nested polymerase chain reaction (PCR). HiScript® II One Step RT-PCR (Nanjing Vazyme, China) was used for first-round PCR operation. The nested PCR was performed with Ace Taq (Nanjing Vazyme, China) in the second-round. Thereafter, the target band was detected by 1% agarose gel electrophoresis. The amplified product was then sent to Chongqing Qingke Biotechnology Co., LTD for Sanger sequencing. PCR primers are listed in Table S1.

Identification of HIV-1 genotypes

The obtained HIV-1 *pol* sequence was spliced and edited by SeqMan software. We then imported the completed HIV-1 *pol* sequence to the NCBI viral genotyping tool (http://www.ncbi.nlm.nih.gov/projects/genotyping/formpage.cgi) and compared it with the reference sequences of

different subtypes and circulating recombinant forms to identify the HIV-1 genotypes.

Drug resistance analysis

We screened the drug resistance mutations (DRMs) and estimated the PDR using the drug resistance database of Stanford University, USA (https://hivdb.stanford.edu/hivdb/by-sequences/). To analyze the degree of drug resistance, we obtained a single score for a specific drug according to the mutation site conferring drug resistance, then accumulated the total score for that particular drug. The degree of drug resistance was judged according to the total score value as follows: susceptible (score 0–9), potential low-level resistance (score 10–14), low-level resistance (score 15–29), intermediate-level resistance (score 30–59), and high-level resistance (score ≥60) [11]. According to the WHO-recommended criteria for PDR [12], PDR is defined as low-level, intermediate-level, or high-level resistance to the following drugs: seven NNRTIs [lamivudine (3TC), abacavir (ABC), zidovudine (AZT), stavudine (D4T), didanosine (DDI), emtricitabine (FTC), tenofovir disoproxil fumarate (TDF)]; five NRTIs [doravirine (DOR), EFV, etravirine (ETR), NVP, rilpivirine (RPV)]; eight PIs [atazanavir/r (ATV/r), darunavir/r (DRV/r), fosamprenavir/r (FPV/r), indinavir/r (IDV/r), lopinavir/r (LPV/r), nelfinavir/r (NFV/r), saquinavir/r (SQV/r), tipranavir/r (TPV/r)].

The prevalence of DRM was defined as the number of participants with at least one detected drug resistance mutation divided by the total number of participants enrolled in our study. The prevalence of PDR was defined as the number of participants with drug resistance to at least one antiretroviral drug divided by the total number of participants enrolled in our study.

Statistical analysis

Statistical Package for the Social Sciences (SPSS) software, Version 24.0 (IBM SPSS Statistics for Windows, IBM Corp., Armonk, N.Y., USA), was used to perform all statistical analyses. Continuous and categorical variables were described as the median with inter-quartile ranges (IQR) and percentages, respectively. Categorical variables were analyzed using the Chi-squared test or Fisher's exact test where necessary. Nine potential risk factors associated with PDR were chosen for univariate logistic regression analysis. Variables with a p-value <0.05 in the univariate logistic regression analysis were incorporated into the multivariate logistic regression model. A forward stepwise approach was used for variable selection in the multivariate regression model. A p-value of <0.05 was considered statistically significant.

RESULTS

Demographic and clinical characteristics of the participants

A total of 1110 participants with HIV-1 were enrolled in this study, none of whom had evidence of initiating ART. The median age of the participants was 51 years, with a predominance of males (80.3%) over females (19.7%), and 84.5% (938/1110) with an education below college level. Most of the participants (59.0%) were married, 22.0% were single, and 19.0% were divorced/widowed. Heterosexual contact (76.9%) was the dominant transmission route, followed by homosexual contact (10.0%) and intravenous drug use (0.7%); the precise transmission route was unknown in the remaining 12.3% (137/1110) of participants. Furthermore, participants with durations between HIV diagnosis and ART initiation of less than/equal to 365 days and more than 365 days represented 90.6% (1006/1110) and 9.4% (104/1110) of all participants, respectively. With respect to the viral and immunological status of the participants, the median HIV viral load (log_{10}) was 5.7 (IQR: 5.2, 6.2) copies/mL, whereas the median $CD4^+$ T-cell count was 52.0 (IQR: 22.0, 14.0) cells/μL (Table 1).

HIV-1 genotype distribution

Nine different HIV-1 genotypes were obtained from the 1110 participants, and the genotype distribution is shown in Figure 1. The dominant genotype was CRF07_BC (55.68%), followed by CRF01_AE (21.44%), CRF08_BC (14.14%), C (3.42%), B (1.89%), CRF55_01B (1.44%), A (0.90%), B+CRF01_AE (0.90%), and CRF85_BC (0.18%). A trend of increasing prevalence was observed for the CRF08_BC genotype, which accounted for 6.80% of all genotypes in 2018 and increased to 18.62% by 2021.

HIV-1 DRMs

Among the 1110 participants, 24.14% (268/1110) exhibited DRMs, with 2.61% (29/1110), 20.27% (225/1110), and 20.27% (225/1110) exhibiting resistance-associated mutation patterns for NRTIs, NNRTIs, and PIs, respectively. We identified 12 NRTI-associated mutation patterns, 14 NNRTI-associated mutation patterns, and nine PI-associated mutation patterns. M184V/I (1.44%) was

TABLE 1 Demographic and clinical characteristics of participants

Characteristics	Total ($n = 1110$)	Genotype CRF07_BC ($n = 618$)	Genotype CRF01_AE ($n = 238$)	Genotype CRF08_BC ($n = 157$)	Other genotypes ($n = 97$)
Median age (years, IQR)	51.0 (38.0, 61.0)	51.0 (37.0, 61.0)	49.0 (33.0, 57.3)	55.0 (46.0, 65.5)	52.0 (37.0, 59.0)
Gender (n, %)					
Male	891 (80.3)	506 (81.9)	187 (78.6)	119 (75.8)	79 (81.4)
Female	219 (19.7)	112 (18.1)	51 (21.4)	38 (24.2)	18 (18.6)
Marital status (n, %)					
Married	655 (59.0)	338 (54.7)	147 (61.8)	113 (72.0)	57 (58.8)
Single	244 (22.0)	156 (25.2)	55 (23.1)	12 (7.6)	21 (21.6)
Divorced/widowed	211 (19.0)	124 (20.1)	36 (15.1)	32 (20.4)	19 (19.6)
Degree of education (n, %)					
Below college	938 (84.5)	520 (84.1)	189 (79.4)	151 (96.2)	78 (80.4)
College and above	172 (15.5)	98 (15.9)	49 (20.6)	6 (3.8)	19 (19.6)
Route of infection (n, %)					
Heterosexual	854 (76.9)	479 (77.5)	177 (74.4)	130 (82.8)	68 (70.1)
Homosexual	111 (10.0)	72 (11.7)	25 (10.5)	0 (0)	14 (14.4)
Intravenous drug user	8 (0.7)	4 (0.6)	2 (0.8)	1 (0.6)	1 (1.1)
Unknown	137 (12.3)	63 (10.2)	34 (14.3)	26 (16.6)	14 (14.4)
Duration between diagnosis and ART (n, %)					
≤365	1006 (93.6)	552 (89.3)	215 (90.3)	148 (94.3)	91 (93.8)
>365	104 (9.4)	66 (10.7)	23 (9.7)	9 (5.7)	6 (6.2)
Median $CD4^+$ T-cell count (cells/μL, IQR)	52.0 (22.0, 114.0)	62.5 (28.8, 124.0)	29.0 (12.0, 72.8)	65.0 (27.0,147.0)	40.0 (16.5,105.5)
Median HIV RNA [log(10), IQR]	5.7 (5.2, 6.2)	5.7 (5.1, 6.2)	5.7 (5.3, 6.1)	5.9 (5.3, 6.3)	5.7 (5.1, 6.1)

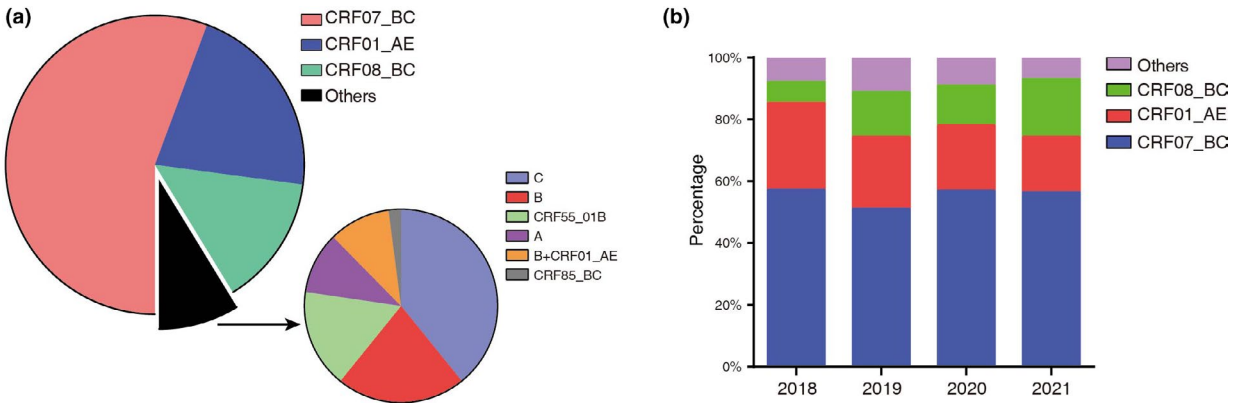

FIGURE 1 (a) Distribution of HIV-1 genotypes among treatment-naïve people living with HIV in Chongqing, China. (b) Proportion of various genotypes of HIV-1 found in treatment-naïve people living with HIV in Chongqing from 2018 to 2021

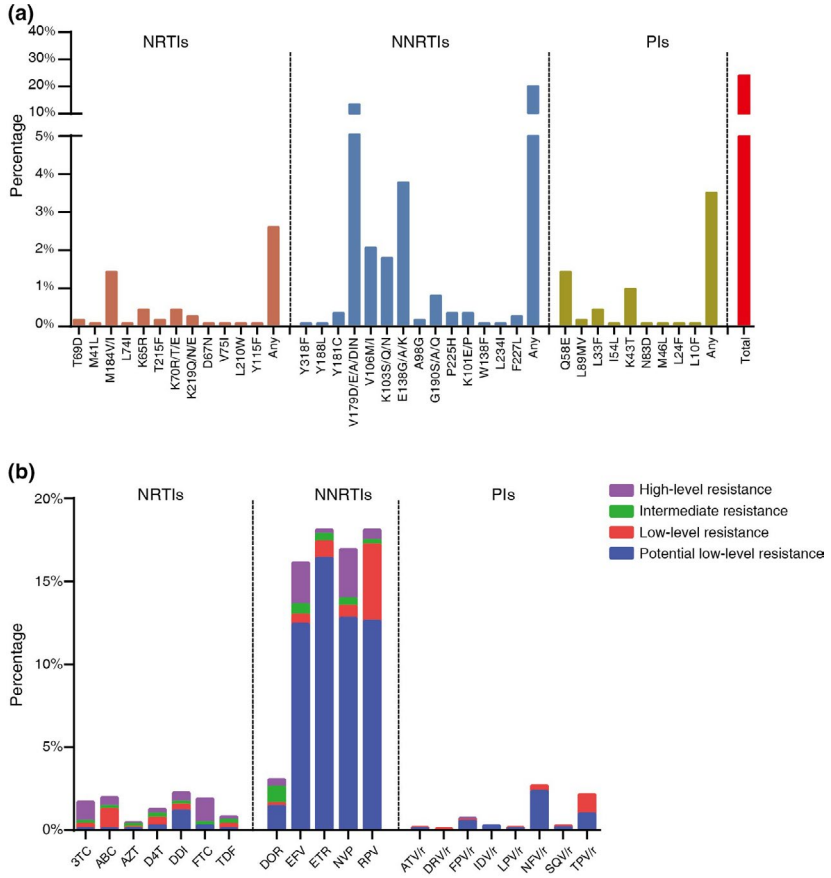

FIGURE 2 (a) Percentage of detected HIV-1 pretreatment drug resistance mutations. (b) Percentage and levels of HIV-1 pretreatment drug resistance to different antiretroviral drugs. 3TC, lamivudine; ABC, abacavir; ATV/r, atazanavir/r; AZT, zidovudine; D4T, stavudine; DDI, didanosine; DOR, doravirine; DRV/r, darunavir/r; EFV, efavirenz; ETR, etravirine; FPV/r, fosamprenavir/r; FTC, emtricitabine; IDV/r, indinavir/r; LPV, lopinavir/r; NFV/r, nelfinavir/r; NNRTIs, non-nucleoside reverse transcriptase inhibitors; NRTIs, nucleoside reverse transcriptase inhibitors; NVP, nevirapine; PIs, protease inhibitors; RPV, rilpivirine; SQV/r, saquinavir/r; TDF, tenofovir disoproxil fumarate; TPV/r, tipranavir/r

the most common NRTI-associated mutation, followed by K65R (0.45%) and K70R/T/E (0.45%). V179D/E/A/DIN (13.60%) was the dominant NNRTI-associated mutation, followed by E138G/A/K (3.78%) and V106 M/I (2.07%). The most prevalent PI-associated mutation was Q58E (1.44%), followed by K43T (0.99%) (Figure 2A).

Overall, 21.98% (244/1110) of individuals exhibited a resistance mutation to a single drug class: the incidence of NRTI-, NNRTI-, and PI-associated mutations was 1.08% (12/1110), 18.11% (201/1110), and 2.79% (31/1110), respectively. The rate of dual-class resistance mutations among participants was 1.44% (16/1110) for NRTIs and

NNRTIs, 0.63% (7/1110) for NNRTIs and PIs, and 0% for NRTIs and PIs. The incidence of resistance mutations to all three ARV drug classes was 0.09% (1/1110) (Figure 3).

The overall DRM rate differed significantly among participants with CRF07_BC (19.4%), CRF01_AE (19.3%), CRF08_BC (37.6%), and other genotypes (44.3%) ($p < 0.001$), as shown in Table 2. Similarly, there were significant differences in DRM rates to NNRTIs among the different genotypes ($p < 0.001$), with the other genotypes exhibiting the highest (39.2%) DRM rate. However, no significant differences were observed in DRM rates to NRTIs or PIs among the different genotypes.

Resistance level to different ARV drugs

The HIV-1 resistance levels and percentage of 20 ARV drugs were analyzed, as shown in Figure 2B. The total prevalence of PDR was 10.54% (117/1110), with NNRTIs (7.66%) showing a higher PDR prevalence than NRTIs (2.43%) and PIs (1.71%) ($p < 0.001$). However, the prevalence of potential low-level resistance (13.60%) was even higher than that of PDR, at 0.09% (1/1110) for NRTIs, 12.61% (140/1110) for NNRTIs, and 1.80% (20/1110) for PIs.

For NRTIs, the resistance to seven NRTIs ranged from 0.36% (4/1110) to 1.89% (21/1110). PDR to AZT (0.36%) was significantly lower than that to 3TC (1.62%) ($\chi^2 = 8.998$, $p = 0.003$), ABC (1.89%) ($\chi^2 = 11.692$, $p = 0.001$), DDI (1.08%) ($\chi^2 = 4.029$, $p = 0.045$), and FTC (1.62%) ($\chi^2 = 8.998$, $p = 0.003$). For NNRTIs, the resistance to five NNRTIs ranged from 1.62% (18/1110) to 5.50% (61/1110), and the PDR prevalence to EFV and NVP was 3.69% (41/1110) and 4.41% (46/1110), respectively. DOR (1.62%) showed the lowest PDR frequency, followed by ETR (1.71%), EFV (3.69%), NVP (4.14%), and RPV (5.50%) ($\chi^2 = 37.968$, $p < 0.001$). The frequencies of PDR to EFV (3.69%), NVP (4.41%), and RPV (5.50%) were all similar ($\chi^2 = 4.596$, $p = 0.100$). For PIs, the prevalence of PDR was low, with only the PDR to TPV/r (1.17%) being more than 0.5% and that to LPV/r being only 0.09% (1/1110).

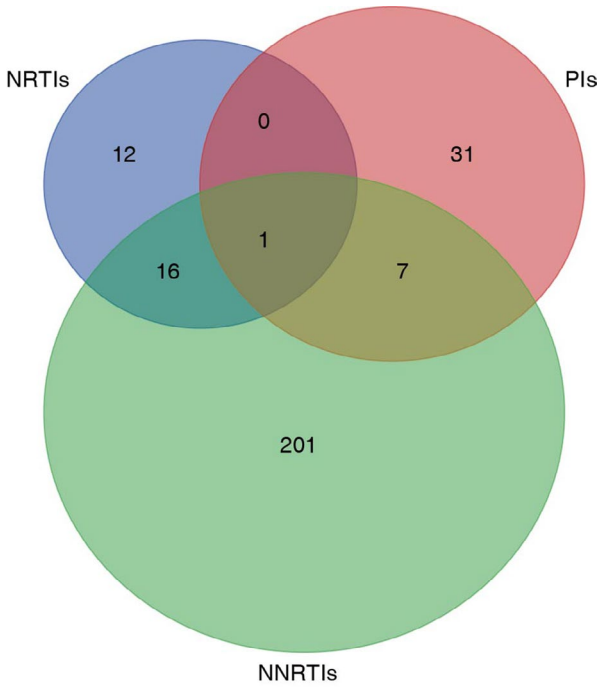

FIGURE 3 Distribution of HIV-1 pretreatment drug resistance mutations

PDR risk factors according to multivariate logistic regression

In order to identify the independent risk factors for HIV-1 PDR, nine potential risk factors were considered as independent variables in the binary logistic regression analysis. The results showed that gender, duration between HIV diagnosis and ART initiation, and HIV-1 genotype were potential factors associated with PDR. Compared with that in males, the odds ratio value for females was 1.944 (95% CI 1.250–3.022, $p = 0.003$). The odds ratio value for a duration between HIV diagnosis and ART initiation of more than 365 days, when compared to a duration of less than or equal to 365 days, was 2.443 (95% CI 1.371–4.354, $p = 0.002$). PDR rates for CRF07_BC and CRF01_AE genotypes were similar. However, compared with those of CRF07_BC, the odds ratio values for genotype CRF08_BC and other genotypes were 4.500 (95% CI 2.741–7.389, $p < 0.001$) and 4.177 (95% CI 2.322–7.514, $p < 0.001$), respectively (Table 3).

DISCUSSION

HIV-1 genotypes vary substantially in different geographical regions and with respect to different demographic factors. A total of nine HIV-1 genotypes were detected among 1110 participants from 2018 to 2021, which indicates that the HIV-1 genotype distribution in Chongqing is diverse and complex. CRF07_BC was the dominant genotype, followed by CRF01_AE and CRF08_BC, which is similar to the results of a study performed by five Chinese blood centers [13] and a nationwide survey [14]. However, our results differed from surveys conducted in Shanghai, Tianjin, Beijing, Jiangsu, Hebei, and Fujian, which found that the dominant genotype was CRF01_AE followed by CRF07_BC [7, 8, 15–18]. This discrepancy may result from the different transmission routes for HIV. Heterosexual contact was the dominant risk factor for HIV transmission

TABLE 2 HIV-1 genotype and drug resistance mutations

	Total ($n = 1110$)	Genotype CRF07_BC ($n = 618$)	Genotype CRF01_AE ($n = 238$)	Genotype CRF08_BC ($n = 157$)	Other genotypes ($n = 97$)	
	n (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*p*-Value
NRTIs	29 (2.6)	13 (2.1)	8 (3.4)	3 (1.9)	5 (5.2)	0.253
NNRTIs	225 (20.3)	97 (15.7)	37 (15.5)	53 (33.8)	38 (39.2)	<0.001
PIs	39 (3.5)	20 (3.2)	6 (2.5)	7 (4.5)	6 (6.2)	0.349
Any	268 (24.1)	120 (19.4)	46 (19.3)	59 (37.6)	43 (44.3)	<0.001

Abbreviations: NNRTIs, non-nucleoside reverse transcriptase inhibitors; NRTIs, nucleoside reverse transcriptase inhibitors; PIs, protease inhibitors.

in our study, whereas homosexual contact was the main transmission route for HIV in the studies conducted in Shanghai, Tianjin, Beijing, Jiangsu, Hebei, and Fujian [7, 8, 15–18]. Furthermore, a recent investigation in Yunnan province revealed CRF08_BC as the dominant strain, followed by unique recombinant forms, CRF01_AE, and CRF07_BC [19]. We further observed that the prevalence of genotype CRF08_BC increased from 2018 to 2021, which is similar to the observations of a previous study [13]. In fact, genotype CRF08_BC appears to have become a distinctive strain earlier in Yunnan province and was rarely observed outside Yunnan province [19]. The distribution of HIV-1 genotypes may have changed with the rapid growth of the migrant population and movement of the HIV-1-infected population within China. This could explain why genotype CRF08_BC has now been identified outside of Yunnan province [1, 7, 8], as well as its apparent increasing trend in our study.

Among the participants, 24.14% exhibited DRMs, which is similar to results reported by a study on Guangxi province (21.2%) [1], lower than the percentage observed in Yunnan province (34.2%) [6], and much higher than the percentages for Asia (4.6%) [20] and Shanghai (17.4%) [7]. We also found that the proportion of participants exhibiting NNRTI-associated mutations (20.27%) was greater than those exhibiting NRTI-associated mutations (2.61%) and PI-associated mutations (3.51%). The dominant NNRTI-associated mutations were V179D/E/A/DIN and E138K/G/A. V179D/E/A/DIN mutations are strongly associated with potential low-level resistance to NNRTIs when existing alone, whereas E138K/G/A mutations confer low- or intermediate-level resistance to RPV. M184V/I and K65R were the two major NRTI-associated mutations in our study. M184V/I causes high-level resistance to 3TC and FTC, and low-level resistance to DDI and ABC, whereas the K65R mutation is strongly associated with resistance to TDF [21]. However, the M184V/I mutation can reduce virological fitness and increase susceptibility to AZT and TDF, whereas K65R increases susceptibility to AZT [21]. We found only two major DRMs for PIs (M46L and I54L) in our study; other DRMs found were minor, and cannot cause drug resistance by themselves, but tend to occur along with major DRMs [21, 22].

However, not all DRMs inevitably result in drug resistance [11, 12]. The level of PDR in Chongqing was moderate (10.54%), which is higher than the national average (6.8%) [9], with 7.66% PDR to NNRTIs, 2.43% PDR to NRTIs, and 1.71% PDR to PIs. The following reasons might explain the higher prevalence of PDR in Chongqing. First, there are many PLWH aged 50 years or older in Chongqing [23], who tend to have poor compliance to ART treatment, which favors the emergence of drug resistance. Second, the delayed detection of ART failure and deferred changes to ART regimens could also lead to the spread of HIV-1 drug resistance strains. Consistent with reports from Shanghai [7], our study also identified a high prevalence of potential low-level resistance (13.60%). However, the clinical significance of potential low-level resistance remains uncertain. Further studies are required to confirm whether this may lead to clinical ART treatment failure.

Notably, compared with the rates for NRTIs (2.43%) and PIs (1.71%), a much higher PDR rate was observed for NNRTIs (7.66%) in our study. Although dolutegravir-containing regimens are recommended as the preferred first-line antiretroviral regimens for PLWH by the WHO [24], two NRTIs in combination with one NNRTI remains the typical first-line regimen in Chongqing due to the prohibitive costs of new antiretroviral drugs, which are not currently provided by the government free of cost. NNRTI-associated resistance is a prominent phenomenon that is increasing worldwide due to the low genetic barrier to resistance [21]. One meta-analysis reported that the annual increase in the odds of pretreatment NNRTI-associated resistance per year was 23% in southern Africa, 17% in western and central Africa, and 11% in Asia [25]. Thus, the WHO recommends that first-line ART regimens should be changed from NNRTI-based to non-NNRTI-based regimens in countries where the levels of PDR to NNRTIs reach the threshold of 10% [5]. Although the present study does not support a change to the current first-line ART regimens in Chongqing, surveillance of PDR prevalence should be regularly

TABLE 3 Univariate and multivariate logistic regression analysis for risk factors of PDR

	Total ($n = 1110$)	Drug resistance n (%)	Univariate			Multivariate		
			OR	95% CI	p	OR	95% CI	p
Gender								
Male	891	81 (9.1)	1.000			1.000		
Female	219	36 (16.4)	1.967	1.287–3.006	0.002	1.944	1.250–3.022	0.003
Age (years)								
<50	527	59 (11.2)	1.000					
≥50	583	58 (9.9)	0.876	0.597–1.286	0.500			
Marital status								
Married	655	72 (11.0)	1.000					
Single	244	24 (9.8)	0.883	0.543–1.438	0.618			
Divorced/ widowed	211	21 (10.0)	0.895	0.536–1.495	0.672			
Degree of education								
Below college	938	98 (10.4)	1.000					
College and above	172	19 (11.0)	1.064	0.632–1.792	0.814			
Route of infection								
Heterosexual	854	88 (10.3)	1.000					
Homosexual	111	11 (9.9)	0.958	0.495–1.854	0.897			
Intravenous drug user	8	1 (12.5)	1.244	0.151–10.225	0.839			
Unknown	137	17 (12.4)	1.233	0.709–2.145	0.458			
Duration between diagnosis and ART (days)								
≤365	1006	99 (9.8)	1.000			1.000		
>365	104	18 (17.3)	1.918	1.108–3.319	0.020	2.443	1.371–4.354	0.002
$CD4^+$ T-cell counts (cells/mm^3)								
≤200	960	102 (10.6)	1.000					
>200	142	14 (9.9)	0.920	0.511–1.658	0.781			
HIV RNA [log(10)]								
<3	9	1 (11.1)	1.000					
3–6	746	74 (9.9)	0.881	0.109–7.141	0.906			
≥6	346	40 (11.6)	1.046	0.127–8.581	0.967			
Genotype								
CRF07_BC	618	41 (6.6)	1.000			1.000		
CRF01_AE	238	18 (7.6)	1.151	0.648–2.047	0.631	1.130	0.632–2.019	0.681
CRF08_BC	157	37 (23.6)	4.339	2.669–7.055	<0.001	4.500	2.741–7.389	<0.001
Others	97	21 (21.6)	3.889	2.182–6.930	<0.001	4.177	2.322–7.514	<0.001

Abbreviations: PDR, pretreatment drug resistance; OR, odds ratio; CI, confidence interval.

conducted to evaluate the effectiveness of existing first-line regimens. One reason for the lower PDR rate of PIs may be that it is not easy to develop resistance to PIs because of the high genetic barrier to resistance [21, 26]. Moreover, PIs have been included in the free ART program in China since 2008 [21, 26] and are mainly used as second-line drugs. However, the more widespread use of PIs will likely increase the prevalence of PDR to PIs in future [21, 26].

We further investigated the risk factors of PDR and concluded that female patients, delays in ART initiation, and the CRF08_BC genotype were positively

associated with PDR. According to the WHO 2019 HIV Drug Resistance Report [2], women have a higher prevalence of PDR, with twice the rate of PDR as men. This phenomenon may be attributable to reasons such as past exposure to antiretroviral drugs for the prevention of mother-to-child transmission of HIV [25, 27], female sex worker occupational status [28], and the effect of "bridging," which refers to women who acquire an HIV infection from men who become infected through sexual contact with ART-treated HIV-infected men who have sex with men [29]. Previous studies have also reported that females have a higher risk of PDR [27, 28], which implies that closer clinical scrutiny of female patients is required. The females in our study might have acquired drug-resistant strains from their partners; however, our data was unable to verify this hypothesis. In addition, delays in ART initiation were also positively correlated with the prevalence of PDR in our study. To explain this phenomenon, we hypothesized that mutations, as well as DRMs, could accumulate over several replication cycles due to continuous natural evolution of the HIV genome [30]. In contrast, a previous study observed that delays in ART initiation did not significantly influence the prevalence of PDR [31]. Therefore, further studies are required to elucidate the reasons behind this observational discrepancy. Moreover, the CRF08_BC genotype was found to confer a higher risk for PDR emergence compared with CRF07_BC, which is consistent with the study by Zhang et al. [17]. CRF08_BC is one of the primary HIV-1 genotypes found among intravenous drug users at the early stage in southwest China; thus, frequent needle sharing and poor ART adherence could likely increase the risk of spread of this drug-resistant strain [17]. However, it remains controversial whether specific HIV-1 genotypes are associated with PDR in PLWH [7, 15, 22, 31]. Further studies of larger cohorts should be performed to adequately explain this putative association.

To the best of our knowledge, this is the first study to investigate the prevalence of PDR and the risk factors associated with PDR development in a large cohort conducted in Chongqing, China. There are two main strengths to our study. First, we focused on the prevalence of PDR in Chongqing, which ranks sixth in the number of PLWH in China; however, little is known about PDR prevalence in Chongqing. Second, our study has a larger sample size than similar studies previously conducted in Chongqing. There are also several limitations to our study. First, as this was a retrospective study, a degree of sample selection bias may exist. For example, our study did not include ART-naïve PLWH who did not undergo HIV-1 PDR testing. Physicians generally recommend only those patients who exhibit a high degree of risk for HIV-1 drug resistance (such as those who have multiple sexual partners) to undergo HIV-1 PDR testing. Second, our analysis did not contain PDR data for integrase inhibitors, which are increasingly being used for HIV management. Last, our findings may not accurately reflect the current state of the HIV epidemic in Chongqing, because some patients in Chongqing are unaware that they are currently infected with HIV-1, as they have not yet been tested. These patients were obviously not included in our study.

CONCLUSION

The distribution of HIV-1 genotypes in Chongqing is diverse and complex, with genotypes CRF07_BC and CRF01_AE being the predominantly prevalent genotypes. The observed prevalence of PDR in Chongqing is moderate and does not currently meet the threshold for initiating a public health response regarding specific changes to current ART regimens. Nevertheless, continuous surveillance of PDR is crucial for estimating the level of PDR and evaluating the effectiveness of current ART regimens in Chongqing.

PATIENT CONSENT STATEMENT

All data were analyzed anonymously, and the requirement for written or oral informed consent was waived.

CLINICAL TRIAL REGISTRATION

This study is not registered.

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CONFLICTS OF INTERESTS

The authors have no conflicts of interest to declare.

ETHICS APPROVAL

This study was approved by the ethics committee of Chongqing Public Health Medical Center (2021-051-01-KY).

ORCID

Min Liu https://orcid.org/0000-0002-4369-057X
Xiao-Qing He https://orcid.org/0000-0002-2134-3809
Ren-Li Deng https://orcid.org/0000-0001-9327-9546
Sheng-Quan Tang https://orcid.org/0000-0002-2762-1056
Vijay Harypursat https://orcid.org/0000-0001-7423-6786
Yan-Qiu Lu https://orcid.org/0000-0002-0342-4381
Kun He https://orcid.org/0000-0002-0587-2313

Qin Huo https://orcid.org/0000-0003-2812-3275
Hong-Hong Yang https://orcid.org/0000-0002-8210-6217
Qian Liu https://orcid.org/0000-0002-1926-4511
Yao-Kai Chen https://orcid.org/0000-0002-3229-0108

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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