

Vital Surveillances

Changes in RPV Resistance-Related Mutations Among Antiretroviral Therapy-Naïve HIV-1-Positive Individuals — China, 2004–2023

Xiang Li¹; Xiu Liu¹; Peixian Xin¹; Dong Wang¹; Jingjing Hao¹; Chang Song¹;
Jing Hu¹; Yi Feng¹; Yuhua Ruan¹; Hui Xing¹; Lingjie Liao^{1,†}

ABSTRACT

Introduction: Rilpivirine (RPV) is currently included in first-line antiretroviral therapy (ART) regimens in China. However, the baseline RPV resistance remains unclear, especially among different HIV-1 subtypes in China.

Methods: HIV-1 *pol* sequences were collected from ART-naïve HIV-infected individuals between 2004 and 2023. RPV resistance-associated mutations (RAMs) were identified and interpreted using the Stanford HIVdb algorithm. The prevalence of RPV resistance was evaluated over a 20-year study period across different HIV-1 subtypes.

Results: In total, 64,429 HIV-1 *pol* sequences from ART-naïve HIV-infected individuals were included in this analysis. The prevalence of RPV resistance increased significantly from 1.5% (2004–2007) to 3.5% (2020–2023) ($P < 0.001$), which was driven by low-level resistance to RPV. CRF65_cpx (37.7%), CRF55_01B (8.5%), and CRF08_BC (5.0%) showed the highest rates of RPV resistance. In RPV-resistant strains, V179D/E/F/L (43.3%) and E138A/G/K/Q/R (38.0%) were the predominant RAMs. The mutations G190A/C/E/Q/S/T (12.5%) and Y181C/F/I/S/V (11.9%) were also commonly detected. Polymorphic and non-nucleoside reverse transcriptase inhibitor (NNRTI) cross-resistant RAMs were identified in 66.8 and 34.8% of RPV-resistant strains, respectively. The polymorphic mutation V179D/E frequently co-occurred with other RAMs, including E138G (14.4%), K103R (7.8%), and V90I (5.5%), and conferred low- or intermediate-level RPV resistance.

Conclusions: Polymorphic RAMs and NNRTI cross-resistance mutations together account for the increased RPV resistance in China. Additional phenotypic data and clinical research are needed to verify the efficacy of RPV-based ART in patients

infected with subtypes CRF65_cpx, CRF55_01B, and CRF08_BC.

Antiretroviral therapy (ART) has improved the prognosis of Human Immunodeficiency Virus (HIV) infection and reduced HIV-related morbidity and mortality. However, the emergence and transmission of HIV drug resistance can undermine ART effectiveness. The prevalence of drug resistance among newly diagnosed ART-naïve HIV-infected adults was 11.4% (1). Furthermore, resistance to non-nucleoside reverse transcriptase inhibitors (NNRTIs) was the most prevalent (7.9% of the cases). The prevalence of resistance to the current and former first-line NNRTIs [efavirenz (EFV) and nevirapine (NVP)] has reached 6.5% (1).

Rilpivirine (RPV), a second-generation NNRTI, exerts antiviral effects by binding to regions adjacent to the active site of HIV reverse transcriptase (RT), suppressing viral replication. The proportion of treatment-naïve individuals receiving RPV as part of an initial ART regimen increased from 0.04% to 1.3% between 2017 and 2019 (2). Owing to its tolerability profile and once-daily dosing, RPV has been included as a first-line ART regimen in China as an alternative to EFV since 2023 (3). The baseline data on RPV resistance among ART naïve HIV-infected individuals are scarce, impeding the optimal use of RPV (4). The present study aimed to analyze the trend of RPV resistance and characterize RPV resistance-associated mutations (RAMs) among ART-naïve HIV-infected individuals in China from 2004 to 2023 and to provide suggestions for optimizing ART strategies.

METHOD

HIV-1 *pol* sequences were obtained from the local

database of the National Center for AIDS/STD Control and Prevention, China CDC (samples collected between 2004 and 2023) and the HIV Database (<https://www.hiv.lanl.gov>), as previously described (5). All sequences were obtained from ART-naïve HIV-infected adults aged ≥ 18 years in China. Duplicated sequences, those without sampling year/provincial-level administrative division information, or sequences with mixed bases exceeding 5% of the sequence length were excluded from the study (Supplementary Figure S1, available at <https://weekly.chinacdc.cn/>). Each sequence covered the coding region for HIV protease (amino acids 10–99) and RT (the first 238 amino acids), corresponding to HXB2 positions 2,280–3,263.

HIV-1 subtypes were determined by phylogenetic analysis using the maximum likelihood method in IQ-Tree (v2.0.7, Integrative Bioinformatics Vienna, Vienna, Austria), with the study sequences aligned with reference sequences retrieved from the HIV gene sequence database (<https://nmdc.cn/hiv/tool/sequence>). RPV RAMs were identified using the Stanford HIV Drug Resistance Database (<http://hivdb.stanford.edu>) and interpreted using the HIVdb (v10.1, Stanford University, Stanford, CA, USA). Low, intermediate, and high resistance levels were classified as RPV resistance, whereas potential low-level resistance and susceptibility to RPV were classified as no resistance. RPV RAMs were divided into NNRTI cross-resistance RAMs (conferring low- or higher-level resistance), polymorphic RAMs (conferring potential low-level/even lower resistance and having $>1\%$ prevalence in at least one HIV-1 subtype, with the exception of E138G, which confers low-level resistance to RPV), the RPV-specific RAM E138K, and other unclassified RAMs.

The samples were divided into five groups based on the sampling period and the corresponding phases of China's National Free Antiretroviral Treatment Program: 2004–2007, the initial period; 2008–2011, the scale-up period; 2012–2015, the period when the CD4 threshold for ART initiation was elevated to 350 cells/ μL ; 2016–2019, the universal treatment period regardless of CD4 cell counts; 2020–2023, the recent study period. EFV and NVP remained the core components of the first-line ART regimens throughout the study period and the latter two periods covered the years after the introduction of RPV. The prevalences of RPV resistance and RAMs were evaluated using the chi-square test. Pairwise comparisons between different periods were performed using Bonferroni correction.

SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA) was used for statistical analyses. A two-sided $P < 0.05$ was considered statistically significant, while a $P < 0.005$ indicated significant differences in pairwise comparisons between the two periods.

RESULT

In total, 64,429 sequences (Supplementary Figure S1) from ART-naïve HIV-infected adults in China (2004–2023) were included in the present analysis (Supplementary Table S1, available at <https://weekly.chinacdc.cn/>). The largest proportion of sequences was obtained from 2012–2015 (33.3%), followed by 2016–2019 (33.2%), 2020–2023 (17.9%), 2008–2011 (13.7%), and 2004–2007 (1.9%). Five predominant HIV-1 subtypes accounted for 94.3% of all the strains [CRF01_AE (38.2%), CRF07_BC (36.5%), CRF08_BC (7.9%), B (7.3%) and CRF55_01B (4.4%)]. A total of 1,538 individuals harbored RPV-resistant strains (overall prevalence rate, 2.4%).

Over the 20-year study period, the prevalence of RPV resistance increased progressively, with rates of 1.5, 1.8, 1.8, 2.6, and 3.5% during 2004–2007, 2008–2011, 2012–2015, 2016–2019, and 2020–2023, respectively ($P < 0.001$; Figure 1). Pairwise comparisons revealed statistically significant differences in prevalence between 2012–2015 and 2016–2019 and between 2012–2015 and 2020–2023. The increase in RPV resistance between 2016 and 2023 was primarily attributed to the rising low-level RPV resistance, which increased from 1.2% in 2016–2019 to 2.2% in 2020–2023, with a statistically significant difference between the two periods ($P < 0.001$). The prevalence of intermediate-level RPV resistance fluctuated over the 20-year period, peaking at 1.1% during 2016–2019, with significant differences found when compared with periods of 2012–2015 and 2020–2023 ($P < 0.001$), whereas high-level RPV resistance remained relatively stable (range, 0.3%–0.5%).

CRF55_01B, CRF08_BC, subtype B, CRF01_AE, and CRF07_BC had RPV resistance prevalences of 8.5%, 5.0%, 3.1%, 1.6%, and 1.5%, respectively (Figure 2A). All five subtypes exhibited an upward trend in RPV resistance during the study period. By 2020–2023, the prevalence of RPV resistance reached 9.3% in CRF55_01B and 7.9% in CRF08_BC.

Additionally, CRF65_cpx showed a high prevalence of RPV resistance (37.7%), increasing from 33.3% in 2008–2011 to 47.8% in 2020–2023 (Figure 2B).

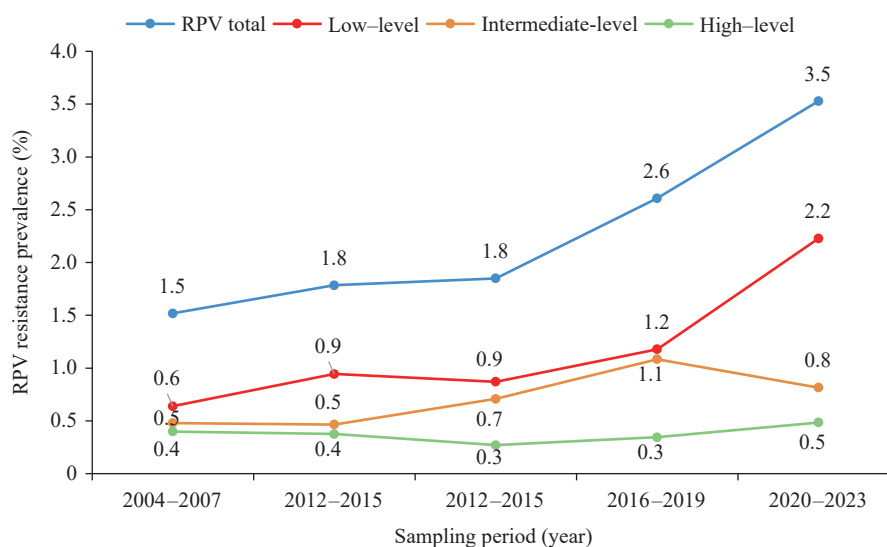


FIGURE 1. The prevalence of RPV resistance among antiretroviral therapy-naïve HIV-infected individuals during the 20-year period in China.

Abbreviation: RPV=rilpivirine; HIV=human immunodeficiency virus; ART=antiretroviral therapy.

Subtype C also exhibited a relatively high RPV resistance rate of 7.0% (31/435), with a rate of 8.8% in 2020–2023.

RPV RAMs were identified at 14 sites within *RT* (Table 1). Among all the HIV strains enrolled, V179D/E/F/L (9.5%) was the most common RPV RAM. Stratified subtype analysis further revealed that these mutations were detected in 95.1% of CRF55_01B isolates and 96.2% of CRF65_cpx isolates, respectively (Supplementary Table S2, available at <https://weekly.chinacdc.cn/>). Additional RAMs with a prevalence >1% included V106A/I (2.5%), E138A/G/K/Q/R (1.7%), and V90I (1.1%). The remaining RPV RAMs exhibited low prevalence (range, 0.1% to 0.3%). Over the 20-year period, three of the four most common RPV RAMs, V179D/E/F/L, E138A/G/K/Q/R, and V90I, showed an increasing incidence (all $P<0.001$), whereas that of V106A/I decreased ($P<0.001$).

Among the 1,538 strains with RPV resistance, V179D/E/F/L was the most frequent (43.3%), followed by E138A/G/K/Q/R (38.0%), G190A/C/E/Q/S/T (12.5%), and Y181C/F/I/S/V (11.9%; Table 2). Polymorphic RAMs (V90I, A98G, V106I, E138A/G, and V179D/E) were identified in 66.8% of the RPV-resistant strains, whereas NNRTI cross-resistance RAMs (L100I/V, K101E/P, Y181C/F/I/S/V, Y188F/L, G190A/C/E/Q/S, F227C, and M230I/L) were found in 34.8% of RPV-resistant isolates. E138K was only detected in 4.0% of the RPV-resistant strains. Furthermore, polymorphic RAMs were predominant

in strains with low-level RPV resistance (80.8%), whereas NNRTI cross-resistance RAMs were present in all strains harboring high-level RPV resistance (100.0%). In RPV resistance strains, V179D/E frequently co-occurred with other mutations [E138G (14.4%), K103R (7.8%), V90I (5.5%), V106I (3.3%), E138A (1.9%), and A98G (1.0%)] which confer low- or intermediate-level RPV resistance. In CRF08_BC, CRF55_01B, and CRF65_cpx, the combinations of V90I with V179D, E138G with V179E, and K103R with V179D accounted for 24.2%, 79.3%, and 93.9% of RPV resistance, respectively (Table 2).

DISCUSSION

In this study, the prevalence of RPV resistance was assessed among 64,429 ART-naïve HIV-infected individuals in China, and a steady increase from 1.5% to 3.5% from 2004 to 2023 was observed. A recent study conducted in southern China reported an RPV resistance rate of 3.4%, consistent with our findings (6). A significant increase in RPV resistance prevalence emerged in 2016–2019 following the introduction of RPV in China. However, E138K, the key specific RPV RAM, accounts for <5% of all RPV-resistant strains, suggesting limited clinical application of this drug. Furthermore, E138K did not show an upward temporal trend among resistant isolates (data not shown), indicating that the elevated prevalence of RPV resistance coincides with the clinical usage of RPV in China.

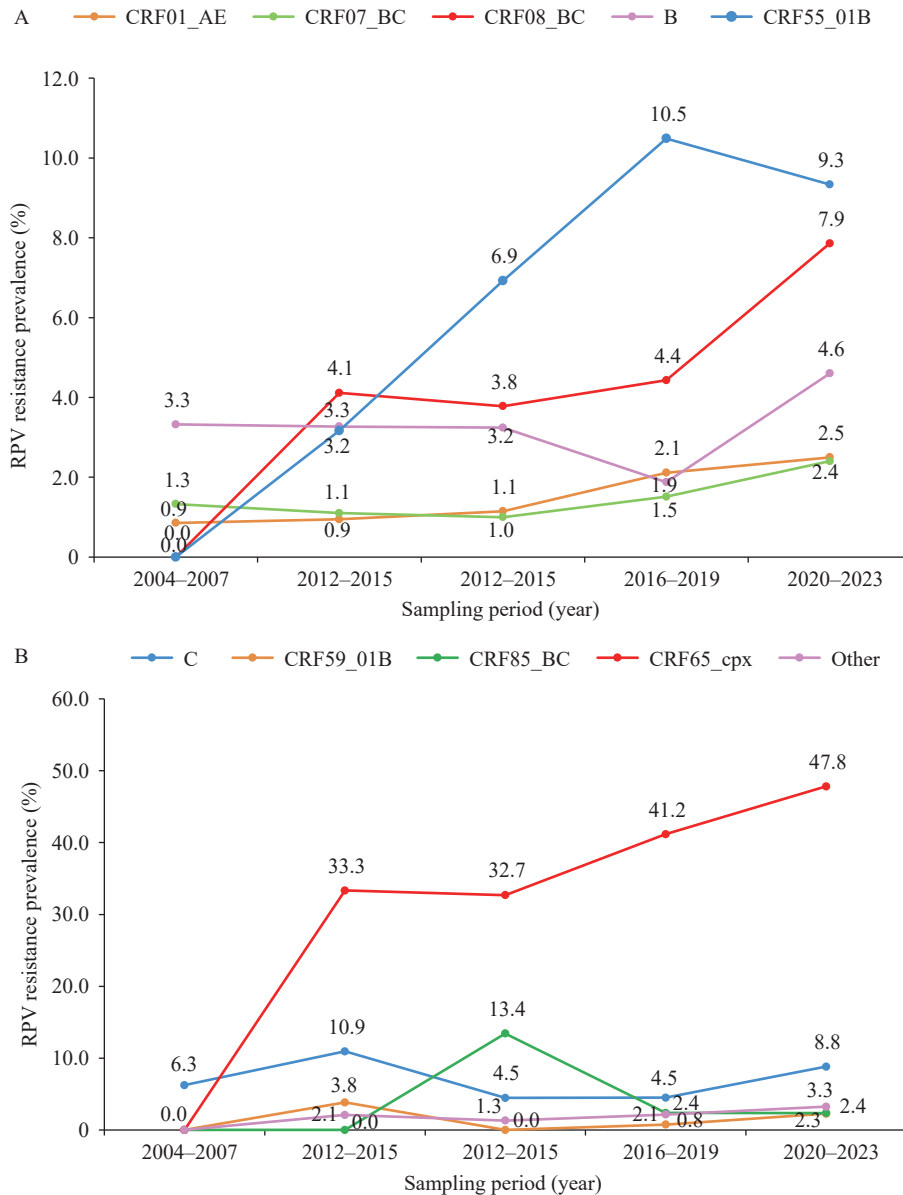


FIGURE 2. The prevalence of RPV resistance in HIV-1 subtypes among antiretroviral therapy-naïve HIV-infected individuals during the 20-year period in China. (A) The prevalence in five major HIV-1 subtypes. (B) The prevalence in HIV-1 subtypes other than the major subtypes.

Abbreviation: RPV=rilpivirine; HIV=human immunodeficiency virus.

In 2020–2023 CRF08_BC and CRF55_01B exhibited RPV resistance rates close to 10%, whereas, CRF65_cpx had a resistance prevalence of <50%. This uneven distribution across subtypes may be attributed to inherent differences in the *RT* genes. E138A (7) is most prevalent in subtypes C (6.1%), F (5.1%), and A (3.3%). In this study, E138A was observed at a much higher frequency in CRF08_BC (7.8%) than in other subtypes. The contribution of this mutation to ART failure remains debatable (8), and it has been reclassified as a RAM, conferring potential low-level resistance to RPV.

RPV resistance observed in this study likely reflects the combined effects of natural polymorphisms and cross-resistance to other NNRTIs. V179D and V179E were the most prevalent mutations at V179 (99.9%). Neither mutation confers low-, intermediate-, or high-level RPV resistance; however, either mutation can do so when co-occurring with other RT RAMs. V179D/E prevalence increased gradually over the 20-year study period and exceeded 10% among the prevailing HIV-1 strains in China (2020–2023). Additionally, V179E and V179D were identified as inherent amino acid polymorphisms in CRF55_01B and CRF65_cpx,

TABLE 1. The prevalence of RPV resistance-associated mutations among ART-naïve HIV-infected individuals during the 20-year period in China.

Drug resistance mutation	2004–2007 (N=1,251) <i>n</i> [§] (%) [¶]	2008–2011 (N=8,795) <i>n</i> (%)	2012–2015 (N=21,461) <i>n</i> (%)	2016–2019 (N=21,389) <i>n</i> (%)	2020–2023 (N=11,533) <i>n</i> (%)	<i>P</i> [*]	Total (N=64,429) <i>n</i> (%)
V90I	13 (1.0)	111 (1.3)	150 (0.7)	185 (0.9)	250 (2.2)	<0.0001	709 (1.1)
A98G	2 (0.2)	17 (0.2)	15 (0.1)	30 (0.1)	43 (0.4)	0.001	107 (0.2)
L100I/V	1 (0.1)	2 (0)	4 (0)	6 (0)	7 (0.1)	0.201	20 (0)
K101E/H/P	1 (0.1)	17 (0.2)	40 (0.2)	45 (0.2)	42 (0.4)	0.003	145 (0.2)
K103R [†]	23 (1.8)	194 (2.2)	533 (2.5)	487 (2.3)	356 (3.1)	<0.0001	1,593 (2.5)
V106A/I	42 (3.4)	340 (3.9)	589 (2.7)	436 (2.0)	220 (1.9)	<0.0001	1,627 (2.5)
V108I	5 (0.4)	26 (0.3)	51 (0.2)	37 (0.2)	32 (0.3)	0.300	151 (0.2)
E138A/G/K/Q/R	22 (1.8)	117 (1.3)	296 (1.4)	416 (1.9)	271 (2.3)	<0.0001	1,122 (1.7)
V179D/E/F/L	34 (2.7)	539 (6.1)	2,038 (9.5)	2,340 (10.9)	1,189 (10.3)	<0.0001	6,140 (9.5)
Y181C/F/I/S/V	7 (0.6)	27 (0.3)	53 (0.2)	62 (0.3)	34 (0.3)	0.701	183 (0.3)
Y188F/L	0 (0)	10 (0.1)	8 (0)	8 (0)	12 (0.1)	0.691	38 (0.1)
G190A/C/E/Q/S/T/V	3 (0.2)	22 (0.3)	51 (0.2)	69 (0.3)	48 (0.4)	0.007	193 (0.3)
H221Y	1 (0.1)	10 (0.1)	23 (0.1)	39 (0.2)	12 (0.1)	0.430	85 (0.1)
F227C/L/Y	3 (0.2)	6 (0.1)	20 (0.1)	43 (0.2)	11 (0.1)	0.258	83 (0.1)
M230I/L	2 (0.2)	4 (0)	7 (0)	25 (0.1)	5 (0)	0.457	43 (0.1)

Abbreviation: HIV=human immunodeficiency virus; ART=antiretroviral therapy; RPV=rilpivirine.

* *P* refers to Pearson's chi-square test. *P*<0.05 is statistically significant.

[†] K103R is not a typical RPV resistance-associated mutation, and this mutation alone cannot reduce susceptibility to rilpivirine.

[§] Number of RPV resistance-associated mutations in ART-naïve individuals

[¶] The detection rate of RPV resistance-associated mutations in ART-naïve individuals infected with HIV.

respectively (9). V179D/E compromises the efficacy of NNRTI-containing regimens either during the first year of ART or in patients with a baseline viral load of at least 100,000 copies/mL (10–11).

According to a national cross-sectional molecular epidemiological survey (2018), CRF01_AE and CRF07_BC together accounted for 76.6% of all HIV-1 strains in China. CRF08_BC and CRF55_01B accounted for 9.0% and 3.9%, respectively, whereas CRF65_cpx accounted for <0.5%. These three subtypes accounted for approximately 14% of all the HIV-1 strains. Despite their relatively low prevalence, these subtypes are concentrated in southern/southwestern China (12–14), regions characterized by frequent population mobility and a historically high proportion of people living with HIV. Caution should be exercised in these regions when incorporating RPV into ART regimens. Further phenotypic and clinical investigations are required to clarify the impact of RPV-based ART in patients infected with the CRF55_01B and CRF65_cpx HIV-1 strains.

Although K103N, the most common RAM linked to EFV, did not confer resistance to RPV, cross-resistance between RPV and EFV and/or NVP was common. This finding was likely due to RAMs at

positions Y181, G190, and K101, consistent with previous studies (15). Among these, Y181C and G190A were the most frequent. Furthermore, they confer high-level resistance to RPV when combined with K101E. Accordingly, RPV-containing regimens are not recommended as salvage therapies following the failure of EFV-based ART (16).

This study has several strengths, including a large number of HIV-1 sequences, long surveillance duration, and nationwide coverage of ART-naïve HIV-infected adults in China, yielding robust data on RPV resistance dynamics. However, this study has some limitations. First, the study relied on retrospective molecular surveillance data, which might have had a sampling bias. The prevalence of HIV-1 subtype B in this study was approximately 10% higher than that reported in a national cross-sectional survey in 2006 (17). Because subtype B exhibited higher RPV resistance rates than the other four subtypes during that period, the prevalence of RPV resistance in the early years was likely overestimated. However, this bias would not alter the overall increasing trend of RPV resistance between 2004 and 2023. Second, the temporal trend in RPV resistance could be confounded by changes in population composition over the study

TABLE 2. The prevalence of RPV resistance-associated mutations in RPV-resistance isolates among different HIV-1 subtypes during the 20-year period in China.

Drug resistance mutation	CRF01_AE (N=394) n** (%)††	CRF07_BC (N=352) n (%)	CRF08_BC (N=256) n (%)	CRF55_01B (N=237) n (%)	B (N=147) n (%)	C (N=31) n (%)	CRF65_cpx (N=49) n (%)	Others (N=72) n (%)	Total (N=1,538) n (%)
V90I	14 (3.6)	21 (6.0)	77 (30.1)	11 (4.6)	8 (5.4)	1 (3.2)	0 (0)	1 (1.4)	133 (8.6)
A98G	34 (8.6)	24 (6.8)	6 (2.3)	12 (5.1)	6 (4.1)	15 (48.4)	0 (0)	10 (13.9)	107 (7.0)
L100I/V	6 (1.5)	5 (1.4)	5 (2.0)	0 (0)	5 (3.4)	0 (0)	0 (0)	0 (0)	21 (1.4)
K101E/H/P	54 (13.7)	45 (12.8)	16 (6.3)	1 (0.4)	13 (8.8)	5 (16.1)	0 (0)	8 (11.1)	142 (9.2)
K103R	73 (18.5)	28 (8.0)	9 (3.5)	1 (0.4)	8 (5.4)	6 (19.4)	46 (93.9)	13 (18.1)	184 (12.0)
V106A/I	39 (9.9)	2 (0.6)	25 (9.8)	8 (3.4)	42 (28.6)	2 (6.5)	1 (2.0)	2 (2.8)	121 (7.9)
V108I	12 (3.0)	3 (0.9)	5 (2.0)	6 (2.5)	5 (3.4)	0 (0)	0 (0)	3 (4.2)	34 (2.2)
E138A/G/K/Q/R	63 (16.0)	176 (50.0)	93 (36.3)	194 (81.9)	34 (23.1)	4 (12.9)	2 (4.1)	19 (26.4)	585 (38.0)
V179D/E/F/L	122 (31.0)	60 (17.0)	125 (48.8)	235 (99.2)	41 (27.9)	9 (29.0)	49 (100.0)	25 (34.7)	666 (43.3)
Y181C/F/I/S/V	74 (18.8)	35 (9.9)	5 (2.0)	5 (2.1)	33 (22.4)	4 (12.9)	0 (0)	22 (30.6)	178 (11.6)
Y188F/L	6 (1.5)	14 (4.0)	3 (1.2)	1 (0.4)	18 (12.2)	0 (0)	0 (0)	1 (1.4)	43 (2.8)
G190A/C/E/Q/S/T/V	108 (27.4)	33 (9.4)	9 (3.5)	2 (0.8)	29 (19.7)	2 (6.5)	0 (0)	9 (12.5)	192 (12.5)
H221Y	14 (3.6)	4 (1.1)	5 (2.0)	3 (1.3)	18 (12.2)	0 (0)	0 (0)	4 (5.6)	48 (3.1)
F227C/L/Y	13 (3.3)	7 (2.0)	42 (16.4)	0 (0)	4 (2.7)	0 (0)	0 (0)	3 (4.2)	69 (4.5)
M230I/L	19 (4.8)	12 (3.4)	3 (1.2)	2 (0.8)	7 (4.8)	0 (0)	0 (0)	0 (0)	43 (2.8)
Categories of RPV RAMs									
NNRTI cross-resistance RAMs*	220 (55.8)	130 (36.9)	41 (16.0)	11 (4.6)	90 (61.2)	10 (32.3)	0 (0)	33 (45.8)	535 (34.8)
Polymorphic RAMs†	200 (50.8)	232 (65.9)	166 (64.8)	236 (99.6)	77 (52.4)	26 (83.9)	49 (100.0)	41 (56.9)	1027 (66.8)
RPV-specific RAM§	18 (4.6)	16 (4.6)	19 (7.4)	1 (0.4)	7 (4.8)	0 (0)	0 (0)	0 (0)	61 (4.0)
Others¶	50 (12.7)	17 (4.8)	72 (28.1)	9 (3.8)	31 (21.1)	0 (0)	0 (0)	19 (26.4)	198 (12.9)
Most frequent RAM combinations involving V179									
V90I, V179DE	5 (1.3)	5 (1.4)	62 (24.2)	9 (3.8)	2 (1.4)	1 (3.2)	0 (0)	0 (0)	84 (5.5)
A98G, V179DE	0 (0)	2 (0.6)	1 (0.4)	12 (5.1)	0 (0)	0 (0)	0 (0)	1 (1.4)	16 (1.0)
K103R, V179DE	47 (11.9)	11 (3.1)	4 (1.6)	0 (0)	0 (0)	3 (9.7)	46 (93.9)	9 (12.5)	120 (7.8)
V106I, V179DE	20 (5.1)	0 (0)	8 (3.1)	6 (2.5)	13 (8.8)	2 (6.5)	1 (2.0)	1 (1.4)	51 (3.3)
E138A, V179DE	0 (0)	4 (1.1)	20 (7.8)	0 (0)	0 (0)	2 (6.5)	1 (2.0)	2 (2.8)	29 (1.9)
E138G, V179DE	5 (1.3)	10 (2.8)	9 (3.5)	188 (79.3)	8 (5.4)	0 (0)	1 (2.0)	1 (1.4)	222 (14.4)

Abbreviation: HIV=human immunodeficiency virus; ART=antiretroviral therapy; RPV=rilpivirine; NNRTI=non-nucleoside reverse transcriptase inhibitor; RAM=resistance-associated mutation.

* NNRTI cross-resistance RAMs include L100I/V, K101E/P, Y181C/F/I/S/V, Y188F/L, G190A/C/E/Q/S, F227C, and M230I/L.

† Polymorphic RAMs include V90I, A98G, V106I, E138A/G, and V179D/E.

§ RPV-specific RAM refers to E138K; this mutation is one of the E138A/G/K/Q/R mutations, along with E138A, E138G, E138Q, and E138R.

¶ Other RPV-RAMs include K101H, V106A, V108I, E138Q/R, V179F/L, G190T/V, H221Y, and F227L/Y.

** Number of RPV-associated drug resistance mutations in RPV resistance isolates among different HIV-1 subtypes.

†† The detection rate of RPV-associated drug resistance mutations in RPV resistance isolates among different HIV-1 subtypes.

period, including shifts in the distribution of HIV-1 subtypes, transmission routes, and demographic characteristics of the tested populations. However, owing to missing data on patient demographic characteristics, the potential correlations between these factors and RPV resistance were not assessed. Third, the phenotypic effect of RPV RAMs on HIV-1 strains prevalent in China was not validated. Thus, further studies are required to provide evidence-based

implications for clinical decision-making.

In conclusion, the prevalence of RPV resistance increased significantly and was mostly driven by low-level RPV resistance. Polymorphic RAMs and NNRTI cross-resistance mutations collectively contribute to increased RPV resistance. Continuous molecular surveillance is crucial for monitoring the evolving RPV resistance trends and identifying emerging and circulating resistant strains. Moreover, caution is

warranted when prescribing RPV-containing regimens to patients infected with HIV-1 strains with high RPV resistance prevalence.

Conflicts of interest: No conflicts of interest.

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* Corresponding author: Lingjie Liao, liao lj@chinaaids.cn.

¹ National Key Laboratory of Intelligent Tracking and Forecasting for Infectious Diseases, National Center for AIDS/STD Control and Prevention (NCAIDS), Chinese Center for Disease Control and Prevention & Chinese Academy of Preventive Medicine, Beijing, China.

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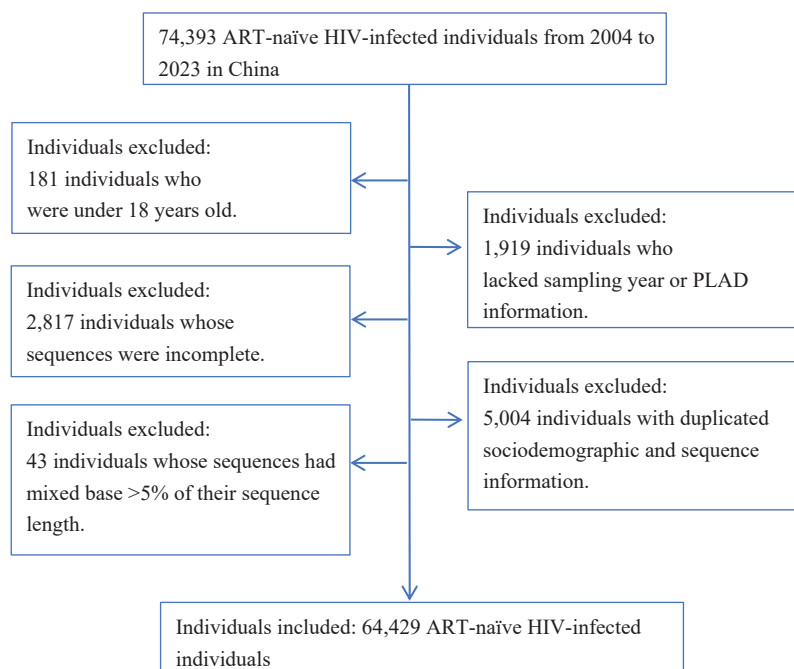
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SUPPLEMENTARY MATERIAL



SUPPLEMENTARY FIGURE S1. Inclusion criteria for study subjects.

Abbreviation: RPV=rilpivirine; HIV=human immunodeficiency virus; ART=antiretroviral therapy, PLAD=provincial-level administrative division.

SUPPLEMENTARY TABLE S1. Distribution of HIV-1 subtypes among ART-naïve HIV-infected individuals during the 20-year period in China.

HIV-1 subtypes	2004–2007		2008–2011		2012–2015		2016–2019		2020–2023		Total	
	<i>n</i> *	Proportion (%)†	<i>n</i>	Proportion (%)	<i>n</i>	Proportion (%)	<i>n</i>	Proportion (%)	<i>n</i>	Proportion (%)	<i>n</i>	Proportion (%)
Total	1,251	100.0	8,795	100.0	21,461	100.0	21,389	100.0	11,533	100.0	64,429	100.0
CRF01_AE	467	37.3	3,912	44.5	9,048	42.2	7,906	37.0	3,280	28.4	24,613	38.2
CRF07_BC	301	24.1	2,540	28.9	7,402	34.5	8,193	38.3	5,077	44.0	23,513	36.5
CRF08_BC	144	11.5	389	4.4	1,190	5.5	2,099	9.8	1,298	11.3	5,120	7.9
B	301	24.1	1,223	13.9	1,634	7.6	959	4.5	565	4.9	4,682	7.3
CRF55_01B	1	0.1	221	2.5	1,170	5.5	1,125	5.3	332	2.9	2,849	4.4
C	16	1.3	128	1.5	112	0.5	111	0.5	68	0.6	435	0.7
CRF85_BC	0	0	0	0	67	0.3	84	0.4	170	1.5	321	0.5
CRF59_01B	2	0.2	26	0.3	100	0.5	132	0.6	44	0.4	304	0.5
CRF79_0107	0	0	19	0.2	47	0.2	66	0.3	62	0.5	194	0.3
CRF65_cpx	0	0	21	0.2	52	0.2	34	0.2	23	0.2	130	0.2
Others	21	1.7	342	3.9	739	3.4	812	3.8	658	5.7	2,572	4.0

Abbreviation: HIV=human immunodeficiency virus; ART=antiretroviral therapy.

* Number of ART-naïve individuals infected with different HIV-1 subtypes.

† The proportion of ART-naïve individuals infected with different HIV-1 subtypes.

SUPPLEMENTARY TABLE S2. The prevalence of RPV resistance-associated mutations among different HIV-1 subtypes during 20-year period in China.

Drug resistance mutation	CRF01_AE (N=24,613) n* (%) [†]	CRF07_BC (N=23,513) n (%)	CRF08_BC (N=5,120) n (%)	CRF55_01B (N=2,849) n (%)	B (N=4,682) n (%)	C (N=435) n (%)	CRF65_cpx (N=130) n (%)	Others (N=3,807) n (%)
V90I	116 (0.5)	157 (0.7)	279 (5.4)	11 (0.4)	83 (1.8)	22 (5.1)	0 (0)	41 (1.3)
A98G	34 (0.1)	24 (0.1)	6 (0.1)	12 (0.4)	6 (0.1)	15 (3.4)	0 (0)	10 (0.3)
L100I/V	6 (0)	5 (0)	5 (0.1)	0 (0)	5 (0.1)	0 (0)	0 (0)	0 (0)
K101E/H/P	54 (0.2)	48 (0.2)	16 (0.3)	1 (0)	13 (0.3)	5 (1.1)	0 (0)	8 (0.3)
K103R	853 (3.5)	314 (1.3)	60 (1.2)	28 (1.0)	151 (3.2)	14 (3.2)	47 (36.2)	126 (4.1)
V106A/I	522 (2.1)	27 (0.1)	123 (2.4)	21 (0.7)	842 (18.0)	3 (0.7)	1 (0.8)	88 (2.9)
V108I	64 (0.3)	25 (0.1)	21 (0.4)	6 (0.2)	27 (0.6)	0 (0)	0 (0)	8 (0.3)
E138A/G/K/Q/R	105 (0.4)	228 (1.0)	460 (9.0)	194 (6.8)	50 (1.1)	14 (3.2)	2 (1.5)	69 (2.2)
V179D/E/F/L	1,310 (5.3)	900 (3.8)	524 (10.2)	2,709 (95.1)	306 (6.5)	31 (7.1)	125 (96.2)	239 (7.7)
Y181C/F/I/S/V	74 (0.3)	35 (0.1)	5 (0.1)	5 (0.2)	33 (0.7)	4 (0.9)	0 (0)	22 (0.7)
Y188F/L	6 (0)	14 (0.1)	3 (0.1)	1 (0)	18 (0.4)	0 (0)	0 (0)	1 (0)
G190A/C/E/Q/S/T/V	108 (0.4)	34 (0.1)	9 (0.2)	2 (0.1)	29 (0.6)	2 (0.5)	0 (0)	9 (0.3)
H221Y	29 (0.1)	14 (0.1)	14 (0.3)	3 (0.1)	18 (0.4)	1 (0.2)	0 (0)	6 (0.2)
F227C/L/Y	19 (0.1)	12 (0.1)	42 (0.8)	0 (0)	5 (0.1)	0 (0)	0 (0)	4 (0.1)
M230I/L	19 (0.1)	12 (0.1)	3 (0.1)	2 (0.1)	7 (0.1)	0 (0)	0 (0)	0 (0)

Abbreviation: HIV=human immunodeficiency virus; RPV=rilpivirine; ART=antiretroviral therapy.

* Number of RPV resistance-associated mutations among different HIV-1 subtypes.

† The detection rate of RPV resistance associated mutations among different HIV-1 subtypes.