Prevalence of acquired drug resistance mutations in antiretroviral-experiencing subjects from 2012 to 2017 in Hunan Province of central South China

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Abstract

Background: There are few data on the prevalence of acquired drug resistance mutations (ADRs) in Hunan Province, China, that could affect the effectiveness of antiretroviral therapy (ART).

Objectives: The main objectives of this study were to determine the prevalence of acquired drug resistance (ADR) the epidemic characteristics of HIV-1-resistant strains among ART-failed HIV patients in Hunan Province, China.

Methods: ART-experienced and virus suppression failure subjects in Hunan between 2012 and 2017 were evaluated by genotyping analysis and mutations were scored using the HIVdb.stanford.edu algorithm to infer drug susceptibility.

Results: The prevalence of HIV-1 ADR were 2.76, 2.30, 2.98, 2.62, 2.23 and 2.17%, respectively, from 2012 to 2017. Overall 2295 sequences were completed from 2932 ART-failure patients, and 914 of these sequences were found to have drug resistance mutation. The most common subtype was AE (64.14%), followed by BC (17.91%) and B (11.50%). Among those 914 patients with drug resistance mutations, 93.11% had NNRTI-associated drug resistance mutations, 74.40% had NRTI drug resistance mutations (DRMs) and 6.89% had PI DRMs. Dual-class mutations were observed in 591 (64.66%) cases, and triple-class mutations were observed in 43 (4.70%) cases. M184V (62.04%), K103N (41.90%) and I54L (3.83%) were the most common observed mutations, respectively, in NRTI-, NNRTI- and PI-associated drug resistance. 93.76% subjects who had DRMs received the ART first-line regimens. CD4 count, symptoms in the past 3 months, and ART adherence were found to be associated with HIV-1 DR.

Conclusions: This study showed that although the prevalence of HIV-acquired resistance in Hunan Province is at a low-level, the long-term and continuous surveillance of HIV ADR in antiretroviral drugs (ARVs) patients is necessary.

Keywords: HIV, Acquired drug resistance, China, Mutation

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Drug resistance can be categorized into ADR mutations in treatment-naive individuals, both of which could cause the failure of the antiretroviral therapy (ART). Ignoring TDR can result in treatment failure of antiretroviral regimens, and ADR is often associated with virological failure (VF) and can increase the burden of treatment [3, 4]. While TDR has been well documented, fewer studies report on the rates of ADR. A systematic review from resource limited settings reports on rates of ADR steadily increased with time on ART [5]. M184V is the most commonly occurring NRTI ADR mutation. In vitro, it causes high-level resistance to lamivudine (3TC). The next most common ADR mutation is the K103N mutation (associated with nucleoside reverse transcriptase inhibitors; NNRTIs) which cause high-level resistance to nevirapine (NVP) and variable resistance to efavirenz (EFV) [6].

Since the first case of HIV infection was found in 1992, there were 29,002 people living with HIV (PLWH) to the end of 2017 in Hunan province, which is in south central China, and has a population of 68 million. More than 25,530 HIV/AIDS patients had received free highly active antiretroviral therapy (HAART) and were still in treatment which is supported by the government by the end of 2017. Although stavudine (D4T) and zidovudine (AZT) are not recommended as first-line therapy in the US and Europe, in much of Asia and other resource-limited settings, the most common first-line regimens for HIV-infected patients still contain either D4T or AZT, which are less costly than tenofovir (TDF) [7–9]. The first line regimen of HAART given to patients in Hunan Province China is a triple therapy choosing from d4T, AZT, TDF, 3TC, Efavirenz (EFV) and nevirapine (NVP), all these generic drugs produced in China. Lopinavir/ritonavir (LPV/r), produce outside China, is the second-line drug. A recent Hunan Province molecular epidemiology survey showed that 4 HIV-1 subtypes in Hunan province reported were CRF_01AE, CRF07_BC, B and C. CRF_01AE was the dominant subtype strain (more than 70%) [10–12].

The emerging ADR and subsequent treatment failure pose a major concern for HIV ART in resource-limited settings where treatment options are limited. The objective of this study is to determine the prevalence and DRMs of HIV-1 virologic failure and acquired drug resistance among ART-experienced adults in Hunan Province, China.

Methods

Ethical statement

The research protocol, approved by the relevant institutional review boards or independent ethics committees, was conducted in accordance with standards for the protection of patient safety and welfare and in compliance with Good Clinical Practices and the principles of the Declaration of Helsinki and its amendments. According to “National Free AIDS ARV guidelines” (China), all participants who receive treatment from AIDS ARV supported by the Chinese government must have viral load testing and genotypic testing, which will be used for this study. Verbal informed consent is given to the doctors at the clinical sites who are responsible for the AIDS patients, and the patients’ names were kept on a list with the doctors’ signature. The study and the verbal consent procedure were approved by the Ethical Committee of Hunan Provincial Center for Disease Control and Prevention (ethical approval number: 201801).

Subjects

The Chinese national free ARV treatment policy guidelines recommend that patients on ART should have an HIV viral load test at least once a year [10–12]. If the viral load is > 1000 copies/ml, a HIV drug resistance genotypic test (Sanger Sequencing based) should be performed. All the patients seen between 2012 to 2017 who had taken ART more than 6 months and had a test for virus load (VL) by the Hunan CDC HIV Laboratory were enrolled in this study. Primary inclusion criteria: 1) HIV-infected; 2) on ART more than 6 months; 3) a minimum of 18 years of age; 4) a plasma HIV RNA > 1000 copies/mL with any CD4+ lymphocyte count. The data were extracted from the Chinese National HIV DR Surveillance database, Questionnaire data from the Chinese HIVDR database on age, ethnicity, gender, profession, education, marital status, route of HIV transmission, initial ART regimen and missed doses in the past month were collected by trained interviewers [13]. The viral load testing and drug resistance testing were done by the Hunan CDC HIV Laboratory.

Laboratory tests

CD4+ T cell abs count and viral load testing

CD4+ T cell abs counts were determined with a FACS Calibur (Becton Dickinson, Mountain View, California, USA) in freshly collected whole blood (within 24 h). Plasma HIV-1-RNA viral loads were determined using the COBAS TaqMan HIV-1 test, version 2.0 (CAP/CTM v2.0) with a lower limit of detection of 40 RNA copies/ml and Abbott m2000 Diagnostics Systems with a lower of detection of 150 RNA copies/ml.

RNA extraction, amplification and sanger sequencing

HIV RNA was then extracted from 140 ul of plasma using the QIAmp Viral RNA mini kit (Qiagen, Germany) according to the manufacturer’s protocol. Approximately 1247 bp pol fragments (HXB2 positions 2057–3304)
were amplified by nested reverse transcriptase polymerase chain reaction (nested RT-PCR). The 1st round PCR using one-step RT PCR kits (Promega, USA) with primers: F1a (5’- TGAARGAITGYACTGARAGRCGGCTAAT – 3’), F1b(5’- ACTGARAGRCGGCTAATTTTTTAG – 3’) and RT-R1(5’-ATCCCCTCATAAATCTGACTTG – 3’) and the cycling conditions were as follows: incubation at 50 °C for 45 min, 94 °C for 2 min, then 35 cycles of 94 °C for 15 s, 50 °C for 20 s, and 72 °C for 2 min 30 s, the 72 °C for 10 min. The 2 nd round was implemented using Taq PCR Mastermix (Tiangen, Beijing, China) with primers: PRT-F2(5’- CTITTCCTCCCTCARATCACT CT – 3’) and RT-R2(5’-CTITCTGTATGTCATTTGAC AGTCC – 3’) and the condition were: 94°C for 4 min, then 30 cycles of 94°C for 15 s,55°C for 20 s, and 72°C for 2 min and then 72°C for 10 min. The PCR products were purified and sequenced by the North Genomics Research Center (Sinogenomax Ltd., Beijing).

**Sequence and mutation analysis**

The results from sequencing were aligned and assembled manually by Bio Edit (7.0.2) and ContigExpress Project, a component of the Vector NTI Suite 6 software, Major drug-resistance mutations were defined according to the IAS-USA 2015 list [14]. The subtype analysis and the level of clinically relevant loss of antiviral activity for each drug was determined using the Stanford HIVdb algorithm (http://hivdb.stanford.edu), with a resistance score of ≥15 for any drug [14, 15]. Statistical analyses were conducted using STATA 15.0 (STATA Corp, Texas, USA). Findings with \( P < 0.05 \) were used to determine statistical significance.

**Results**

**Demographics characteristics**

For the years 2012 to 2017, there were 1520, 3650, 6749, 882 copies/ml (IQR 5215–63,000), and the median CD4 was 189 cells/ml (IQR:81–277). (Table 1.)

**Prevalence of HIV drug resistance mutation**

Among the 2932 ART failures, 2295 pol area complete sequences (1200 bp) were obtained by Sanger sequencing. The primary subtype was AE (64.14%, 1472/2295), followed by BC (17.91%, 411/2295) and B (11.50%, 264/2295) (Table 2). Although the AE subtype was the most common strain in most cities in Hunan Province, in Zhangjiajie City subtype B was the main strain.

Nine hundred and fourteen of 2295(39.83%) had at least one primary drug resistance mutation (DRM) according to the Stanford HIVdb algorithm SDRM list of mutations [16]. The annual acquired drug resistance (ADR) rates for 2012 to 2017 were 2.76%(42/1520), 2.30%(84/3650), 2.98%(201/6749), 2.62%(215/8215), 2.23%(209/9380), and 2.17%(163/7524). Ninety-three percent (851/914) of cases had NNRTI-associated drug resistance mutations; 680 cases (74.40%) had mutations associated with NRTI resistance, and 63 had PI DRMs (6.89%). Dual-class mutations were observed in 591 (64.66%) cases: 583 NRTI + NNRTI, four NRTI + PI and four NNRTI + PI. Triple-class mutations were observed in 43 (4.70%) sequences (Table 2). The most common primary NRTI mutations detected included M184V (62.04%), K65R (20.24%), K70R (15.01%), T215D/S (9.41%), Y115F (7.22%) and M41L (6.35%). The most frequent mutations to NNRTIs were K103N (41.90%), Y181C (28.12%), G190A (26.48%), K101E (11.71%), V106M/A (10.94%), V108I (7.44%), 221Y (7.22%) and Y188N (5.91%). The proportion of PI-associated DRMs showed a tendency to increase from 2012 to 2017(0, 0, 0.22, 0.98, 1.86 and 3.81%). The major mutations were I54L (3.83%), V82L (2.84%) and M41L/1 (1.53%). All of these can lead to LPV/r resistance (Table 3). The NNRTI (t)-associated mutation G190A emerged in 92.24% (202/219) of the subtype CRF01AE sequences and in 7.76% of other subtypes (\( p < 0.05 \)). Y181C also emerged frequently in subtype CRF01AE sequences (71.60% vs 28.40% for other subtypes, \( p < 0.05 \)).

Eight hundred and fifty-seven of 914 subjects (93.76%) who had DRMs received the ART first-line regimens that include two NRTIs and one NNRTI. All the regimens contain 3TC, 468 patients took AZT + NVP/EFV, 102 took D4T + NVP/EFV and 287 took TDF + NVP/EFV. 6.13% of patients (56/914) received the second-line regimens which were made with LPV/r and two NRTIs/NNRTIs. Only 1 patient took the Raltegravir (RAL) + 3TC+ Abacavir (ABC). Among the 1381 no DRMs subjects, 882(63.87%, 882/1381) took the first-line regimens: 424 took AZT + NVP/EFV, 62 took D4T + NVP/EFV and 396 took TDF + NVP/EFV; 499(36.13%, 499/1381)
received the second-line regimens (LPV/r or RAL or ABC + 3TC + NVP).

**Factors associated with the detection of ADRs**

Nine potential risk factors were considered in the multivariate logistic regression analysis (Table 4). Of these factors, the CD4+ T cell count, symptoms in recent three months and ART non-adherence (missed doses in the prior 7 days) were related to HIV-1 DR ($p < 0.05$). The odds of having a drug resistance mutation were 3.15 times higher for a patient who had low-level CD4 count ($\leq 200$ cells/mm$^3$) than for a patient who had a high-level CD4 count (>200 cells/mm$^3$) (95% CI:2.54–3.89). The patients who had symptoms in the last 3 months had a higher risk for developing drug resistance mutation compared with those with no symptoms in the last 3 month (OR:1.48, 95%CI:1.18–1.85). There were 59.63% (545/914) DRMs detected subjects who reported missing a dose in the last 7 days, and compared to 37.29% (515/1381) no mutation detected subjects who missed a dose in the last 7 days (OR:2.94, 95% CI:2.38–3.63). The patients taking regimens containing TDF or LPV/r had fewer resistance mutations compared with the patients on regimens containing AZT ($P < 0.05$). The age, gender, subtype, WHO clinical stage and routes of infection did not show significantly differences between DRMs identified and no DRM identified group ($P > 0.05$).

**Discussion**

Our study aimed to elucidate the prevalence of HIV ADRs in ART VF subjects, since ADR is one major problem with the efficacy of ART as it affects the clinical outcomes of treatment. In this study, the annual ADR rate was lower than the threshold low-incidence rate defined by WHO of 5% [17] (from 2012 to 2017: 2.76, 2.44, 2.98, 2.68, 2.24 and 2.19%). This situation is mainly

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**Table 1** Demographic Characteristics of the ART-Failure patients in Hunan Province from 2012 to 2017

|                | 2012 (n = 130) | 2013 (n = 303) | 2014 (n = 585) | 2015 (n = 675) | 2016 (n = 703) | 2017 (n = 536) | Total (n = 2932) |
|----------------|---------------|---------------|---------------|---------------|---------------|---------------|----------------|
| **Gender**     |               |               |               |               |               |               |                |
| Male           | 83            | 208           | 401           | 492           | 520           | 395           | 2099           |
| Female         | 47            | 95            | 184           | 183           | 183           | 141           | 833            |
| **Age**        |               |               |               |               |               |               |                |
| < 14           | 3             | 3             | 14            | 5             | 6             | 5             | 36             |
| 15–29          | 16            | 37            | 85            | 111           | 125           | 96            | 470            |
| 30–44          | 60            | 134           | 229           | 210           | 192           | 171           | 996            |
| 45–59          | 35            | 80            | 141           | 196           | 208           | 129           | 789            |
| ≥ 60           | 16            | 49            | 116           | 153           | 172           | 135           | 641            |
| Median and IQR | 41 (33–49)    | 43 (32–54)    | 42 (33–52)    | 45 (34–58)    | 46 (33–59)    | 44 (31–56)    | 44 (33–52)     |
| **Route of Infection** |       |               |               |               |               |               |                |
| Blood transmission | 6          | 10            | 7             | 5             | 5             | 8             | 41             |
| PWID           | 25            | 50            | 67            | 69            | 47            | 25            | 283            |
| MSM            | 3             | 19            | 45            | 58            | 76            | 80            | 281            |
| Heterosexual   | 88            | 214           | 431           | 506           | 537           | 391           | 2167           |
| Vertical transmission | 3          | 3             | 14            | 5             | 6             | 8             | 39             |
| Unknown        | 5             | 7             | 21            | 32            | 32            | 24            | 121            |
| **Marital Status** |           |               |               |               |               |               |                |
| Single         | 25            | 70            | 136           | 146           | 189           | 159           | 725            |
| Married/cohabitation | 76        | 156           | 319           | 376           | 349           | 263           | 1539           |
| Divorced/separated | 14        | 41            | 86            | 101           | 109           | 75            | 426            |
| Widowed        | 15            | 36            | 39            | 49            | 52            | 36            | 227            |
| Unknown        | 0             | 0             | 5             | 3             | 5             | 3             | 16             |
| **HIV RNA levels (Median, IQR)** | 14,950 (5000–63,500) | 26,405 (6813–74,022) | 29,361 (6791–79,763) | 18,344.5 (5162–68,838) | 14,631 (4315–51,518) | 15,717 (4760–51,048) | 18,882 (5215–63,000) |
| **CD4 levels (Median, IQR)** | 167 (74–247) | 182.5 (89–257) | 174.5 (76–255) | 188.5 (81–275) | 200.5 (81–287) | 208 (89.5–300.5) | 189 (81–277) |

HIV RNA levels expressed in copies/ml, CD4 levels expressed in cells/mm$^3$

PWID Persons who inject drugs, MSM Man have sex with man, IQR Interquartile range
due to the expansion of National Free Antiretroviral Treatment Program (NFATP) in Hunan Province in recent years. Early antiviral treatment can control virus replication as early as possible, and reduce the possibility of drug resistance [13, 18]. The result showed that CRF01 AE is still the most dominance subtype in Hunan province (64.14%), followed by subtype BC, B and C, the finding corresponding to the last years of the Hunan Province molecular epidemiology survey (2009–2015) results [10–12, 19]. The prevalence of HIV-1 subtype B was significantly higher in Zhangjiajie City compared with other areas in Hunan province. Most of the HIV infected people were older people, who lived same area. Previous studies have had similar findings [20]. A HIV epidemic outbreak of subtype B occurred in former plasma donors (FPDs) in central China in 1990s [21, 22]. Some of the FPDs came from Zhangjiajie city, and as a result the B strains were introduced into Zhangjiajie. Due to the economic backwardness of the region, traffic congestion, limited emigration, and open sex, the B strain became a localized epidemic.

In addition to the common subtypes, other subtypes including combined subtype CRF06cpx, CRF09cpx, CRF18cpx and CRF45cpx were found in the last 2 years (2016 and 2017). This may indicate a more complicated and diverse trend of HIV-1 epidemiology emerging in Hunan province.

Table 2 The subtype distribution of the ART failures patients from 2012 to 2017, and the different drug resistance classes (single/ double/ Triple drug resistance) in these patients

| Year | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 | Total |
|------|------|------|------|------|------|------|-------|
| ART Failure | 130 | 303 | 585 | 675 | 703 | 536 | 2932 |
| Obtained sequence | 112 | 239 | 508 | 511 | 528 | 397 | 2295 |
| Subtype | | | | | | | 
| AE | 75 (66.96%) | 163 (68.20%) | 355 (69.88%) | 365 (71.43%) | 294 (55.68%) | 220 (55.42%) | 1472 (64.14%) |
| BC | 11 (9.82%) | 26 (10.88%) | 80 (15.75%) | 106 (20.74%) | 115 (21.78%) | 73 (18.39%) | 411 (17.91%) |
| B | 21 (18.75) | 37 (15.48%) | 52 (10.24%) | 30 (5.87%) | 66 (12.50%) | 58 (14.61%) | 264 (11.50%) |
| C | 4 (3.57) | 13 (5.44%) | 20 (3.94%) | 9 (1.76%) | 23 (4.53%) | 12 (3.02%) | 81 (3.53%) |
| Other | 1 (0.89%) | 0 | 1 (0.20%) | 1 (0.20%) | 30 (5.68%) | 34 (8.56%) | 67 (2.92%) |
| DRMs | | | | | | | 
| Only PI | 42 | 84 | 201 | 215 | 209 | 163 |
| Only NRTI | 0 | 0 | 1 | 3 | 4 | 2 | 10 |
| Only NNRTI | 1 | 1 | 13 | 6 | 21 | 7 | 49 |
| PI and NRTI | 6 | 23 | 57 | 56 | 50 | 29 | 221 |
| PI and NNRTI | 0 | 0 | 0 | 1 | 1 | 2 | 4 |
| NRTI and NNRTI | 35 | 60 | 129 | 144 | 121 | 94 | 583 |
| PI and NRTI and NNRTI | 0 | 0 | 1 | 5 | 9 | 28 | 43 |

Note: ART Antiretroviral therapy DRMs Drug resistance mutations PI Protease inhibitor NRTI Nucleoside reverse transcriptase inhibitors NNRTI Non-nucleoside reverse transcriptase inhibitors

The most frequently identified ADR present in NRTI was M184V/I (61.27%), followed by K65R (19.96%). The elevated presence of M184V/I is expected and arises as a consequence of the use of 3TC as part of all the first-line regimens in China [23]. Although M184V/I causes high-level in vitro resistance to 3TC, M184V/I is not a contraindication to continued treatment with 3TC because it increases susceptibility to AZT, and in addition, it is associated with clinically significant reductions in HIV-1 replication [14, 24, 25]. Similar to our study finding, a high prevalence of M184 V/I mutation has been reported in Asia, Sub-Saharan Africa and Latin America, but a lower prevalence in western Europe [26–31]. This difference can be explained by the more frequent use of 3TC in low- and middle-income countries than in West European countries. K65R is the signature mutation associated with TDF resistance [32, 33]. This study showed that K65R codon mutation had a significant increase from 2014 (71.4, 6.74, 16.42, 22.73%, 20.48 and 30.91%, respectively, from 2012 to 2017), due to the widespread use of TDF replacing AZT and D4T as a part of the first-line treatment from 2014 in China following WHO recommendations [34].

NNRTIs are notorious for rapidly triggering the emergence of drug-resistant HIV-1 variants. One NNRTI drug mutation can lead to multiple and high-level NNRTI-associated drug resistance [35]. The prevalence
of NNRTIs DRMs (91.91%) was higher than NRTIs (74.00%) and PIs (7.44%) in this study. This is due to the fact that NNRTIs have a low genetic barrier to resistance and a single major mutation was often leads to multiple and high-level resistance to NNRTIs drugs [30, 35, 36], which is why the second-line therapy did not include NNRTIs. K103N/S (41.32%) was the most frequently observed resistance mutation in NNRTIs, followed by Y181C (27.83%) and G190A (26.21%), This is a consequence of the frequent use of NNRTI-based (EFV/NVP) first-line therapy for more than a decade in China, and these results are similar to the data from low- and middle-income countries [27, 37–39]. K103N/S was strongly associated with failure against NVP and EVP. Y181C reduces susceptibility to NVP by >50-fold and to EFV by 2-fold. G190A caused high-level resistance to NVP and intermediate resistance to EFV and could result in attenuation of the resistance that occurs with

### Table 3 The major acquired drug resistance mutations found in this study. The drug resistance mutations were identified using the Stanford University HIVdb algorithm

| Mutations | 2012 (n=42) | 2013 (n=84) | 2014 (n=201) | 2015 (n=215) | 2016 (n=209) | 2017 (n=163) | Total (n=914) |
|-----------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| PI        |             |             |             |             |             |             |             |
| M46I/L    | 0           | 4           | 2           | 4           | 5           | 4           | 14 (1.53%)  |
| I84V      | 0           | 0           | 1           | 1           | 1           | 1           | 4 (0.44%)   |
| L76V      | 0           | 0           | 1           | 3           | 5           | 2           | 11 (1.20%)  |
| L90M      | 0           | 0           | 1           | 1           | 1           | 0           | 3 (0.33%)   |
| F53L      | 0           | 0           | 0           | 1           | 0           | 0           | 1 (0.11%)   |
| I50V      | 0           | 0           | 0           | 2           | 0           | 3           | 5 (0.55%)   |
| I54L      | 0           | 0           | 0           | 3           | 7           | 25          | 35 (3.83%)  |
| V82L      | 0           | 0           | 0           | 3           | 6           | 17          | 26 (2.84%)  |
| NRTI      |             |             |             |             |             |             |             |
| M184V/I   | 36          | 53          | 124         | 129         | 118         | 108         | 567 (62.04%)|
| T69S/D/N  | 1           | 3           | 4           | 15          | 9           | 4           | 35 (3.83%)  |
| T215D/S   | 9           | 16          | 20          | 17          | 18          | 7           | 86 (9.41%)  |
| M41L      | 2           | 7           | 6           | 6           | 18          | 16          | 58 (6.35%)  |
| K70Q/R    | 10          | 12          | 21          | 34          | 36          | 26          | 138 (15.10%)|
| K65R      | 3           | 6           | 33          | 50          | 43          | 51          | 185 (20.24%)|
| L210W     | 4           | 6           | 6           | 3           | 9           | 5           | 32 (3.50%)  |
| K70R/E    | 10          | 13          | 20          | 34          | 36          | 26          | 138 (15.10%)|
| Y115F     | 3           | 1           | 11          | 17          | 22          | 13          | 66 (7.22%)  |
| L74I      | 1           | 2           | 11          | 15          | 20          | 10          | 58 (6.35%)  |
| NNRTI     |             |             |             |             |             |             |             |
| K103N/S   | 14          | 42          | 85          | 91          | 75          | 76          | 383 (41.90%)|
| K101E     | 9           | 11          | 22          | 26          | 22          | 17          | 107 (11.71%)|
| Y181C     | 11          | 26          | 69          | 64          | 49          | 39          | 257 (28.12%)|
| 221Y      | 6           | 12          | 17          | 9           | 16          | 6           | 66 (7.22%)  |
| Y188H     | 2           | 7           | 14          | 13          | 8           | 10          | 54 (5.91%)  |
| G190A     | 14          | 23          | 53          | 61          | 55          | 37          | 242 (26.48%)|
| P225H     | 2           | 5           | 15          | 12          | 8           | 6           | 48 (5.25%)  |
| A98       | 2           | 3           | 12          | 14          | 9           | 10          | 50 (5.47%)  |
| V108I     | 3           | 10          | 17          | 12          | 17          | 9           | 68 (7.44%)  |
| V106M/A   | 2           | 2           | 17          | 37          | 25          | 17          | 100 (10.94%)|
| L100I     | 0           | 0           | 4           | 5           | 9           | 3           | 31 (3.39%)  |
| M230L     | 0           | 1           | 6           | 11          | 6           | 7           | 31 (3.39%)  |

Note: HIV drug resistance mutations were identified using the Stanford University HIVdb algorithm

A98 regimens
Table 4 Nine potential risk factors were considered the association with incidence of HIV-1 DRMs among participants experiencing treatment failure

| Variable                     | Mutation identified (n = 914) | No mutations identified (n = 1381) | OR    | P       | 95%CI     |
|------------------------------|-------------------------------|-----------------------------------|-------|---------|----------|
| Gender                       |                               |                                   |       |         |          |
| Male                         | 654                           | 994                               | 0.95  | 0.68    | 0.76–1.20 |
| Female                       | 260                           | 387                               | 1     |         |          |
| Age                          |                               |                                   |       |         |          |
| < 14                         | 20                            | 12                                | 1     |         |          |
| 15–29                        | 146                           | 224                               | 0.22  | 0.32    | 0.11–4.37 |
| 30–44                        | 314                           | 461                               | 0.21  | 0.31    | 0.01–4.22 |
| 45–59                        | 255                           | 374                               | 0.18  | 0.26    | 0.01–3.61 |
| ≥ 60                         | 179                           | 310                               | 0.20  | 0.30    | 0.01–4.06 |
| Regimen                      |                               |                                   |       |         |          |
| AZT + 3TC + NVP/EFV          | 468                           | 424                               | 1     |         |          |
| D4T + 3TC + NVP/EFV          | 102                           | 62                                | 1.39  | 0.10    | 0.94–2.05 |
| TDF + 3TC + NVP/EFV          | 287                           | 396                               | 0.40  | 0.00    | 0.32–0.51 |
| Second line regimens         | 57                            | 499                               | 0.08  | 0.00    | 0.06–0.11 |
| Subtype                      |                               |                                   |       |         |          |
| AE                           | 607                           | 866                               | 1     |         |          |
| BC                           | 126                           | 285                               | 0.62  | 0.00    | 0.48–0.81 |
| B                            | 103                           | 160                               | 0.80  | 0.16    | 0.59–1.09 |
| C                            | 36                            | 45                                | 1.18  | 0.55    | 0.69–2.00 |
| Other                        | 42                            | 25                                | 1.36  | 0.28    | 0.77–2.39 |
| CD4 abs count (cells/mm³)    |                               |                                   |       |         |          |
| ≤ 200                        | 685                           | 705                               | 3.16  | 0.00    | 2.55–3.91 |
| > 200                        | 229                           | 676                               | 1     | 1.00    |          |
| WHO Stage                    |                               |                                   |       |         |          |
| I                            | 449                           | 695                               | 1     |         |          |
| II                           | 237                           | 358                               | 0.9   | 0.41    | 0.70–1.16 |
| III                          | 129                           | 214                               | 0.81  | 0.20    | 0.59–1.12 |
| IV                           | 99                            | 114                               | 1.08  | 0.68    | 0.74–1.59 |
| Route of transmission        |                               |                                   |       |         |          |
| BLOOD transmission           | 12                            | 18                                | 1     |         |          |
| PWID                         | 79                            | 148                               | 1.02  | 0.96    | 0.42–2.50 |
| MSM                          | 90                            | 128                               | 1.44  | 0.43    | 0.58–3.58 |
| Horizontal transmission      | 676                           | 1024                              | 1.27  | 0.58    | 0.55–2.95 |
| Vertical transmission        | 21                            | 13                                | 0.49  | 0.64    | 0.02–9.80 |
| Unknown                      | 36                            | 50                                | 1.32  | 0.58    | 0.50–3.49 |
| Recently 7 days number of doses missed |                   |                                   |       |         |          |
| 0                            | 369                           | 866                               | 1     |         |          |
| ≥ 1                          | 545                           | 515                               | 2.94  | 0.00    | 2.38–3.63 |
| Symptoms in recent three months |                           |                                   |       |         |          |
| No                           |                               |                                   | 1     |         |          |
| Yes                          |                               |                                   | 1.48  | 0.00    | 1.18–1.85 |

Note: OR Odds ratio; CI Confidence interval
P-Values in bold are statistically significant at the 0.05 significance level
K103N alone, and G190A had a higher frequency (83.47%) of drug resistance in the HIV-1 CRF 01AE subtype in this study.

The overall rate of resistance to PIs was low (7.44%) in our study. This might be explained by the low PI coverage in China as a second-line regimen; and since PIs have a much higher barrier to resistance, patients receiving PI-based regimens are less likely to develop resistance. Our study results showed increasing trends in PIs mutation year by year from 2012 to 2017 (from 0 to 8.31%), associated with the increasing use of second-line regimens in recent years. Therefore, it is advisable to include surveillance of PIs mutation in the coming years.

Our study results revealed that the patients' low-level CD4 count (≤200 cells/mm³), clinical symptoms and bad medication compliance (missing drugs) could more easily lead to HIV-1 drug-resistance mutation among participants experiencing treatment failure. These phenomena are a reminder that monitoring resistant strains using CD4 counts and clinical symptoms in the course of ART as well as evaluating patients' therapy compliance are very important. TDF has been commonly used as the first-line regimen since 2014, and LPV/r has always been used as the second-line regimen. The regimens containing TDF or LPV/r resulted in less drug resistance compared with AZT-contained ARVs because they were not used as much as AZT in Hunan Province. But this study result showed that the PI-related mutation had an tendency to increase year by year from 2012 to 2017. This means that in the future we should pay more attention to second-line regimens drug resistance surveillance.

Several limitations of this study should be noted. First, in this study we focused on PI- and RT-associated mutations as the most commonly used ARV classes in Hunan province up to 2017. However, some patients have used integrase inhibitor drugs at their own expense in recent years, and the picture of integrase-associated DRMs remains blank and warrants further study. A second deficiency in our research is that a moderate proportion of subjects did not return for CD4 count or viral load monitoring, and the surveillance frequency of HIV virus load (VL) was relatively low (free VL testing once a year) due to local government policy, all of which may have reduced our power to detect associations between ADR and virologic outcome.

Conclusions
In contrast with our previous research, we focused on HIV ADR in Hunan Province during the last 6 years of this study. Although free antiviral therapy has been widely practiced in Hunan Province for more than 15 years, the prevalence of HIV-acquired resistance in Hunan Province is at a low-level. However, the resistant strains are common among those with viral suppression failure. Therefore, long-term and continuous surveillance of HIV ADR in ARV patients is necessary.

Abbreviations
3TC: Lamivudine; ABC: Abacavir; ADR: Acquired drug resistance; AOR: Acquired drug resistance mutations; ART: Antiretroviral therapy; AZT: Zidovudine; D4T: Stavudine; DR: Drug resistance; DRMs: Drug resistance mutations; EFV: Efavirenz; HAART: Highly active antiretroviral therapy; LPV/r: Lopinavir/ritonavir; NVP: Nevirapine; PLWH: People living with HIV; RAL: Raltegravir; TDF: Tenofovir; TDR: Transmitted drug resistance; VF: Virological failure; VL: Virus load

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Authors' contributions
Xiaobai Zou: Writing the paper, performance of experiment, data analysis and submitting paper. Jianmei He: Writing the paper and data analysis. Jun Zheng: data analysis and review the manuscript. Roberta Malmgren: review the manuscript. Wei Li: Data analysis. Xiuqing Wei: Data collection. Guoqiang Zhang: Performance of experiment. Xi Chen: Conceived and designed the experiments, reviewed the manuscript and submit it. The author(s) read and approved the final manuscript.

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Availability of data and materials
The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request.

Ethics approval and consent to participate
The research protocol, approved by the relevant institutional review boards or independent ethics committees, was conducted in accordance with standards for the protection of patient safety and welfare and in compliance with Good Clinical Practices and the principles of the Declaration of Helsinki and its amendments. According to "National Free AIDS ARV guidelines" (China), all participants who receive treatment from AIDS ARV supported by the Chinese government must have viral load testing and genotypic testing, which will be used for this study. Verbal informed consent is given to the doctors at the clinical sites who are responsible for the AIDS patients, and the patients' names were kept on a list with the doctors' signature. The study and the verbal consent procedure were approved by the Ethical Committee of Hunan Provincial Center for Disease Control and Prevention (ethical approval number: 201801).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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