Original Article

HIV-1 Subtype Diversity and Factors Affecting Drug Resistance among Patients with Virologic Failure in Antiretroviral Therapy in Hainan Province, China, 2014–2020*

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Abstract

Objective This study aimed to determine the HIV-1 subtype distribution and HIV drug resistance (HIVDR) in patients with ART failure from 2014 to 2020 in Hainan, China.

Methods A 7-year cross-sectional study was conducted among HIV/AIDS patients with ART failure in Hainan. We used online subtyping tools and the maximum likelihood phylogenetic tree to confirm the HIV subtypes with *pol* sequences. Drug resistance mutations (DRMs) were analyzed using the Stanford University HIV Drug Resistance Database.

Results A total of 307 HIV-infected patients with ART failure were included, and 241 available *pol* sequences were obtained. Among 241 patients, CRF01_AE accounted for 68.88%, followed by CRF07_BC (17.00%) and eight other subtypes (14.12%). The overall prevalence of HIVDR was 61.41%, and the HIVDR against non-nucleoside reverse transcriptase inhibitors (NNRTIs), nucleotide reverse transcriptase inhibitors (NRTIs), and protease inhibitors (PIs) were 59.75%, 45.64%, and 2.49%, respectively. Unemployed patients, hypoimmunity or opportunistic infections in individuals, and samples from 2017 to 2020 increased the odd ratios of HIVDR. Also, HIVDR was less likely to affect female patients. The common DRMs to NNRTIs were K103N (21.99%) and Y181C (20.33%), and M184V (28.21%) and K65R (19.09%) were the main DRMs against NRTIs.

Conclusion The present study highlights the HIV-1 subtype diversity in Hainan and the importance of HIVDR surveillance over a long period.

Key words: HIV-1 subtypes; Antiretroviral therapy; Virological failure; Drug resistance

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INTRODUCTION

Antiretroviral therapy (ART), also known as highly active ART (HAART), has been widely used in the treatment of human immunodeficiency virus (HIV) infection. The roll-out of ART has dramatically reduced HIV-related morbidity, mortality, and complications and increased life expectancy, making acquired immune deficiency syndrome (AIDS) a manageable chronic disease[1]. Viral suppression by ART leads to a decline in HIV transmission at the individual and population levels[2]. Therefore, ART is highly effective in reducing the risk of HIV transmission and is currently the most effective treatment for AIDS.

In 2003, China's government launched a National Free ART program[3]. The first guideline for diagnosing and treating HIV/AIDS was issued in 2005, stating that zidovudine (or stavudine) plus lamivudine plus efavirenz (ZDV/d4T+3TC+EFV) was the recommended first-line ART regimen for treatment-naïve adults[4]. In 2008, the first-line ART regimen was updated to azidothymidine (or stavudine) plus lamivudine plus nevirapine (or efavirenz) (AZT/d4T+3TC+NVP/EFV). In 2012, it was changed to tenofovir disoproxil fumarate (or azidothymidine) plus lamivudine plus nevirapine (or efavirenz) (TDF/AZT+3TC+EFV/NVP)[5]. As of 2020, 978,000 people living with HIV received prescribed therapy[5], accounting for 92.2% of people living with HIV[6].

Drug resistance mutations (DRMs) appear in HIV strains under treatment pressure, leading to viral rebound and treatment failure[7]. Furthermore, drug-resistant variants can be transmitted to treatment-naïve individuals, which may limit treatment options and is a significant issue for the effective treatment of HIV infection[7]. Two recent systematic reviews on HIV drug resistance (HIVDR) indicated China's national transmitted drug resistance (TDR) ranged from 3.0% to 9.3%[8,9], and acquired drug resistance (ADR) prevalence over 17 years (2001–2017) was 44.7%[8]. Among patients with failed ART, the prevalence of HIVDR was 64.1%, 39.8%, and 51.9% in south China[10], central south China[11], and north China[12], respectively. However, the risk factors related to DR have varied in previous studies. For example, previous studies have demonstrated antiretroviral adherence[13], marital status and the duration from HIV diagnosis to initiating ART[14], age and initial regimens[15], high viral load and HIV-syphilis co-infection[16], duration on ART and educational level[17] are associated with HIVDR. The World Health Organization (WHO) guidelines recommend routine viral load monitoring and expanded DR testing[18].

Hainan Island, China's southernmost province, has a pleasant tropical season and is one of the country's most popular tourist destinations. In addition to many tourists, many people have migrated to Hainan Island, especially from the northeastern provinces, due to Hainan's tropical climate and environment[19-20]. Hainan is considered one of the provinces with a low HIV prevalence in China. By the end of 2019, 3,711 HIV-infected patients in Hainan had received ART[21]. The situation has been challenged by tourism and immigration. For example, most HIV diagnoses in Hainan Island occurred among men who have sex with men (MSM) in recent years[22], which is generally consistent with the situation in the northeastern provinces of China[23-25]. However, neither HIV-1 epidemic subtypes in Hainan nor the prevalence of HIVDR (or DRMs) in patients receiving ART has been adequately studied in the last ten years. We conducted a 7-year (2014–2020) cross-sectional study to address these questions. Most importantly, we assessed for the first time the characteristics of HIV-1 subtypes and the prevalence of HIVDR and DRMs in patients with ART failure and identified the factors associated with HIVDR in Hainan Province, China.

PATIENTS AND METHODS

Study Site and Participants

A cross-sectional study was conducted on HIV/AIDS patients with ART failure from 2014 to 2020 at the Fifth People's Hospital of Hainan Province, the largest HIV/AIDS clinical treatment center in Hainan Province. According to China's national guidelines for HIV/AIDS management (2018), *virologic failure* is defined as plasma HIV-RNA ≥ 200 copies/mL after 48 weeks of initial ART (initiation or modification); or virologic rebound; or HIV-RNA appearing ≥ 200 copies/mL after complete virologic inhibition. In this study, the inclusion criteria were as follows: (1) age ≥ 18 years, (2) confirmed diagnosis (enzyme-linked immunosorbent assay and Western blot) of HIV-1 infection, (3) virologic failure with HIV-RNA load ≥ 200 copies/mL after 48 weeks of initial ART, or virologic rebound, or HIV-RNA ≥ 200 copies/mL after complete virologic inhibition.

Baseline and follow-up clinical data, including

demographic characteristics (sex, age, risk factors for HIV infection, occupation, marital status, ethnicity, education, and city of residence), CD4+T cell count, plasma viral load, HIV diagnosis date, co-infection with hepatitis B/C, ART initiation date, first-line ART regimen, the time between HIV diagnosis and ART initiation were obtained from the individual's medical records.

Initial Treatment Regimens

The recommended first-line ART regimens in Hainan comprised two nucleotide reverse transcriptase inhibitors (NRTIs) and one non-nucleoside reverse transcriptase inhibitor (NNRTI). In the present study, the two NRTIs were 3TC plus either TDF, d4T, or AZT, whereas the NNRTI was either EFV, NVP, or RPV. The second-line regimens comprised two NRTIs (3TC plus TDF or AZT) sequentially selected based on which NRTIs were used as the first-line and a boosted protease inhibitor (PI), lopinavir-ritonavir (LPV/r). The initial regimens were as follows: (1) TDF+3TC+EFV (56.4%, 136/241), (2) AZT+3TC+NVP (20.3%, 49/241), (3) TDF+3TC+NVP (10.8%, 26/241), (4) AZT+3TC+EFV (6.2%, 15/241), (5) d4T+3TC+NVP (3.3%, 8/241), and (6) other regimens (2.9%, 7/241).

Laboratory Testing

Approximately 10 mL of peripheral blood was collected from participants in ethylenediaminetetraacetic acid (EDTA) vacutainer tubes and immediately processed to separate plasma by centrifugation at 3,000 rpm for 15 min. Plasma samples were frozen at −80 °C until tested for HIV-1 RNA viral load and DR. Viral RNA extraction and HIV-1 *pol* amplification were performed at the Guangxi Key Laboratory of AIDS Prevention and Treatment (Guangxi Medical University, Guangxi, China). The HIV-1 *pol* sequence (1,300 base-pairs) that encodes HIV protease and HIV reverse transcriptase (RT, amino acids 1–335) was sequenced by Sangon Biotech Company. The primers to amplify the HIV *pol* region using nested RT-PCR were as described previously[26].

HIV Subtyping and Antiretroviral Resistance Analysis

HIV-1 *pol* sequences were assembled by Sequencher v5.1.4.6 and aligned using the online HIV align tool (http://www.hiv.lanl.gov/content/sequence/viralign.html) by the MAFFT model and HXB2 reference sequence. HIV-1 subtypes were determined using the automated tool COMET (https://comet.lih.lu/) and Recombinant Identification Program for preliminary classification and identified by the maximum likelihood phylogenetic tree (ML tree) with reference sequences (subtypes A-K+Recombinants) downloaded from the Los Alamos sequence database (http://www.hiv.lanl.gov/). The ML tree was constructed with the general time-reversible substitution model with a gamma-distributed rate variation and proportion of invariant sites (GTR+F+R10) using IQ tree v1.6.12 choosing the best-fit model according to Akaike Information Criterion. Subtype O.CM was set as an outgroup. The stability of the ML tree topology was tested using ultra-fast bootstrap (1,000 replicates). Ultra-fast bootstrap values ≥ 0.8 were considered significant. Finally, the tree was visualized using Fig tree v1.4.4.

DR and DRMs were assessed using the HIVDR Database online platform at Stanford University (http://hivdb.stanford.edu). The database employs the list of major standardized HIV-1 DRMs. Cases were classified as susceptible or having low-, intermediate-, or high-level drug resistance in the three drug classes analyzed (PIs, NNRTIs, and NRTIs).

Statistical Analysis

The data analyses were performed using IBM SPSS v21.0. For data description, the numeric variables were displayed with medians and interquartile ranges (IQRs), whereas the categorical variables were presented as proportions and analyzed with χ^2 test or Fisher's exact test. Multivariate logistic regression models assessed associations between considered variables and HIVDR. If variables in the final multivariate logistic regression model with $P < 0.05$ were considered statistically significant and reported descriptively with a 95% confidence interval (95% *CI*) of adjusted odds ratio (a*OR*).

RESULTS

The Trend of HIV-1 Virologic Failure

From 2014 to 2020, the annual number of patients who received ART was 687, 942, 1,467, 1,905, 2,408, 2,642, and 3,094, respectively. A total of 307 patients with virologic failure after ART were recruited. The annual prevalence of virologic failure in patients after ART was 2.62% (18/687), 2.87% (27/942), 1.50% (22/1,467), 0.58% (11/1,905), 1.54% (37/2,408), 1.97% (52/2,642), and 4.52% (140/3,094), respectively (Figure 1).

Of the 307 patients with virologic failure after ART, 66 patients were excluded due to a lack of personal information or failure of HIV-1 sequencing. Finally, a total of 241 (78.5%, 241/307) HIV-1 available *pol* sequences and corresponding medical records were analyzed in this study. From 2014 to 2020, the annual proportion of sequences was 7.05% (17/241), 9.13% (22/241), 7.47% (18/241), 3.73% (9/241), 12.45% (30/241), 17.84% (43/241), and 42.32% (102/241), respectively. Moreover, from 2014 to 2020, the annual proportion of sequences among patients on ART varied from 0.47% to 3.33% (Figure 1).

HIV-1 Subtypes and Patients Characteristics

A closer inspection of the ML tree (Figure 2) revealed the HIV-1 subtype diversity in the Hainan Province. Among 241 patients, CFR01_AE accounted for 68.88% (166/241), followed by CFR07_BC (41/241, 17.01%) and CRF55_01B (10/241, 4.14%). In addition, eight CRF65_CPX strains, eight CRF08_BC strains, three subtype B strains, two CRF57_BC strains, one CRF59_01B strain, one CRF104_0107 strain, and one subtype C strain were detected.

The demographic characteristics of 241 patients are described in Table 1. Among them, the median age was 32 (IQR: 26–42) years, 83.4% were male, and 67.63% were single. Ninety-nine cases (41.08%) had a middle school edducation. The main routes of HIV-1 infection were heterosexual transmission (119, 49.38%) and homosexual transmission (86, 35.68%). The majority of infections (77%) occurred between 2017 and 2020. Nearly half of the patients (49.79%) had baseline CD4 cell counts lower than 200 cells/mm^3, 67.22% used TDF+3TC+EFV/NVP, and 62.66% received ART within seven months after diagnosis. The median VL was 32,529 copies/mL (IQR: 9,071–101,453 copies/mL).

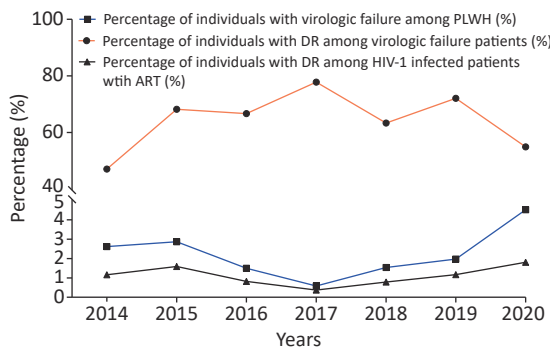

Figure 1. The prevalence of virologic failure and drug resistance (DR) over time from 2014 to 2020. PLWH, people living with HIV-1. ART, antiretroviral therapy.

Prevalence of HIV Drug Resistance among Patients with Virologic Failure

Table 2 shows the prevalence of HIVDR among the 241 patients with virologic failure. The overall prevalence of HIVDR to antiretroviral drugs was 61.41% (148/241). From 2014 to 2020, the annual prevalence of HIVDR was 47.06%, 68.18%, 66.67%, 77.78%, 63.33%, 72.09%, and 54.90%, respectively. The prevalence of HIVDR remained stable over time (χ^2 = 8.824, P = 0.218, Figure 1).

Among the 241 patients, 59.34% were high-level DR, 1.24% were intermediate-level DR, and 0.83% were low-level DR. The prevalence of HIVDR to NRTIs, NNRTIs, and PIs was 45.64% (110/241), 59.75% (144/241), and 2.49% (6/241), respectively (Figure 3). For NRTI drugs, the prevalence of HIVDR against ABC was the highest (45.23%, 109/241), followed by FTC and 3TC (106/241, 43.98%). NVP (59.75%, 144/241), EFV (59.35%, 143/241), and doravirine (DOR) (43.57%, 105/241) were the most common HIVDR drugs to NNRTIs. For PIs, the highest prevalence of HIVDR was 1.66% (NFV, 4/241) (Figure 3). Among the 241 patients, four cases (1.66%) showed triple drug resistance to NRTIs, NNRTIs, and PIs, and 108 cases (44.81%) were resistant to both NRTIs and NNRTIs (Figure 4D).

Factors Associated with HIV Drug Resistance

In the univariate model, male patients had a higher prevalence of HIVDR than female patients (66.17% *vs*. 37.50%), while those aged 40–49 had the lowest prevalence (35.90%). Single participants had a higher prevalence than others (66.87% *vs*. 50.00%), and patients who acquired HIV through homosexual behavior and whose baseline CD4 cell counts were lower than 200 cells/mm^3 had the highest prevalence of HIVDR (73.26% and 71.67%). In addition, patients infected with HIV-1 CRF01_AE strain and hypoimmunity or opportunistic infections had higher HIVDR prevalence than other patients (Table 2).

In the multivariate model, sex, initial therapeutic regimen, age, HIV-1 subtype, patient occupation, sampling time, and hypoimmunity or opportunistic infections were independently associated with HIVDR (Table 2). Compared with male patients, the a*OR* for female patients was 0.11 (95% *CI*: 0.03–0.38). HIVDR was more common in patients on TDF-based regimens than on AZT-

based regimens (a*OR*: 0.15, 95% *CI* = 0.04−0.48). HIVDR was discovered less frequently in patients with CRF07 BC than in those with CRF01 AE (a*OR*: 0.14, 95% *CI*: 0.05−0.40) and in patients aged 40–49 years than in patients aged 19–29 years (a*OR*: 0.27, 95% *CI*: 0.08−0.94). Unemployed patients were more likely to be HIVDR than farmers (a*OR*: 4.32, 95% *CI*: 1.38−13.51). Similar to the samples from 2014 to 2016, the samples from 2017 to 2020 had a higher prevalence of HIVDR (a*OR*: 3.26, 95% *CI*: 1.12−9.47). We also found that patients with hypoimmunity or opportunistic infections had a lower prevalence of HIVDR (a*OR*: 0.32, 95% *CI*: 0.101−0.996).

Drug Resistance Mutations in Patients with Virologic Failure in Antiretroviral Therapy

Regarding DRMs against NRTIs, the most common DRM was M184V, causing high-level resistance to 3TC and FTC, found in 68 of 241 patients (28.22%); K65R, causing high-level resistance to FTC, was detected in 46 of 241 patients (19.09%) (Figure 4A). Against NNRTIs, the K103N, leading to high-level resistance against NVP, was the most common and was found in 53 of 241 patients (21.99%); Y181C and V106M were observed in 20.33% (49/241) and 12.45% (30/241) of the patients, respectively (Figure 4B). M46I and Q58E

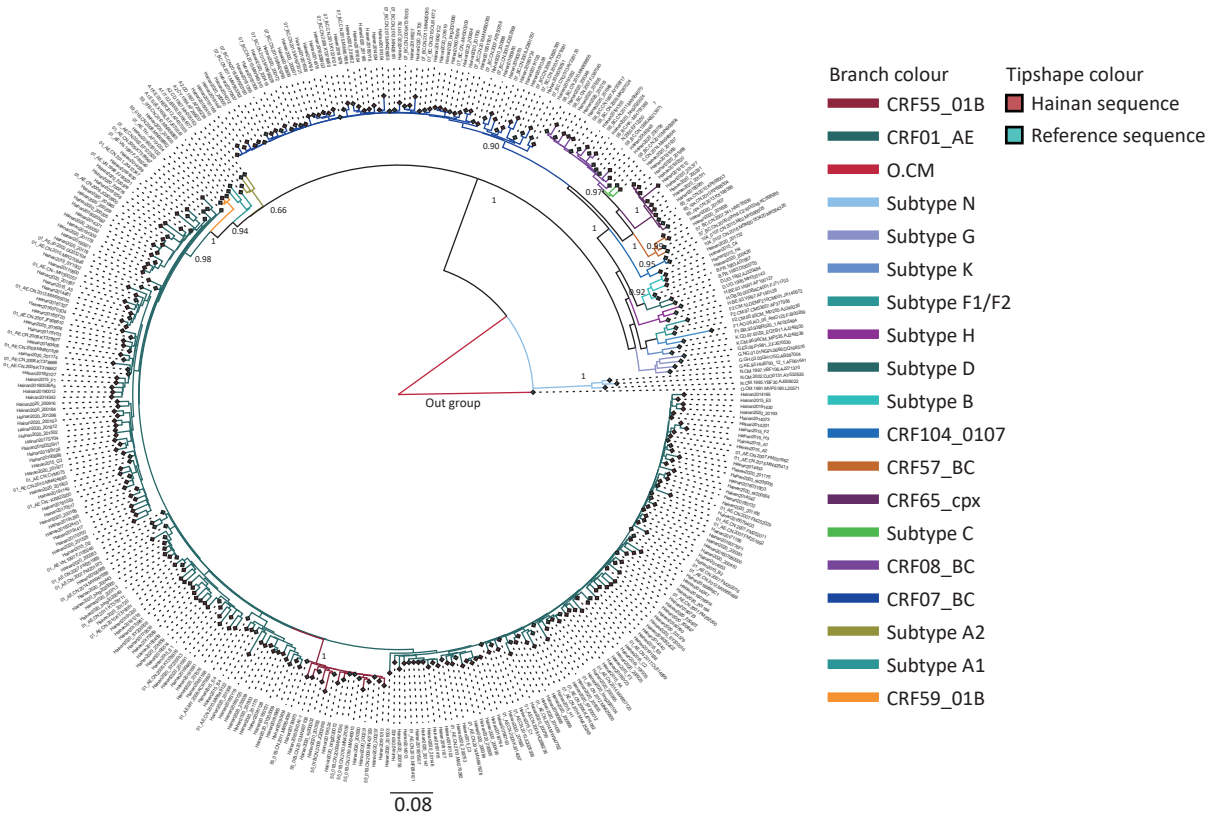

Figure 2. Phylogenetic tree of HIV-1 *pol* sequences obtained from patients with ART-failure in Hainan Province. The maximum likelihood phylogenetic tree (ML tree) was constructed using 365 HIV-1 *pol* sequences, including 241 Hainan sequences and 124 reference sequences. A total of 166 HIV-1 CRF01_AE query sequences branched with 47 HIV-1 CRF01_AE reference sequences (dark green color), bootstrap value was 0.98. Forty-one HIV-1 CRF07_BC query sequences branched with 22 reference sequences (dark blue color), bootstrap value was 0.90. Ten HIV-1 CRF55_01B query sequences were identified (dark red color), bootstrap value was 1.0. Eight HIV-1 CRF08_BC (lilac color) and eight HIV-1 CRF65_cpx (deep purple color) query sequences were identified (bootstrap value = 0.97 and 1, respectively) with nine and three reference sequences, respectively. Meanwhile, CRF57_BC (brown color), CRF59_01B (orange color), CRF104_0107 (light blue color), subtype B (blue-green color) and subtype C (bright green color) were detected. The green diamond of tip shape corresponds to Hainan sequences, and red diamond corresponds to reference sequences.

Table 1. Characteristics of HIV-infected patients with virologic failure from 2014 to 2020 in Hainan Province, China

Variables	Number (*N*)	Percent (%)
Total	241	100
Sex		
Female	40	16.60
Male	201	83.40
Age, years: median 32, IQR (26, 42)		
19–29	97	40.25
30–39	73	30.29
40–49	39	16.18
≥ 50	32	13.28
Marital Status		
Married and cohabiting	78	32.37
Single	163	67.63
Ethnics		
Han	203	84.23
Others	32	13.28
Unknown	6	2.49
Region		
Northern Hainan	92	38.17
Eastern Hainan	22	9.13
Southern Hainan	63	26.14
Western Hainan	39	16.18
Central Hainan	25	10.37
Education		
Primary school or lower	46	19.09
Secondary school	99	41.08
High school or above	90	37.34
Unknown	6	2.49
Occupation		
Farmer	83	34.44
Unemployment	64	26.56
Others	79	32.78
Unknown	15	6.22
Risk factors		
Homosexual	86	35.68
Heterosexual	119	49.38
Others	36	14.94
Sampling time, year		
2014–2016	57	23.65
2017–2020	187	77.59

Continued

Variables	Number (*N*)	Percent (%)
Basic line CD4 cell count, cells/mm^3: median 195, IQR: 80–324		
< 200	120	49.79
200−350	70	29.05
301−500	24	9.96
> 500	18	7.47
Unknown	9	3.73
HIV-1 subtype		
CRF01_AE	166	68.88
CRF07_BC	41	17
Other (B/C/CRF08_BC/CRF59_01B/CRF55_01B/CRF65_cpx /CRF57_BC/CRF104_0107)	34	14.12
Initial therapeutic regimen		
AZT+3TC+EFV/NVP	64	26.56
TDF+3TC+EFV/NVP	162	67.22
Others	15	6.22
Hypoimmunity or opportunistic infections		
Yes	76	31.54
No	165	68.46
The duration from diagnosis to ART, month: median 3, IQR: 0–20.5		
< 7	151	62.66
≥ 7	90	37.34
Time on ART, month: median 20, IQR: 10–39.5		
< 13	82	34.02
13−25	58	24.07
> 25	101	41.91
Co-infection hepatitis B		
Yes	34	14.11
No	127	52.70
Unknown	80	33.20
Co-infection hepatitis C		
Yes	30	12.45
No	112	46.47
Unknown	99	41.08
Viral load, $\log_{10}$ copies/mL		
Viral load median: 32,529 copies/mL, IQR: 9,071−101,453 copies/mL		
4.00−4.99	116	48.13
≥ 5.00	62	25.73
≤ 3.99	63	26.14

Note. IQR, interquartile range; ART, antiretroviral therapy; CRF, circulating recombinant form; AZT, zidovudine; 3TC, lamivudine; EFV, efavirenz; NVP, nevirapine; TDF, tenofovir.

Table 2. Factors associated with drug resistance among HIV-1 patients with virologic failure from 2014 to 2020 in Hainan Province, China

Variables	*N*	Drug resistance		Univariate		Multivariate	
		Number (*N*)	Percent (%)	χ^2	P_1	a*OR* (95% *CI*)	P_2
Total	241	148	61.41				
Sex							
Male	201	113	66.17	11.571	0.001	Ref.	
Female	40	15	37.50			0.11 (0.03, 0.38)	**0.001**
Ages, years							**0.077**
19–29	97	67	69.07			Ref.	
30–39	73	50	68.49	15.587	0.001	1.31 (0.49, 3.46)	0.590
40–49	39	14	35.90			0.27 (0.08, 0.94)	0.039
≥ 50	32	17	53.13			0.51 (0.13, 2.05)	0.343
Marital status							
Single	163	109	66.87	6.336	0.012	Ref.	
Married and cohabiting	78	39	50.00			0.53 (0.21, 1.32)	0.172
Ethnics							
Han	203	123	60.59			Ref.	
Others	32	24	75.00	2.450	0.118[a]	3.386	0.083
Unknown	6	1	16.67				
Region							**0.467**
Central Hainan	25	12	48.00			Ref.	
Northern Hainan	92	59	64.13			1.87 (0.45, 7.75)	0.39
Eastern Hainan	22	11	50.00	4.128	0.389	0.63 (0.11, 3.75)	0.61
Southern Hainan	63	42	66.67			2.09 (0.45, 9.76)	0.347
Western Hainan	39	24	61.54			2.08 (0.39, 11.20)	0.394
Education							**0.183**
Primary school or lower	46	26	56.52			Ref.	
Secondary school	99	62	62.63	1.061	0.588[a]	1.34 (0.45, 4.01)	0.607
High School or above	90	59	65.56			0.48 (0.12, 1.87)	0.291
Unknown	6	1	16.67				
Occupation							**0.034**
Farmer	83	48	57.83			Ref.	
Unemployment	64	48	75.00	4.873	0.087[a]	4.32 (1.38, 13.51)	0.012
Others	79	49	62.03			1.51 (0.54, 4.19)	0.433
Unknown	15	3	20.00				
Risk factors							**0.321**
Homosexual	86	63	73.26			Ref.	
Heterosexual	119	61	51.26	10.685	0.005	0.54 (0.17, 1.66)	0.282
Others	36	24	66.67			0.24 (0.04, 1.68)	0.152
Sampling time, year							
2014–2016	57	35	61.40	< 0.001	0.999	Ref.	
2017–2020	187	116	62.03			3.26 (1.12, 9.47)	**0.030**

Continued

Variables	*N*	Drug resistance		Univariate		Multivariate	
		Number (*N*)	Percent (%)	χ^2	P_1	a*OR* (95% *CI*)	P_2
Basic line CD4 cell count, cells/mm^3							0.419
< 200	120	86	71.67			Ref.	
200–350	70	38	54.29			0.44 (0.14, 1.34)	0.148
301–500	24	13	54.17	15.914	0.001[a]	0.42 (0.10, 1.82)	0.246
> 500	18	5	27.78			0.35 (0.07, 1.83)	0.214
Unknown	9	6	66.67				
HIV-1 subtype							**0.001**
CRF01_AE	166	115	69.28			Ref.	
CRF07_BC	41	14	34.14	17.634	< 0.001	0.14 (0.05, 0.40)	< 0.001
Others	34	19	55.88			0.26 (0.07, 0.88)	0.031
Initial therapeutic regimen							**0.006**
AZT+3TC+EFV/NVP	64	43	67.19			Ref.	
TDF+3TC+EFV/NVP	162	96	59.26	1.230	0.541	0.15 (0.04, 0.48)	0.002
Others	15	9	60.00			0.46 (0.07, 2.92)	0.410
Hypoimmunity or opportunistic infections							
Yes	76	60	78.95	14.405	< 0.001	Ref.	
No	165	88	53.33			0.32 (0.10, 0.99)	**0.049**
The duration from diagnosis to ART, month							
< 7	151	93	61.59	0.005	0.941	Ref.	
≥ 7	90	55	61.11			0.87 (0.34, 2.24)	0.769
Time on ART, month							**0.610**
< 13	82	53	64.63			Ref.	
13–25	58	34	58.62	0.594	0.743	0.89 (0.29, 2.72)	0.843
> 25	101	61	60.40			0.60 (0.21, 1.74)	0.348
Co-infection hepatitis B							**0.689**
Yes	34	21	61.76			Ref.	
No	127	78	61.42	0.003	0.999	0.59 (0.17, 2.07)	0.407
Unknown	80	49	61.25			0.61 (0.15, 2.56)	0.501
Co-infection hepatitis C							**0.361**
Yes	30	18	60.00			Ref.	
No	112	70	62.50	0.108	0.947	0.29 (0.05, 1.59)	0.155
Unknown	99	60	60.61			0.35 (0.06, 2.13)	0.256
Viral load, $\log_{10}$ copies/mL							**0.935**
4.00–4.99	116	69	59.48			Ref.	
≥ 5.00	62	43	69.35	2.317	0.314	0.97 (0.36, 2.65)	0.955
≤ 3.99	63	36	57.14			0.84 (0.33, 2.14)	0.717

Note. aOR, adjusted odd ratio; ART, antiretroviral therapy; CRF, circulating recombinant form; AZT, zidovudine; 3TC, lamivudine; EFV, efavirenz; NVP, nevirapine; TDF, tenofovir; P_1, significant values of univariate analysis; P_2, significant values of multivariate logistic regression analysis; [a], among the corresponding independent variables, the number of the last group is too small to be included in the analysis.

mutations, which PIs selected, occurred in 0.83% (2/241) (Figure 4C).

DISCUSSION

This study first investigated the prevalence of virologic failure in patients with ART in Hainan Province, China. The results showed that from 2014 to 2020, the prevalence of virological failure ranged from 0.58% to 4.52%. The highest prevalence of virologic failure was 4.52% in 2020, lower than 11.8% in China in 2014[27]. In addition, some studies found that the replacement of the ART regimen[28], male sex[29], illiteracy[30], level of baseline CD4 cell count below 100 cells/mm^3, and adherence[31] were associated with a higher likelihood of virologic failure

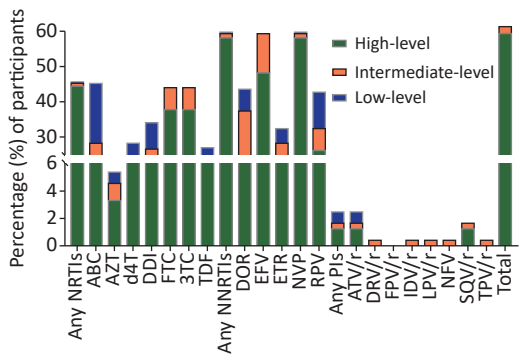

Figure 3. Drug-resistant levels against antiretroviral drugs among ART-failure individuals in Hainan Province from 2014 to 2020. Among the 241 participants, 59.34% acquired high-level drug resistance, 1.24% was intermediate-level drug resistance, and 0.83% was belong to low-level drug resistance (DR). For antiretroviral drugs, 45.29% of patients had DR to ABC, which belongs to NRTIs. About 59% of patients was DR to NVP and EFV belonging to NNRTIs. NRTIs, nucleoside reverse transcriptase inhibitors; NNRTIs, non-nucleoside reverse transcriptase inhibitors; PIs, boosted protease inhibitors. ABC, abacavir; AZT, zidovudine; d4T, sanilvudin; DDI, dideoxynosine; FTC, emtricitabine; 3TC, lamivudine; TDF, tenofovir; DOR, doravirine; EFV, efavirenz; ETR, etravirine; NVP, nevirapine; RPV, rilpivirine; ATV/r, atazanavir with ritonavir; DRV/r, darunavir with ritonavir; FPV/r, fosamprenavir with ritonavir; IDV/r, indinavir with ritonavir; LPV/r, lopinavir with ritonavir; NFV, nelfinavir; SQV/r, saquinavir with ritonavir; TPV/r, tipranavir with ritonavir.

on ART. Although the prevalence of virologic failure in Hainan Province is low, the current results suggest increasing the medication guidance, strengthening the management of treatment follow-up, and following the prescribed dose[32].

In previous years, Wei Deng et al. found that CRF01_AE was the dominant HIV-1 subtype in Hainan, accounting for 84.3% of HIV-positive patients, followed by the B' variant (9.6%)[33]. In the present study, our results showed that CRF01_AE remained the most prevalent subtype. Although the proportion decreased from 2009 to 2020 (68.9% *vs*. 84.3%), many novel CRFs appeared for the first time, such as CRF55_01B, CRF57_BC, CRF65_cpx, and CRF59_01B. Our study further highlights the high genetic diversity of HIV-1 in Hainan, which drives the local HIV epidemic. As a major tourism province, Hainan has attracted many tourists and immigrants, which may have contributed to the wide diversity of HIV subtypes. In addition, previous research has found that subtypes were associated with the progression of HIV/AIDS[34]. The diversity of subtypes has challenged the prevention and control of HIV/AIDS. Therefore, understanding the HIV-1 subtype is essential for guiding targeted HIV control efforts.

HIVDR remains one of the major obstacles to ART efficacy and AIDS treatment, especially in countries with limited access to ART. Among HIV-infected people on ART, between 2014 and 2020, in Hainan Province, the highest prevalence of HIVDR was 1.81% in 2020, which did not reach the threshold of low prevalence, according to the definition of WHO (5%)[35]. From 2014 to 2020, the overall prevalence of HIVDR among the patients with virologic failure was 61.41% in Hainan Province, higher than in Sichuan (45.3%)[36], Guangxi (32.4%), and the national level (51.56%)[37] China. However, it was notably lower than in KwaZulu-Natal Province (92.2%) in South Africa[38], Brazil (84.1%)[39], Ethiopia (74.4%)[40], and Russia (72.5%)[41].

Several factors contributed to HIVDR among patients with ART failure in this study, including sex, initial therapeutic regimen, HIV-1 subtype, occupation, sampling time, and hypoimmunity or opportunistic infections. In this study, males are more likely to be HIVDR than females. The higher proportion of male HIV-positive patients[21] and more male patients with virologic failure than females in Hainan Province can explain the higher odds of HIVDR among males. In addition, adherence to treatment plays a crucial role in the prevalence of DR, and previous research confirmed that men[42] or

unemployed patients[43] have poor adherence to ART. This is in line with our finding that unemployed patients are more likely to have HIVDR than farmers. This study also found that TDF regimens had a lower HIVDR prevalence than AZT-based regimens. It may be because TDF has been used for antiviral therapy for a relatively short period and was included in first-line regimens from 2015, supported by Margot's and Etiebet's studies[44,45]. In addition, the HIV-1 subtype and sampling time were related to HIVDR in the present study. Patients infected with the CRF01_AE strain had a higher prevalence than CRF07_BC, which can be explained by CRF01_AE being the most prevalent subtype in Hainan. As Gao Xiaoli found in Shanxi Province, the most prevalent CRF07_BC had the highest prevalence of HIVDR in patients with failed ART[46]. Our results showed that samples from 2017 to 2020 had a higher prevalence of HIVDR than those from 2014 to 2016, which was associated with prolonged treatment time[36].

Of note, we observed that patients who were hypoimmunity or with opportunistic infections had a higher prevalence of HIVDR. HIV patients might have insufficient physical resistance due to hypoimmunity or opportunistic infections, leading to viral suppression failure and drug resistance, which may contribute to the higher prevalence of HIVDR. The results showed that drug resistance monitoring for HIV-infected patients with hypoimmunity or opportunistic infections should be strengthened. In this study, another finding was that the HIVDR was not associated with CD4 count and viral load. However, previous studies found varying results regarding the relationship between viral load, baseline CD4 count, and the presence of HIVDR in ART[11,47]. Some studies, including the present study, found that age and infection route were unrelated to HIVDR for HIV-1 infected patients with ART failure[48].

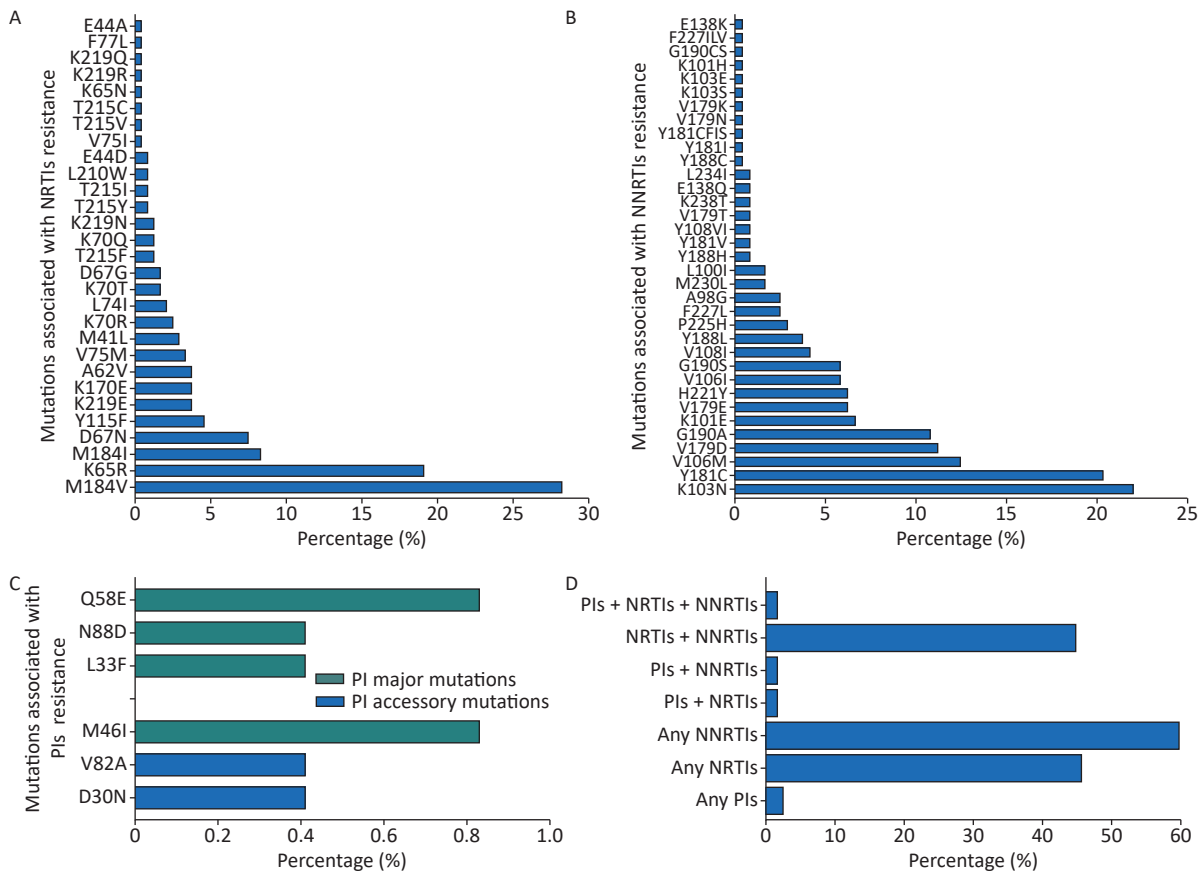

Figure 4. Frequency of drug resistance mutations (DRMs) and drug resistance prevalence among 241 patients with virologic failure after ART in Hainan Province, 2014–2020. (A) Frequency of DRMs to nucleoside reverse transcriptase inhibitors (NRTIs). (B) Frequency of DRMs to non-nucleoside reverse transcriptase inhibitors (NNRTIs). (C) Frequency of DRMs to boosted protease inhibitors (PIs). (D) Drug resistance prevalence by ART drugs.

Consistent with studies conducted in other areas of China[49], the prevalence of HIVDR to NNRTIs was substantially higher than that of NRTIs and PIs among patients with ART failure in Hainan. The first-line regimens in Hainan Province consist of two NRTIs and one NNRTI. In this study, the main regimens were TDF+3TC+NVP/EFV or AZT+3TC+NVP, which account for more than 85.0%. Under the pressure of drug selection, DRMs associated with NNRTIs, and NRTIs were dominant.

NNRTIs have a low genetic barrier to resistance, and one primary mutation of NNRTIs often leads to multiple and high-level resistance to NNRTIs drugs[50]. In our study, we observed that K103N (22%) was the most common resistance mutation to NNRTIs. K103N, a nonpolymorphic mutation selected by NVP and EFV[51], can reduce NVP and EFV susceptibility[52] and cause high resistance to NVP. We also observed that the prevalence of DR to NVP was the highest in all NNRTIs drugs in this study. This study also found that ABC, FTC, and 3TC were the most critical NRTIs drugs responsible for high drug resistance. The major DR-associated mutations to NRTI were M184V and K65R. M184V, the most prevalent, is selected due to the wide use of 3TC as a first-line therapy in China[53]. The M184V mutation causes high-level resistance to 3TC and FTC and also causes low-level resistance to ABC[54].

Nevertheless, M184V could increase the susceptibility to AZT, d4T, and TDF and slow the emergence of resistance to AZT, d4T, and TDF[55]. Therefore, 3TC has been widely used in China until now[56]. K65R is selected by TDF, ABC, and 3TC, decreasing viral susceptibility to these drugs[57]. The increasing and preferential usage of TDF in clinical practice, including in a context of a failing regimen, could be the primordial reason for the significant expansion of K65R, as other studies show a higher prevalence of this mutation in patients failing ART treatment[58]. Another finding is that 2.49% of participants exhibited HIVDR to PIs in this study, indicating that PIs still work well in our settings.

Of note, 44.81% of the patients were resistant to both NRTIs and NNRTIs, and 1.66% were resistant to triple NRTIs, NNRTIs, and PI in this study. Previous studies have confirmed that multi-drug resistance can reduce susceptibility to almost all drugs, making it challenging to optimize therapy to halt viral replication in these patients. In addition, multi-drug resistance is associated with an increased risk of clinical progression and death[59]. Managing patients infected with multi-drug resistance strains are among the critical issues in HIV therapy[60].

There were several limitations to this study. First, the sample size is not large. However, these annual samples were from the vast majority of cities in Hainan Province, accounting for 78.5% of ART-failed patients in a drug resistance surveillance program, which could represent the population of HIV-1 positive patients with ART failure. Second, HIVDR could not be identified as ADR or TDR because the samples were collected after ART. Third, the year of ART failure might differ from the year of HIVDR testing. In this study, we excluded samples collected repeatedly in different years, which may underestimate the results of HIVDR.

In conclusion, we highlighted the diversity of HIV-1 subtypes and reported the prevalence of virologic failure for the first time in Hainan Province, and illustrated that the HIVDR was low in Hainan during the rapid expansion of ART from 2014 to 2020. Of note, we found that patients with hypoimmunity or opportunistic infections were more likely to develop HIVDR, suggesting that drug resistance monitoring of these patients should be strengthened in the future. Meanwhile, this study showed that NNRTIs and NRTIs resistance developed rapidly among patients with virologic failure, and the PI-based treatment regimen might be superior to NNRTIs. Our results support that HIVDR testing should be universal and mandatory as it is the best way to promote personalized selection of the most optimized ART regimen.

ETHICAL CONSIDERATIONS

This study was approved by the Human Research Ethics Committee of Guangxi Medical University under protocol 20220207. Written informed consent was obtained from all participants prior to enrolment in the study.

CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this study.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

SEQUENCES DATA

The GenBank accession numbers of the 241

Hainan HIV-1 sequences are OP830908-OP831148.

AUTHORS' CONTRIBUTIONS

Yu DE and Xu YJ conceived the study; Yang Y, Zhong SM, Qin C, and Li M designed the study; Xu YJ, Zhu KK, Li DW, Pang Y, Lan YN, Yu JP, Qin XQ performed the experiments; Zhu KK, Yu DE, and Liang HY generated and analyzed the data; Yu DE and Liang BY wrote the first draft; Liang BY and Ye L supervised the study at all stages. All co-authors participated in writing, reviewing, and approving the final manuscript.

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