Drug Resistance and Its Related Factors in ART-failure Patients From 2018 to 2019 in Liaoning Province, China

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Research

Keywords: HIV, Antiretroviral therapy, Drug resistance, ART failure

Posted Date: January 4th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-136514/v1

Abstract

Background:

To understand the prevalence of HIV-1 drug resistance and the mutation patterns in ART-failure individuals in Liaoning Province, China, we conducted a cross-sectional survey.

Patients and methods: Plasma samples were collected from HIV-1-positive individuals who experienced ART failure in Liaoning Province between April 2018 and September 2019.Genotype resistance test was performed using an in-house assay on these collected samples. Factors associated with drug resistance were identified by logistic regression analysis.

Results: A total of 256 HIV-1-positive individuals experiencing ART failure were tested for drug resistance from April 2018 to September 2019. Of these, the most predominant genotype was CRF01_AE, accounting for 77.73%. The resistance rate to any of the three classes of antiretroviral drugs (NNRTIs, NRTIs, and PIs) was 64.84%. Among 256 ART-failure patients, 62.89% showed drug resistance to NNRTIs, 50.39% to NRTIs, and 3.13% to PIs. G190S (31.25%) and Y181C (25.78%) mutations were the most common NNRTIs resistance mutations, and K65R (29.69%), M184V (28.52%) were the most common NRTIs resistance mutations. Factors associated with drug resistance included current ART regimen, viral load.

Conclusion: The high drug resistance rate among ART-failure individuals in Liaoning Province needs more attention. Corresponding strategies for the risk factors associated with HIV drug resistance can better control and prevent the prevalence of resistance.

Introduction

The acquired immunodeficiency syndrome (AIDS) is a chronic infectious disease caused by human immunodeficiency virus (HIV) infection. Since the first case was reported in 1981, the number of people living with HIV/AIDS has increased year by year, which has been one of the serious diseases endangering public health. The highly active antiretroviral therapy (HAART) can suppress virus replication, promote body immune reconstruction, and significantly reduce HIV/AIDS associated morbidity and mortality [1, 2]. Given the potential benefits of HAART, with the support of the "Four Frees and One Care" policy, China has gradually implemented free antiretroviral therapy (ART) program nationwide since 2002 [3]. This policy has made great achievement in providing the necessary care and treatment to HIV-infected people in China.

According to the official report, the coverage of the ART program has been increasing since

the introduction of free ART in China in 2002. The number of people receiving antiviral treatment increased from 171,000 in 2012 to 610,000 million in 2017. And the coverage rate of ART was 80.4% and the treatment success rate remained above 90% in 2017 [4].

However, with the increasing coverage of antiretroviral therapy, HIV drug resistance (HIVDR) has become increasingly serious. According to the 2017 HIV drug resistance report released by the WHO, the prevalence of HIV drug resistance has increased from 11% to 29% since the global rollout of ART in 2001 [5]. In China, a prospective cohort study conducted in four urban sentinel sites finds that the proportions of drug resistance to nucleoside reverse transcriptase inhibitors (NNRTIs), non-nucleoside reverse transcriptase inhibitors (NRTIs) and protease

inhibitors (PIs) were 100%, 81.8% and 0% after a year of first-line ART [6] . The enhanced drug resistance is a great challenge to the effectiveness and sustainability of ART, which may directly lead to a decrease availability of the future drug options in resource-limited countries. And drug resistance is considered to be one of the important factors leading to the failure of ART [7, 8]. Continuing using of failed treatment regimens may lead to more complex mutation patterns, the development of cross-resistance, and the increasing occurrence of transmitted HIV drug resistance (TDR). To ensure the long-term effectiveness of the ART, the WHO recommends that drug resistance should be monitored and assessed in these receiving HIV ART individuals.

Liaoning lies in the northeast of China, separated from North Korea by a river and across the sea from Japan and South Korea. As the gateway to the opening up of the Northeast region, understanding the prevalence and mutation patterns of HIV-1 drug resistance is conducive to controlling the spread of HIV drug-resistant strains. Nationwide, the prevalence of HIV/AIDS was at a low level in Liaoning Province [9, 10]. A ten-year cross-sectional study in Liaoning's capital city of Shenyang showed that the average reported incidence of HIV/AIDS was 7.51 per 100,000, and the main route of infection was sexual intercourse, especially MSM [11]. Zhao Yan's study revealed that the drug resistance rate of 239 patients who failed ART in Liaoning province in 2018 was 61.9% [12]. Therefore, it is particularly necessary to grasp and understand the prevalence in

ART-failure patients in Liaoning Province. This study aims to understand the prevalence and mutation patterns of HIV-1 drug resistance among HIV-infected people experiencing ART failure from 2018 to 2019 in Liaoning Province as well as to determine its related factors.

Material And Methods

Ethical statement

Each of the participants in the study signed an informed consent prior to the enrollment. This study was approved by the Ethics Committee of Liaoning Provincial Center for Disease Control and Prevention (ethical approval number: 2020-006).

Study design and participants

The National Free Antiretroviral Treatment Handbook (Version 4) recommends that HIV- positive patients receiving ART for more than 12 months should perform a HIV drug resistance genotypic test [13]. We conducted a cross-sectional study on HIV- positive individuals who experienced ART failure from April 2018 to September 2019. All the samples were collected through the routine report and then genotyped at Liaoning Provincial Center for Disease Prevention and Control. Samples were obtained from 14 cities in Liaoning Province. The local centers for disease control and prevention (CDC) were responsible for the collection of plasma specimens and transporting them to Liaoning Provincial Center for Disease Prevention and Control. And then the virus load (VL) testing and drug resistance testing were performed in the Liaoning Provincial AIDS Confirmatory Laboratory. All the testing results were uploaded to the HIV/AIDS integrated prevention and control data information management system of China. This system contained the demographic information, the self-reported transmission route, antiretroviral therapy information and HIV testing results and so on. The basic information of participants in this study was derived from this system. Samples with VL≥1000 copies/mL after 12 months of ART were defined as ART-failure individuals. The ART-failure individuals confirmed between April 2018 and September 2019 were enrolled in the

study. The inclusion criteria were: 1) HIV-infected; 2) a minimum of 18 years of age; 3) on ART more than 12 months; 4) a plasma HIV RNA≥1000 copies/ mL ;5) consent and willingness to participate in the study.

Laboratory analysis

The virus load testing and drug resistance testing were performed at the Liaoning Provincial AIDS Confirmatory Laboratory. The plasma HIV-1 virus load was tested by using the COBAS Ampliprep TaqMan 96 (Roche) detection system. And the lowest detection limit value was 20 copies / mL. Samples with VL≥1000 copies/mL were selected for HIV drug resistance testing by using an in-house method developed in core laboratories in China. The HIV-1 RNA was extracted from the plasma using the QIAamp Viral RNA Mini Kit. The 1,300 bp of pol protease region (PR) and transcriptase region (RT) gene regions were amplified by nested PCR. And the amplified regions included the full length of PR (amino acids1-99) and RT region (amino acids 1-300).

All the primers, PCR reaction system and amplifying condition was described in previous studies [14, 15]. The resulting sequences were spliced together by the software (Chromaspro, Conting Express, BioEdit) and analyzed for drug resistance mutations by Stanford HIV Drug Resistance Database (http://hivdb.stanford.edu/). And the Stanford HIV Resistance Database will give the corresponding resistance levels and resistance mutation sites for various antiviral drugs.

The COMET HIV-1 software (https://comet.lih.lu /) and HIV BLAST tool were used for genotyping.

In this study, we included mutation results conferred low-level, intermediate-level and high-level resistance [16]. All experimental protocols were carried out in accordance with the manufacturer's instructions, including any relevant details.

Statistical analysis

The database was established by excel, and SPSS 24.0 (IBM Corp., Armonk, NY, USA) software was used for statistical analysis. The demographic variables were analyzed using descriptive statistics. Logistic regression model was used to analyze the risk factors of drug resistance in ART-failure individuals. A stepwise approach was used for variable selection in the multivariate regression model. Factors with $p<0.05$ in the univariate models were included in the multivariate logistic regression model, and a two-sided $p<0.05$ was defined as statistically significant.

Results

Basic characteristics of the participants

We collected a total of 468 ART-failure individuals who met the inclusion criteria through routine reporting, and the pol gene sequence in the plasma sample of 256 participants were successfully amplified and analyzed. Of these, 166 were considered drug resistant and 90 were drug sensitive

(Table 1). Of these,91.41% were men; the median age was 35.61 years (IQR:28.73-48.67); and more than half of the participants (55.86%) were unmarried, 28.13% were married or cohabiting, and 16.02% were divorce or

separation; the most common infection routes were mainly homosexual contact (56.25%) and heterosexual contact (30.86%).

Of 256 participants experiencing treatment failure, 46.88% were WHO stage I patients, followed by WHO stage IV patients (20.31%), WHO stage II patients (18.36%), and WHO stage III patients (14.45%); the median time of ART was 33.15 months (IQR:25.41-49.83); the initial ART regimen for most patients was 3TC+TDF+EFV (82.03%); the current ART regimen with a large proportion were LPV/r+3TC+AZT (33.98%), EFV+3TC+TDF (29.69%) and LPV/r+3TC+TDF (23.44%), respectively. The median viral load was 36205 copies/ml (IQR:11234-96047).

Genetic subtypes

Among the 256 successfully amplified samples, CRF01_AE (77.73%) was the predominant genotype, followed by subtype B (9.77%), unique recombinant forms (URFs) (5.08%), CRF07_BC (3.52%), subtype A (1.95%), subtype C (1.56%), and CRF08_BC (0.39%) (Figure 1). In addition, the two URFs recombination patterns were CRF01_AE/B and BC. The most common subtype for these infected by MSM was CRF01_AE, followed by subtype B, and for those infected by heterosexual contact, the most common subtype was also CRF01_AE, followed by URFs (Table 2).

Table 2 Prevalence of genotypes among ART-failure patients infected by different route of transmissions

Routes of infection	CRF01_AE	B	CRF07_BC	A	C	CRF08_BC	URFs	Total
MSM	114(79.17)	15(10.42)	5(3.47)	1(0.69)	3(2.08)	0(0.00)	6(4.16)	144
Heterosexual	61(77.22)	5(6.33)	2(2.53)	3(3.80)	1(1.27)	1(1.27)	6(7.59)	79
Others	24(72.73)	5(15.15)	2(6.06)	1(3.03)	0(0.00)	0(0.00)	1(3.03)	33
Total	199(77.73)	25(9.77)	9(3.52)	5(1.95)	4()1.56	1(0.39)	13(5.08)	256

HIV-1 drug resistance analysis

Of the 256 ART-failure cases, 64.84% had drug resistance to any of the three classes of antiretroviral drugs; 50.39% were dual resistant to NRTIs and NNRTIs; and the percentages of resistance to NNRTIs, NRTIs, and PIs was 62.89%,50.39% and 3.13%, respectively (Figure 2). The percentages of resistance to ABC, 3TC, FTC, ddI, d4T, and TDF drugs were 50.39%, 49.22%, 49.22%, 43.36%, 37.11%, and 36.33%, respectively. Only 0.39% showed drug resistance to AZT. The percentage of resistance to NNRTIs was high. 62.89% of participants showed resistance to EFV and NVP;50.00% showed resistance to RPV; and 38.28% showed resistance to ETR. The resistance rate to the only PIs drug (NFV) was 3.13% (Figure 3).

The percentage of drug resistance associated mutations in NNRTIs, NRTIs, and PIs among 256 ART-failure patients are listed as follows (Table 3). The most common mutations associated with drug resistance to NNRTIs were G190S (31.25%), Y181C (25.78%), K101E (17.19%), V179D (15.23%), and K103N (12.89%). As well, K65R (29.69%), M184V (28.52%), and Y115F (10.55%) were found as the most frequent mutations to NRTIs. There were only three drug resistance associated mutations to PIs, which were M46I (3.52%), L33F (1.95%) and K20KT (0.39%), respectively.

Factors Associated with Drug Resistance

Ten potential risk factors were considered in the univariate logistic regression analysis (Table 4). Of these factors, duration of ART, current ART regimen, viral load, gene subtype, and WHO clinical stages significantly were correlated with drug resistance (p□0.05). To identify the risk factors associated with HIV-1 drug resistance, these five statistically significant factors were included in the multivariate logistic regression model. Two factors were found to be significantly associated with the developing of HIV-1 DR in ART-failure participants: current ART regimen

(compared to 3TC +TDF+ EFV regimen: the other regimen AOR 0.99,95% CI 0.40-2.45; LPV/r+3TC+TDF regimen AOR 0.17,95% CI 0.07 -0.40; EFV+3TC+TDF regimen AOR 0.50,95% CI 0.18-1.37;), and viral load (compared to VL□ 80000:VL 0-40000 AOR 18.34, 95% CI 3.42- 98.40; VL 40001-80000 AOR 2.09, 95% CI 0.57-7.68).

Discussion

In this study, we find that the most common infection route was sexual transmission, accounting for 87.11%, of which MSM accounted for 56.25% of transmission. A national systematic review showed that the HIV prevalence in MSM increased from 1.77% in 2000 to 5.98% in 2010 [17]. Actually, the transmission route of HIV/AIDS in China has undergone great changes since 2007: the main transmission route has changed from the injection drug use route to the sexual transmission route, and the proportion of MSM transmission increased significantly [18] [12]. MSM has a higher risk of HIV infection due to their multiple sexual partners and unprotected anal intercourse behavior [19]. Furthermore, study showed that only 20% of MSM used condoms with their wives [20], which increased the risk of HIV acquisition among their female partners. Therefore, comprehensive intervention should be expanded and taken to reduce the prevalence of HIV among MSM, which would be beneficial to both MSM groups and their female groups. The most dominance subtype in our study was CRF01_AE (77.73%). CRF01_AE has been the absolutely dominant genotype in Northeast China in recent years (≥60%) [21], suggesting that the prevalence of CRF01_AE was relatively stable in this region. Moreover, the distribution of HIV-1 strain was closely related to the route of transmission. Here we found that the most common subtype among the ART failure who were infected by MSM and heterosexual was CRF01_AE. Since the first CRF01_AE was detected among commercial sex workers of Yunnan province of China in 1994 [22], the prevalence of CRF01_AE had increased from 9.6% in 1998 to 42.5% in 2016 [23]. Study showed that there were four major clusters of HIV-1 CRF01_AE strains caused a real epidemic in China, while cluster 4 was epidemic among homosexuals in Jilin and Liaoning

province of China[22]. Importantly, it was reported that the high proportion of CXCR4 usage for CRF01_AE strains may result in the loss of susceptibility to maraviroc (CCR5 antagonists) [24], which would reduce antiretroviral regimen options for ART-failure patients..

The prevalence of HIVDR among ART-failure individuals in this study was much higher than the results of previous studies among other HIV-infected populations [25, 26]. It suggested that failure of antiretroviral therapy was closely related to the occurrence of HIVDR, and should be paid more attention on clinical treatment. The prevalence of resistance to NNRTIs was high, which was 62.89% for efavirenz and nevirapine and 50.00% for rilpivirine. The high prevalence of DR in NNRTIs may related to widespread use of efavirenz in the initial regimen in this study. Of concern, 50.39% participants in our study showed dual-class resistance (NNRTIs and NRTIs), which was similar to the patterns of studies reported from other studies from low and middle-income countries [27-29]. In addition, we noted that the prevalence of resistance to PIs was significantly lower than that of NNRTIs and NRTIs. Several studies have found that mutations in gag protein cleavage sites and other sites may itself contribute to PIs resistance [30-32], while this study we only concentrated on the pol gene, the prevalence of PIs resistance may be underestimated.

K65R and M184V were the most prevalent NRTIs mutations identified in this study. M184V, the most common discriminatory mutations (DAM), was selected by 3TC and FTC and could reduce susceptibility to these drugs >100-fold[33]. The mutation also caused low-level resistance to ABC and ddI [34, 35], and increased susceptibility to AZT, d4T and TDF [36-38]. K65R was known to reduce susceptibility to TDF, ABC, d4T, ddI, of note, a retrospective study showed that patients who developed ART failure on a TDF-containing regimen were at a somewhat higher risk of developing K65R if they were infected with a subtype C virus [39]. Although the prevalence of subtype C was lower in our study, the more importance should be attached to the drug resistance associated with K65R in the high prevalence of subtype C region. The major NNRTIs mutations identified in our study were G190S, Y181C andK101E.G190S reduced susceptibility to NVP and EFV [40]. Y181C was an uncommon nonpolymorphic mutation selected by NVP and EFV. It caused high-level resistance to NVP and EFV.K101E usually occurred in combination with other NNRTIs-resistance mutations. All the mutations were in accordance with the two NNRTIs drugs selected in our first-line therapy regimen. The frequencies of major or minor DRM of PIs were very low in our study.M46I were associated with decreased susceptibility to ATV, FPV, IDV, LPV and NFV. In this study, only 3.13% of the participants showed drug resistance to NFV. Fortunately, no one showed drug resistance to LPV/r, which was the core component in our second-line regimens.

Our study revealed that viral load and current ART regimen were associated with drug resistance among participants experiencing treatment failure. First, compared with the higher VL (>80,000 copies/ml), the lower VL has a higher risk of developing HIV-1 DR, which was consistent with the study in Yunnan province [41]. Some studies had proved that compared with the wild-type HIV virus, the HIV resistant strains had poor replication adaptability during a course of viral DNA synthesis [42] [43]. Therefore, some patients may have a high viral load without resistance. Second, the individuals using LPV/r+3TC+TDF in current ART regimen had a lower risk for developing drug resistance compared with those using EFV+3TC+TDF regimen. It was recommended that the medical staff should make timely judgments and change to the second-line treatment regimen to reduce the risk of DR when drug resistance occurred in patients with first-line regimen. Although the rate of DR to PIs was relatively low in this study, the second-line regimens DR surveillance should attract more attention with the increasing use of the second-line regimen.

Limitations

We also acknowledge there are several limitations in our study. First, of the 468 ART-failure participants, only 256 were successfully sequenced, and 212 individuals didn't have sequence analysis data, meaning sample size was relatively small. The HIVDR rate in this study may

not better represent the true drug resistance rate. Second, due to the lack of baseline HIVDR data, we are unable to determine whether the mutant variants pre-existed in the patients. Third, good medication adherence can reduce the occurrence of drug resistance, which is critical for people living with HIV. Regrettably, due to some objective reasons, there is no discussion on adherence in our study.

Conclusion

This study aims to illustrate the prevalence of HIV-1 DR of ART-failure individuals in Liaoning Province, China. The genotype distribution of patients experiencing treatment failure is diverse, with CRF01_AE as the predominant genotype. We observe that these patients show high resistance to NRTIs and NNRTIs, meanwhile, there are multiple DR mutation sites in NRTIs and NNRTIs. In the end, the prevalence of HIV-1 DR in the ART-failures is high. Long-term and ongoing surveillance of HIV DR is necessary, which will help us to better grasp the prevalence of resistance and guide the clinical treatment.

Abbreviations

AIDS: The acquired immunodeficiency syndrome; HIV: Human immunodeficiency virus;

HAART: The highly active antiretroviral therapy; ART: Antiretroviral therapy; HIVDR: HIV drug resistance; TDR: Transmitted HIV drug resistance; CDC: Centers for disease control and prevention; VL: Virus load; NRTIs: Nucleoside reverse transcriptase inhibitors; NNRTIs: Non-nucleoside reverse transcriptase inhibitors; PIs: Protease inhibitors; IQR: Interquartile range; 3TC: Lamivudine; EFV: Efavirenz; TDF: Tenofovir; AZT: Zidovudine; NVP: Nevirapine; LPV/r: Lopinavir + Ritonavir; ABC: Abacavir; FTC: Emtricitabine; ddI: Didanosine; d4T: Stavudine; ETR: Etravirine; RPV: Rilpivirine; MSM: Men who have sex with men; URFs: Unique recombinant forms; OR: Odds ratio; CI: Confidence interval.

Declarations

Acknowledgments

Our study was supported by the Natural Science Fund of Liaoning Province (No.20180550715). The funders had no role in study design, data collection and analysis, decision to publish, and preparation of the manuscript. The authors thank the staff of local Centers for Disease Control and Prevention in Liaoning Province for the help of data input in this study.

Ethics approval and consent to participate

Each of the participants in the study signed an informed consent prior to the enrollment. This study was approved by the Ethics Committee of Liaoning Provincial Center for Disease Control and Prevention (ethical approval number: 2020-006).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

Our study was supported by the Natural Science Fund of Liaoning Province (No.20180550715). The funders had no role in study design, data collection and analysis, decision to publish, and preparation of the manuscript.

Authors' contributions

QW, LW, SP and SY conceived of the study, participated in the design and coordination. YZ, XK and YL performed the experiments. YZ, XK, SP and SY collected the data. QW analyzed the data and drafted the manuscript. LW and YL reviewed this manuscript. All authors read and approved the final manuscript.

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Tables

Table 1 Basic characteristics among ART-failure patients in Liaoning Province

Variables	Number	Percentage(n%)
Total	256	
Age(years)		
19~38	154	60.16
39~58	75	29.30
59~78	27	10.55
Median (IQR)	35.61(28.73-48.67)	
Gender		
Male	234	91.41%
Female	22	8.59%
Marital status		
Unmarried	143	55.86%
Married or cohabiting	72	28.13%
Divorce or separation	41	16.02%
Routes of infection		
MSM	144	56.25%
Heterosexual	79	30.86%
Others	33	12.89%
Duration of ART (months)		
13-24	54	21.09%
25-36	98	38.28%
37-48	35	13.67%
49-60	17	6.64%
≥61	52	20.31%
Median (IQR)	33.15(25.41-49.83	
Initial ART regimen		
3TC+TDF+EFV	210	82.03%
3TC+AZT+NVP	20	7.81%
AZT + 3TC + EFV	9	3.52%
Others	17	6.64%
Current ART regimen		
LPV/r+3TC+AZT	87	33.98%

EFV+3TC+TDF	76	29.69%
LPV/r+3TC+TDF	60	23.44%
3TC+AZT+NVP	8	3.13%
AZT + 3TC + EFV	7	2.73%
Others	18	7.03%
Viral load(copies/ml）		
1000-40000	132	51.56%
40001-80000	49	19.14%
＞80000	75	29.30%
Median (IQR)	36205(11234-96047)	
WHO clinical stages		
I	120	46.88%
II	47	18.36%
III	37	14.45%
IV	52	20.31%

Table 2 Prevalence of genotypes among ART-failure patients infected by different route of transmissions

Routes of infection	CRF01_AE	B	CRF07_BC	A	C	CRF08_BC	URFs	Total
MSM	114(79.17)	15(10.42)	5(3.47)	1(0.69)	3(2.08)	0(0.00)	6(4.16)	144
Heterosexual	61(77.22)	5(6.33)	2(2.53)	3(3.80)	1(1.27)	1(1.27)	6(7.59)	79
Others	24(72.73)	5(15.15)	2(6.06)	1(3.03)	0(0.00)	0(0.00)	1(3.03)	33
Total	199(77.73)	25(9.77)	9(3.52)	5(1.95)	4()1.56	1(0.39)	13(5.08)	256

Table 3 Percentage of drug resistance associated mutations in NNRTIs, NRTIs, and PIs among ART-failure patients

NRTIS		NNRTIS		PIS	
Mutation	Frequency(n%)	Mutation	Frequency(n%)	Mutation	Frequency(n%)
K65R	76(29.69)	G190S	80(31.25)	M46I	9(3.52)
M184V	73(28.52)	Y181C	66(25.78)	L33F	5(1.95)
Y115F	27(10.55)	K101E	44(17.19)	K20KT	1(0.39)
A62V	14(5.47)	V179D	39(15.23)		
K70E	12(4.69)	K103N	33(12.89)		
M184I	11(4.30)	V106 M	22(8.59)		
D67N	10(3.91)	F227L	8(3.13)		
K219E	10(3.91)	G190A	7(2.73)		
D67G	6(2.34)	V179E	7(2.73)		
L74I	6(2.34)	P225H	7(2.73)		
V75I	6(2.34)	V108I	7(2.73)		
V75M	6(2.34)	G190C	6(2.34)		
K219N	4(1.56)	V179T	4(1.56)		
K219R	4(1.56)	Y188L	4(1.56)		
L74V	3(1.17)	A98G	4(1.56)		
K219Q	2(0.78)	V106I	3(1.17)		
K65KR	1(0.39)	Y188C	3(1.17)		
K219KE	1(0.39)	H221Y	3(1.17)		
K70KT	1(0.39)	G190E	2(0.78)		
K70Q	1(0.39)	V106A	2(0.78)		
L74LI	1(0.39)	H221HY	2(0.78)		
M41L	1(0.39)	E138K	2(0.78)		
M184MI	1(0.39)	M230L	2(0.78)		
A62AV	1(0.39)	G190GA	1(0.39)		
Y115TF	1(0.39)	Y181V	1(0.39)		
D67DN	1(0.39)	Y181YC	1(0.39)		
D67GISV	1(0.39)	V179VAIT	1(0.39)		
K70KQ	1(0.39)	V179VF	1(0.39)		
		K101HQ	1(0.39)		
		K103H	1(0.39)		

	V106VI	1(0.39)
	E138A	1(0.39)
	E138Q	1(0.39)
	M230ML	1(0.39)
	M230I	1(0.39)
	L100I	1(0.39)
	D190S	1(0.39)

Table 4 The factors associated with drug resistance among ART-failure individuals

Variables	Virologic failure	Drug resistance (n%)	Crude OR （95%CI）	p^1	Adjusted OR （95%CI）	p^2
Total	256	166(64.84)				
Gender						
Male	22	11(50.00)	1.96(0.82-4.72)	0.13		
Female	234	155(66.24)				
Age(years)						
19~	154	151(98.05)	0.81(0.34-1.91)	0.62		
39~	75	15(20.00)	1.21(0.47-3.09）	0.70		
59~	27	0(0.00)				
Marital status						
Unmarried	143	86(60.14)	0.70(0.34-1.47)	0.35		
Married or cohabiting	72	52(72.22)	1.207(0.52-2.79)	0.66		
Divorce or separation	41	28(68.29)				
Routes of infection						
MSM	144	96(66.67)	1.47(0.68-3.19)	0.33		
Heterosexual	79	51(64.56)	1.34(0.59-3.08)	0.49		
Others	33	19(57.58)				
Duration of ART (months)						
13-	152	115(75.66)	3.48(1.80-6.73)	0.01	0.22(0.04-1.29)	0.09
37-	52	26(50.00)	1.00(0.46-2.16)	1.00	0.79(0.24-2.55)	0.69
≥61	52	25(48.08)				
Initial ART regimen						
3TC+TDF+EFV	210	144(68.57)	1.56(0.68-3.59)	0.29		
3TC+AZT+NVP	20	8(40.00)	0.49(0.15-	0.24		

			1.60)			
Others	26	14(53.85)				
Current ART regimen						
LPV/r+3TC+AZT	87	77(88.51)	1.48(0.67-3.28)	0.34	0.99(0.40-2.45)	0.99
LPV/r+3TC+TDF	76	30(39.47)	0.22(0.10-0.46)	0.01	0.17(0.07-0.40)	0.01
Others	33	14(42.42)	0.51(0.21-1.28)	0.15	0.50(0.18-1.37)	0.18
3TC +TDF+ EFV	60	45(75.00)				
Viral load						
0-40000	132	77(58.33)	5.48(2.93-10.26)	0.01	18.34(3.42-98.40)	0.01
40001-80000	49	34(69.39)	1.79(0.87-3.71)	0.12	2.09(0.57-7.68)	0.27
80000	75	55(73.33)				
Gene subtype						
CRF01_AE	199	136(68.34)	2.45(1.15-5.21)	0.02	1.94(0.78-4.84)	0.15
B	25	15(60.00)	1.70(0.59-4.90)	0.33	1.75(0.51-6.06)	0.38
Others	32	15(46.88)				
WHO clinical stages						
I	120	66(55.00)	0.19(0.08-0.46)	0.01	0.37(0.14-0.99)	0.47
II	47	28(59.57)	0.23(0.09-0.62)	0.01	0.53(0.17-1.68)	0.28
III	37	27(72.97)	0.42(0.14-1.23	0.12	0.46(0.14-1.57)	0.22
IV	52	45(86.54)				

Notes: [1]Univariate analysis; [2]Multivariate analysis.

P-Values are statistically significant at the 0.05 significance level

Figures

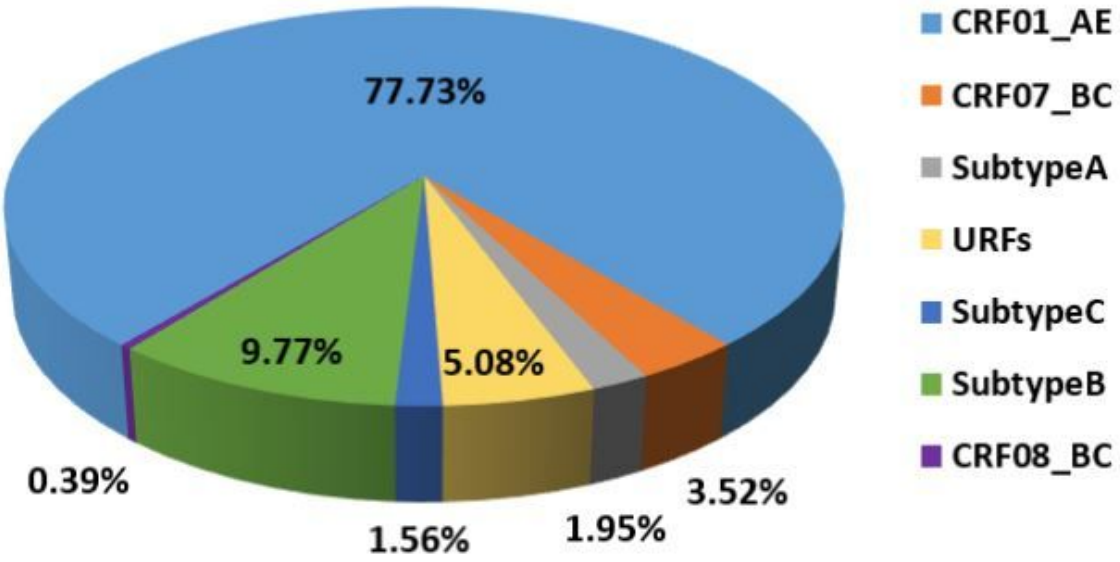

Figure 1

Distribution of HIV-1 genotypes among ART-failure patients in Liaoning Province.

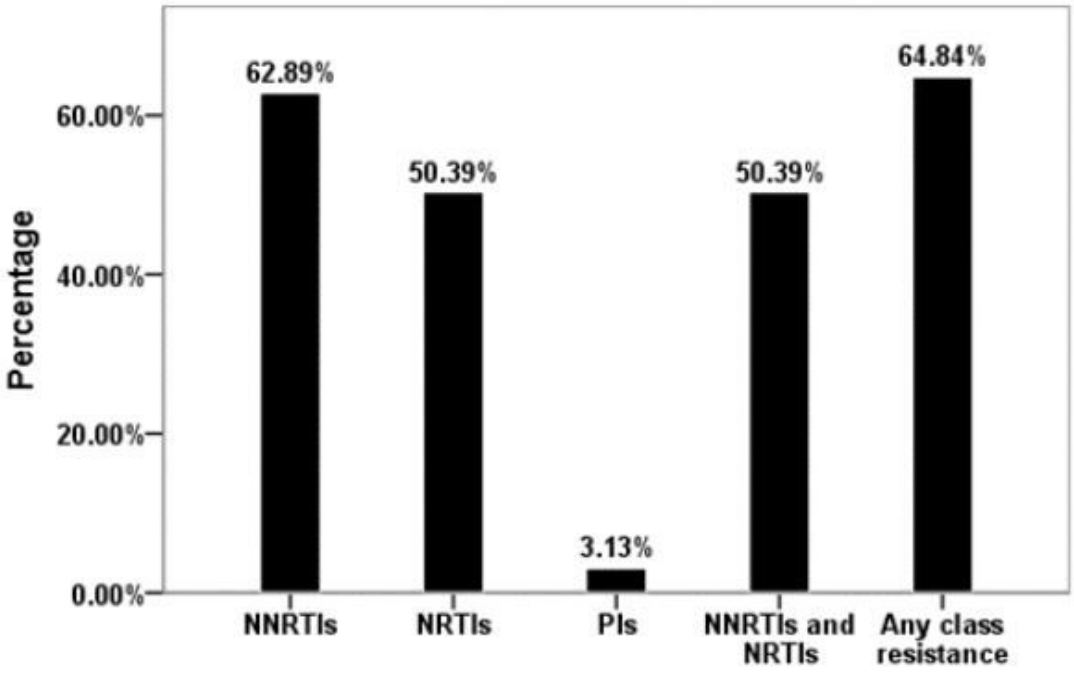

Figure 2

The proportion of drug resistance in ART-failure individuals according to drug classes.

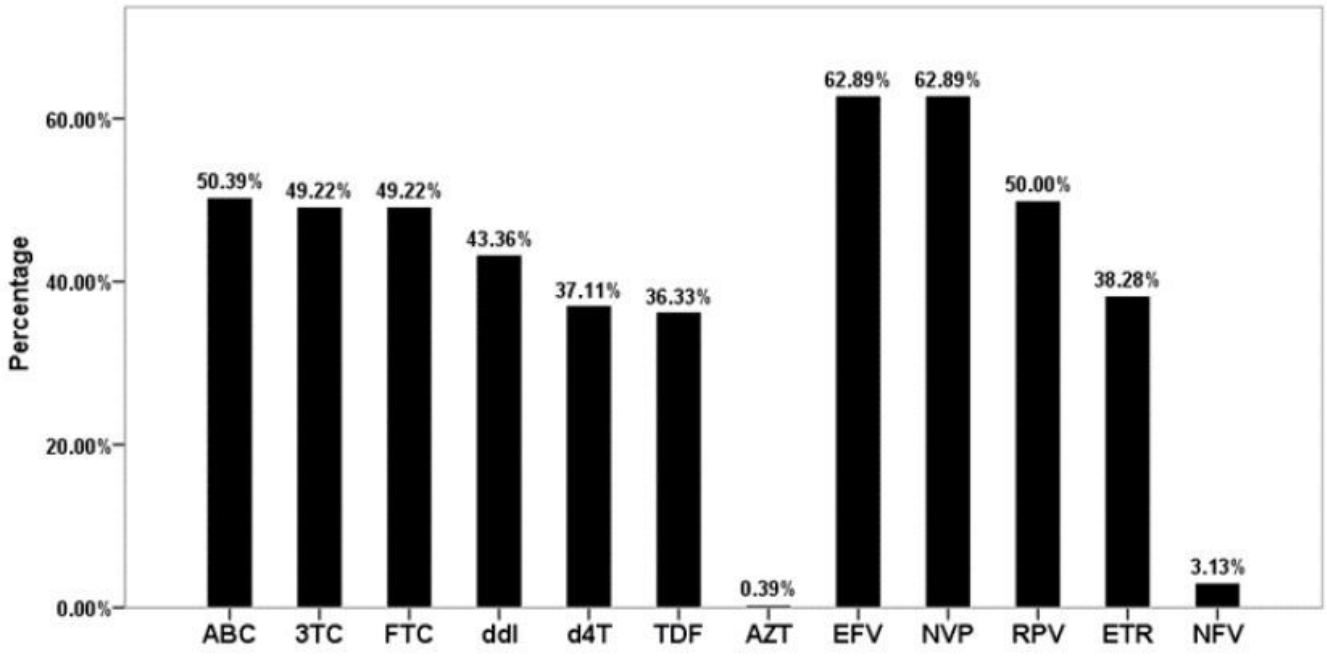

Figure 3

Percentage of resistance to three classes of antiretroviral drugs among ART-failure patients.