Infection and Drug Resistance

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ORIGINAL RESEARCH

Prevalence of HIV Transmitted Drug Resistance in Nanjing from 2018 to 2021

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Background: Transmitted drug resistance (TDR) is a major challenge in the clinical management of acquired immunodeficiency syndrome (AIDS). Therefore, this study aimed to investigate the epidemic characteristics of and risk factors for human immunodeficiency virus (HIV)-1 TDR in Nanjing from 2018 to 2021 to provide support for clinical management.

Methods: The HIV-1 Pol gene was amplified by nested reverse transcription polymerase chain reaction from venous blood of 1190 HIV-infected patients who did not receive antiviral therapy, and the amplified product was sequenced using an in-house sequencing method. The sequencing result was compared with the HIV drug resistance database from Stanford University to elucidate the rates of antiviral drug resistance and distribution of drug-resistant mutation sites. Factors associated with TDR were evaluated using a logistic regression model.

Results: Detection of drug resistance at the gene level was successful in 1138 of 1190 HIV-1-infected patients (95.6%), and the overall 4-year drug resistance rate was 8.2% (93/1138). The drug resistance rate was higher for non-nucleoside reverse transcriptase inhibitors (NNRTIs; 6.7%) than for nucleoside reverse transcriptase inhibitors (NRTIs; 2.5%) or protease inhibitors (PIs; 0.1%) (χ^2 = 83.907, P<0.0001). The most common NNRTI-related mutation was V179D/E followed by K103N. M184V was the dominant NRTI-associated mutation, and M46L/I was the most prevalent PI-associated mutation. A $CD4^+$ T cell count of <50 cells/μL was significantly associated with an increased risk of TDR (OR=3.62, 95% CI: 1.38–9.51, P=0.009).

Conclusion: The prevalence of TDR in the city of Nanjing from 2018 to 2021 was at a moderate epidemic risk according to World Health Organization standards. Continuous monitoring of TDR can inform clinical diagnosis and treatment. Patients with advanced disease and a low $CD4^+$ T lymphocyte count are more likely to have TDR in Nanjing.

Keywords: HIV-1, transmitted drug resistance, mutation sites

Introduction

Since the first case of acquired immunodeficiency syndrome (AIDS) was discovered in the United States in 1981, AIDS has spread around the world and become a serious public health concern. The Joint United Nations Program on HIV/AIDS (UNAIDS) estimated that there were 38.4 million people were living with HIV, 1.5 million new cases, and about 650,000 deaths from HIV-related diseases in 2021.[1] Presently, antiretroviral therapy (ART) is the main method of treatment for AIDS. The preferred regimen is two nucleoside reverse transcriptase inhibitors (NRTIs) plus one non-nucleoside reverse transcriptase inhibitor (NNRTI)/protease inhibitor (PI)/integrase inhibitor (INSTI).[2] The recommended regimens in China were tenofovir plus lamivudine or emtricitabine as NRTIs, efavirenz or ripiverin as NNRTIs, ripiverin/ritonavir as PIs and dolutegravir or raltegravir as INSTIs.[2]

The National Free Antiretroviral Treatment Program (NFATP) was launched in six provinces in central China in 2003 and subsequently expanded to 31 provinces and autonomous regions in 2006, improving the access of patients living with HIV to antiretroviral treatment.[3,4] Previous research showed that the use of ART in China reduced AIDS-related deaths from 39.3% in 2002 to 14.2% in 2009.[5] However, the continuous use of ART in China has led to an increase in the drug resistance rate in

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newly-treated patients living with HIV,[6] especially the resistance to NNRTIs.[7] A nationwide cross-sectional survey conducted in 2015 found that the average prevalence of transmitted drug resistance (TDR) was 3.7%,[8] and a significant increase in TDR in different regions was reported by other studies during subsequent years.[9,10] The presence of drug resistance in patients before treatment results in a reduced viral suppression rate, impaired immune recovery, further accumulation of drug resistance, and increases in the number of new HIV infections and risk of death.[11,12] Furthermore, resistant mutations in pregnant women need prompt diagnosis and treatment because they can be passed from mother to child if the mother is not virologically suppressed, which complicates management of the infant.[13] Therefore, close monitoring and early detection of TDR in ART initiators is crucial to avoid antiviral treatment failure and other negative effects. Additionally, a prior study found that the changes in HIV drug resistance rates over time varied between different regions.[14] Previous investigations reported that the prevalence of TDR was 6.0% in sub-Saharan Africa,[15] 7.8% in Greece,[16] 10.7% in Hungary,[17] and 14.0–17.5% in the United States.[18,19] However, the prevalence of TDR in the city of Nanjing in recent years is unknown. Therefore, this study aimed to estimate the prevalence of TDR in Nanjing during 2018–2021 and elucidate the factors associated with TDR to provide up-to-date information that may help support clinical decision making.

Methods

Study Design and Participants

This study included outpatients attending the Second Hospital of Nanjing from January 2018 to December 2021. The inclusion criteria were: (i) outpatient attending the Second Hospital of Nanjing; (ii) HIV infection confirmed by an HIV antibody test; (iii) had not received any previous antiviral treatment; and (iv) genotypic drug resistance was detected before treatment. Baseline data such as sex, age, marital status, route of transmission, $CD4^+$ T lymphocyte count, and viral load (HIV ribonucleic acid [RNA] level) were collected from the patients who were included in the study. All patients provided written informed consent, and the study was approved by the Medical Ethics Committee of the Second Hospital of Nanjing (approval number: 2018-LY-kt027; approval date: 9 May, 2018). The study was conducted in accordance with the Declaration of Helsinki.

Detection of HIV Genotype Resistance

A 10-mL sample of peripheral blood was collected from the HIV-positive patients, and plasma was separated by centrifugation. Viral load was evaluated using a commercial kit (COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test version 2.0; Roche Diagnostics International, Risch-Rotkreuz, Switzerland). HIV-1 RNA was extracted using an automated nucleic acid extraction system (Shuoshi Company, Taizhou, Jiangsu, China). Using the complete genome (9719 bp) of the international standard strain of HXB2 as a reference, PCR was used to amplify the full length of the protease gene (codons 1–99) and the first 300 amino acids (codons 1–300) of the reverse transcriptase gene. The primers used for the first round of PCR were: lateral upstream primer, F1a:5′-TGAARGAITGYACTGARAGRCAGGCTAAT-3′ and F1b:5′-ACTGARAGRCAGGCTAATTTTTTAG-3′; lateral downstream primer, RT-R1: 5′-ATCCCTGCATAAATCTGACTTGC-3′. The primers used for the second round of PCR were: medial upstream primer, PRT-F2: 5′-CTTTARCTTCCCTCARATCACTCT-3′, medial downstream primer, RT-R2: 5′-CTTCTGTATGTCATTGACAGTCC-3′. The reaction conditions for the first round of PCR were: 50°C for 45 min; 94°C for 2 min; 50 cycles of 94°C for 15s, 55°C for 20s and 72°C for 2 min; 72°C for 10 min; and holding temperature of 4°C. The reaction conditions for the second round of PCR were: 94°C for 4 min; 40 cycles of 94°C for 15s, 55°C for 20s and 72°C for 2 min; 72°C for 10 min; and holding temperature of 4°C. The amplified products were sequenced using an in-house method based on traditional Sanger sequencing,[20] and the sequences were corrected and spliced using CExpress software. The resulting full sequences were analyzed using the Stanford HIVDB software provided on the Stanford University HIV Drug Resistance Database (http://HIVDB.stanford.edu/) to confirm the genotyping and to identify the drug resistance mutations. The resistance level of HIV-1 to each antiviral drug was divided into five grades according to the scoring criteria from the Stanford website: sensitive, potentially low resistance, low resistance, moderate resistance, and high resistance. In this study, patients with low, medium or high drug resistance (according to the Stanford website) were classified as having TDR, in accordance with the World Health Organization's (WHO's) HIV Drug Resistance Report 2021.[21]

Statistical Analysis

The data were analyzed using SPSS 26.0 (IBM Corp., Armonk, NY, USA). Quantitative data are described as absolute numbers and rates. Univariate and multivariate logistic regression analyses were used to identify factors associated with HIV-1 TDR, and the χ^2 test was used to analyze drug resistance. A P-value <0.05 was considered statistically significant.

Results

Demographic Characteristics

From 2018 to 2021, a total of 2151 HIV-infected outpatients attended the Department of Infectious Diseases of the Second Hospital of Nanjing and started antiretroviral therapy. The genotypic resistance testing rates in these 2151 patients during 2018, 2019, 2020 and 2021 were 8.5%, 46.1%, 74.7%, and 83.7%, respectively. In total, 1190 of the 2151 HIV-1-infected patients (55.3%) were tested for genotype resistance, and successful sequencing was achieved in 1138 of these 1190 cases (95.6%, 1138/1190). Therefore, the final analysis included 1138 HIV-infected patients (Table 1). The majority of the 1138 newly-treated patients

Table 1 Demographic Characteristics of 1138 Newly-Treated Patients Living with HIV Who Successfully Underwent Genotype Resistance Testing

	Total n=1138 (100)	Years				χ^2	P-value
		2018 n=38 (3.3%)	2019 n=237 (20.8%)	2020 n=414 (36.4%)	2021 n=449 (39.5%)		
Gender						3.600	0.308
Male	1065 (93.6%)	38 (100%)	220 (92.8%)	384 (92.8%)	423 (94.2%)		
Female	73 (6.4%)	0	17 (7.2%)	30 (7.2%)	26 (5.8%)		
Age (years)						10.278	0.328
<20	30 (2.6%)	1 (2.6%)	7 (3.0%)	10 (2.4%)	12 (2.7%)		
20–40	737 (64.8%)	28 (73.7%)	155 (65.4%)	248 (59.9%)	306 (68.2%)		
40–60	267 (23.5%)	8 (21.1%)	56 (23.6%)	110 (26.6%)	93 (20.7%)		
≥60	104 (9.1%)	1 (2.6%)	19 (8.0%)	46 (11.1%)	38 (8.5%)		
Route of transmission						37.228	<0.001
Homosexual transmission	780 (68.5%)	28 (73.7%)	155 (65.4%)	275 (66.4%)	322 (71.7%)		
Heterosexual transmission	268 (23.6%)	6 (15.8%)	52 (21.9%)	106 (25.6%)	104 (23.2%)		
IDU	9 (0.8%)	0	5 (2.1%)	2 (0.5%)	2 (0.4%)		
Others*	6 (0.5%)	2 (5.3%)	0	2 (0.5%)	2 (0.4%)		
Unknown	75 (6.6%)	2 (5.3%)	25 (10.5%)	29 (7.0%)	19 (4.2%)		
Marital status						22.381	0.008
Married/cohabiting	337 (29.6%)	8 (21.1%)	65 (27.4%)	147 (35.5%)	117 (26.1%)		
Single	652 (57.3%)	26 (68.4%)	131 (55.3%)	217 (52.4%)	278 (61.9%)		
Divorced/widowed/separated	115 (10.1%)	3 (7.9%)	35 (14.8%)	41 (9.9%)	36 (8.0%)		
Unknown	34 (3.0%)	1 (2.6%)	6 (2.5%)	9 (2.2%)	18 (4.0%)		
$CD4^+$ T lymphocyte count (cells/μL)						4.715	0.858

(*Continued*)

Table 1 (Continued).

	Total n=1138 (100)	Years				χ^2	P-value
		2018 n=38 (3.3%)	2019 n=237 (20.8%)	2020 n=414 (36.4%)	2021 n=449 (39.5%)		
<200	362 (31.8%)	11 (28.9%)	80 (33.8%)	136 (32.9%)	135 (30.1%)		
200–500	591 (51.9%)	23 (60.5%)	113 (47.7%)	212 (51.2%)	243 (54.1%)		
>500	178 (15.6%)	4 (10.5%)	42 (17.7%)	64 (15.5%)	68 (15.1%)		
Unknown	7 (0.6%)	0	2 (0.8%)	2 (0.5%)	3 (0.7%)		
HIV-RNA (copies/mL)						8.711	0.727
<1000	18 (1.6%)	0	3 (1.3%)	5 (1.2%)	10 (2.2%)		
1000–9999	169 (14.9%)	7 (18.4%)	29 (12.2%)	65 (15.7%)	68 (15.1%)		
10,000–100,000	605 (53.2%)	21 (55.3%)	127 (53.6%)	221 (53.4%)	239 (53.2%)		
>100,000	324 (28.5%)	10 (26.3%)	70 (29.5%)	118 (28.5%)	123 (27.4%)		
Unknown	22 (1.9%)	0	8 (3.4%)	5 (1.2%)	9 (2.0%)		

Note: *Others included transfusion-transmitted infection, mother-to-child transmission, and occupational exposure.
Abbreviation: IDUL, intravenous drug use.

with HIV-1 were male (93.6%). Most of the participants were aged 20–40 years-old (64.8%), with only 13 patients (1.1%) aged <18 years-old, and the median age was 31 years-old. The main route of transmission was homosexual transmission (68.5%), but there was an increase in the rate of heterosexual transmission over time (from 15.8% in 2018 to 23.2% in 2021). The median $CD4^+$ T lymphocyte count was 288 cells/μL, and the median HIV RNA (viral load) was 41,800 copies/mL.

TDR Prevalence

Among the 1138 patients who were tested for genotypic resistance, the overall TDR prevalence rate was 8.2% (93/1138), and the drug resistance rate was significantly higher for NNRTIs (6.7%, 76/1138) than for NRTIs (2.5%, 29/1138) or PIs (0.1%, 1/1138) (χ^2=83.907, P<0.0001). Resistance to both NNRTIs and NRTIs was observed in 1.1% (13/1138) of the patients, and simultaneous resistance to all three classes of drugs (NNRTIs, NRTIs and PIs) was not identified in this study population.

The TDR prevalence increased progressively from 2018 to 2020, reaching a peak of 9.4%, before falling in 2021 (Figure 1). Among the different types of antiviral drug, NNRTIs had the highest drug resistance rate in all four years

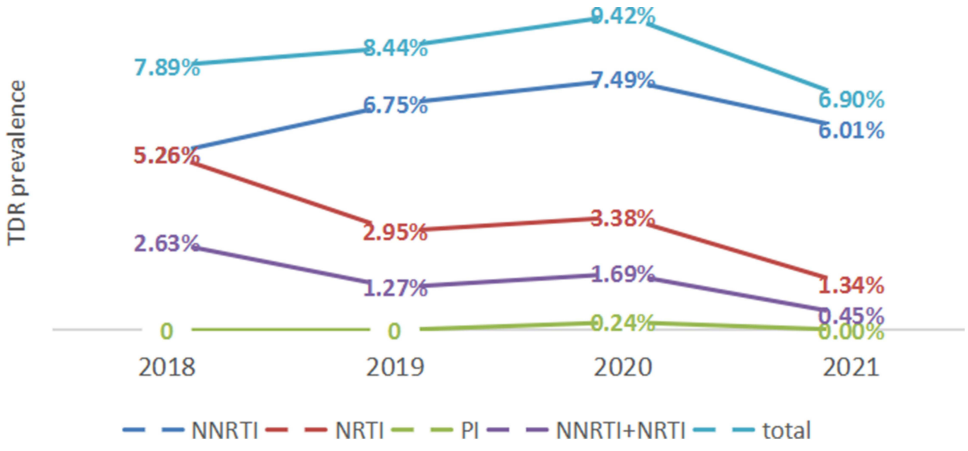

Figure 1 Epidemic trend of the different types of antiviral TDR from 2018 to 2021.

analyzed, reaching a peak prevalence of 7.49% in 2020 (Figure 1). The NRTI drug resistance rate fell during the four-year period and remained below 4% from 2019 to 2021 (Figure 1). PIs had the lowest drug resistance rates among the different types of antiviral drug (Figure 1).

Analyses of Drug Resistance Levels and Drug Resistance Mutation Sites

Drug resistance levels were evaluated for 13 commonly used antiviral drugs. These drugs included five NNRTIs, namely efavirenz (EFV), etravirine (ETR), nevirapine (NVP), ripiverin (RPV) and doravirine (DOR); five NRTIs, namely abacavir (ABC), zidovudine (AZT), emtricitabine (FTC), lamivudine (3TC) and tenofovir (TDF); and three PI drugs, namely atazanavir/ritonavir (ATV/r), denavir/ritonavir (DRV/r) and ripiverin/ritonavir (LPV/r).

The drug resistance level and TDR prevalence for each of these antiviral drugs are shown in Figure 2 and Table 2. EFV and NVP had the highest drug resistance rates among the NNRTI drugs. Of the 76 patients, 40.8% (31/76) developed resistance to the NNRTI class drugs, EFV, NVP, and RPV, and the resistance rates for these three agents were similar (χ^2=4.572, P=0.102). Relatively large proportions (89.8% and 82.8%) of patients had drug resistance to EFV and NVP at a medium to medium-high level, while 69.8% of patients exhibited resistance to RPV at a low level. ETR had the lowest drug resistance rate (1.49%, 17/1138) among the NNRTI drugs, and the resistance rate for ETR was significantly lower than that for NVP (5.6%, 64/1138) (P<0.0001). The overall drug resistance rate for NRTIs was lower than that for NNRTIs, with ABC having the highest drug resistance rate of 1.4% (16/1138) followed by AZT (1.2%, 14/1138) and FTC (1.1%, 13/1138). The drug resistance rate of 3TC, which is commonly used in Nanjing, was 1.1% (12/1138), and this would be considered medium-high drug resistance. Only one patient had high-level resistance to AZT, and the rest exhibited low-level resistance. Resistance to PIs was detected in only one patient in the study and manifested as both low resistance to ATV/r and moderate resistance to LPV/r. No DRV/r resistant strains were detected in this study.

Fifteen NRTI-related mutation sites, 14 NNRTI-related mutation sites, and 8 PI-related mutation sites were identified in this study (Figure 3). The drug-resistant mutation rate was 18.5% (210/1138), and the most common NNRTI-related mutation site was V179D/E with a mutation frequency of 7.8% (89/1138), followed by K103N (1.7%, 20/1138), V106I (1.5%, 17/1138), and G190A (0.6%, 7/1138). M184V (0.5%, 6/1138), D67DAG (0.4%, 5/1138) and K65R (0.4%, 5/1138) were the main NRTI-related mutations, while M46L/I (0.5%, 6/1138) was the main PI-related mutation.

Risk Factors Associated with TDR

Factors independently associated with HIV-1 TDR were evaluated using univariate and multivariate logistic regression analyses, using gender, age, marital status, route of transmission, $CD4^+$ T lymphocyte count and HIV RNA as

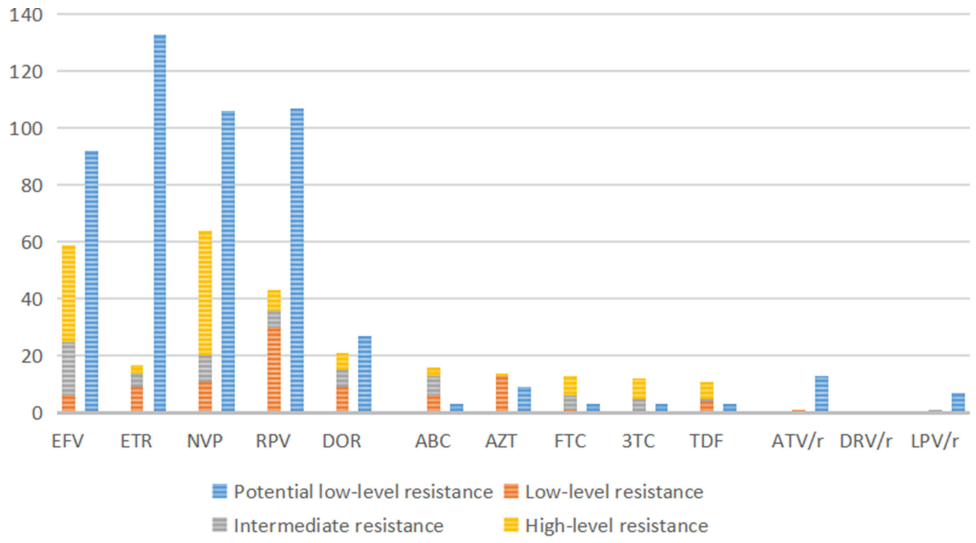

Figure 2 Frequency and degree of resistance of different antiviral drugs.

Table 2 Drug Resistance and TDR Prevalence for Different Antiviral Drugs

	EFV	ETR	NVP	RPV	DOR	ABC	AZT	FTC	3TC	TDF	ATV/r	DRV/r	LPV/r
Low-level resistance	6	9	11	30	9	6	13	1	0	4	1	0	0
Intermediate resistance	19	5	9	6	6	7	0	5	5	1	0	0	1
High-level resistance	34	3	44	7	6	3	1	7	7	6	0	0	0
Total	59	17	64	43	21	16	14	13	12	11	1	0	1
TDR prevalence	5.2%	1.5%	5.6%	3.8%	1.8%	1.4%	1.2%	1.1%	1.1%	1.0%	0.1%	0%	0.1%

independent variables. Gender, age, route of transmission, marital status and HIV RNA showed no significant differences among the groups. Patients with a $CD4^+$ T lymphocyte count <50 copies/mL were more likely to have TDR than patients with a $CD4^+$ T lymphocyte count ≥500 copies/mL (P=0.009) (Table 3).

Discussion

A total of 1138 newly-treated HIV patients with no prior history of ART who attended our institution during 2018–2021 were successfully genotyped for baseline drug resistance, and the overall 4-year drug resistance rate was 8.2%. The resistance rates in Nanjing were 7.9%, 8.4%, 9.4% and 6.9% in 2018, 2019, 2020 and 2021, respectively, which were higher than those in Jiangsu province during 2009–2011[22] (2.1% in 2009, 4.0% in 2010, and 4.1% in 2011). The resistance rate was also higher than those in other regions of China during the same period, such as 6.7% in Beijing,[23] 4.6% in Guangdong,[24] and 4.9% in Shenyang.[25] According to WHO guidelines, HIV drug resistance rates of <5%, 5–15% and >15% are considered low, medium, and high prevalence, respectively.[26] Therefore, the overall TDR prevalence rate of 8.2% in Nanjing would be considered moderate prevalence but is far higher than the overall TDR level in South China.[27] Since prior studies have reported that the prevalence of TDR differs between geographical regions and is increasing,[27] a likely reason for the higher rate in Nanjing than in other geographical regions is that our study evaluated TDR prevalence more recently than previously published investigations.

In this study, the drug resistance rate was highest for NNRTIs among the three types of antivirals. The trend in the drug resistance rate for NNRTIs between 2018 and 2021 was similar to that for the overall TDR rate, indicating that the

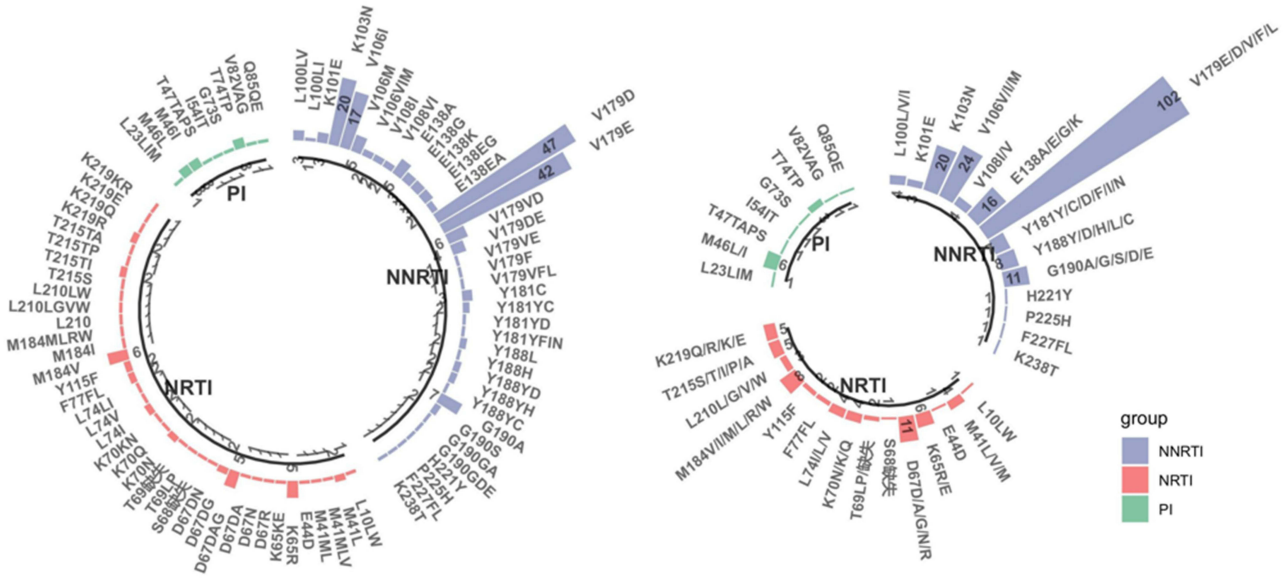

Figure 3 The drug-resistant mutation sites detected in newly-treated patients living with HIV.

Table 3 Analysis of the Factors Associated with HIV-1 Transmissible Drug Resistance

	Univariate			Multivariate		
	OR	**95% CI**	**P-value**	**OR**	**95% CI**	**P-value**
Gender						
Male	0.53	0.25–1.11	0.093	0.50	0.21–1.18	0.115
Female	1.00			1.00		
Age (years)						
<20	1.00			1.00		
20–29	1.06	0.21–4.68	0.936	0.95	0.21–4.25	0.949
30–39	1.06	0.23–4.87	0.938	0.93	0.19–4.47	0.926
40–49	1.91	0.41–8.92	0.409	1.41	0.26–7.57	0.687
50–59	1.49	0.31–7.07	0.619	1.08	0.19–6.09	0.929
≥60	1.85	0.39–8.84	0.440	1.73	0.31–9.74	0.534
Marital status						
Married/cohabiting	1.00			1.00		
Single	0.58	0.36–0.91	**0.018**	0.75	0.36–1.56	0.434
Divorced/widowed/separated	0.59	0.27–1.31	0.193	0.65	0.29–1.49	0.310
Unknown	0.25	0.03–1.90	0.180	0.23	0.03–1.81	0.161
Route of transmission						
Homosexual transmission	1.00			1.00		
Heterosexual transmission	1.09	0.70–1.44	0.969	0.73	0.40–1.33	0.301
IDU	<0.01	(<0.01->99.99)	0.999	<0.01	(<0.01->99.99)	0.999
Others*	<0.01	(<0.01->99.99)	0.999	<0.01	(<0.01->99.99)	0.999
Unknown	1.334	0.58–3.06	0.497	0.93	0.38–2.31	0.880
$CD4^+$ T lymphocyte count (cells/μL)						
<50	3.16	1.30–7.65	**0.011**	3.62	1.38–9.51	**0.009**
50–200	1.67	0.70–4.01	0.250	1.59	0.63–4.00	0.325
200–350	2.10	0.94–4.70	0.070	1.94	0.86–4.40	0.112
350–500	2.11	0.92–4.88	0.079	2.04	0.88–4.76	0.098
≥500	1.00			1.00		
HIV-RNA (copies/mL)						
<5000	1.00			1.00		
5000–10,000	1.57	0.48–5.15	0.458	1.21	0.36–4.05	0.755
10,000–50,000	1.99	0.76–5.18	0.161	1.65	0.62–4.38	0.316
50,000–100,000	1.62	0.56–4.66	0.369	1.16	0.39–3.45	0.794
≥100,000	1.66	0.62–4.47	0.316	0.98	0.34–2.87	0.977

Notes: *Others included transfusion-transmitted infection, mother-to-child transmission, and occupational exposure. Bold indicates statistically significant values.
Abbreviations: CI, confidence interval; IDU, intravenous drug use; OR, odds ratio.

prevalence of TDR was dominated by drug resistance to NNRTI drugs. The overall TDR prevalence and NNRTI resistance rate both exhibited a downward trend between 2020 and 2021. Since the decrease only occurred in 2021, it is impossible to judge whether the downward trend is a long-term phenomenon, and continuous monitoring of drug resistance in newly-treated patients living with HIV in this region should be recommended. Additionally, with the emergence of a new-generation integrase inhibitors such as dolutegravir (DTG), more patients are adopting INSTI-based antiviral regimens. The use of INSTI drugs in the Nanjing area has increased gradually in recent years (10.5% in 2018, 25.3% in 2019, 36.7% in 2020, and 54.4% in 2021 in this study population). DTG has a high resistance barrier and can reduce the transmission of HIV.[28] However, integrase-related drug resistance mutations were not evaluated in the present research, and this is one of the limitations of our study. As antiviral treatment regimens continue to improve, more attention should be paid to INSTI drug resistance in newly-treated patients.

NNRTIs have a low resistance barrier, and NNRTI drug resistance has exhibited a rising trend in Africa, Asia and other regions.[29] The WHO recommends switching first-line antiretroviral therapy from NNRTI-based antiviral regimens to non-NNRTI-based regimens if the NNRTI drug resistance rate is ≥10%.[30] Although the NNRTI resistance rate in Nanjing did not reach a high level, NNRTIs should be considered with caution when initiating treatment in this region given the currently limited selection of NNRTI drugs in China. The main NNRTI-related mutation site was V179D/E, which is consistent with previous survey results in southwest China.[31] The existence of this mutation site can lead to potential low resistance to multiple NNRTI drugs. The combination of V179D and K103R significantly reduces the sensitivity to EFV and NVP, which are widely used as first-line NNRTI drugs in Nanjing. The most common NRTI-related mutation in this study was M184V, which results in high resistance to 3TC and FTC and low resistance to ABC.[32] Although the resistance rate to 3TC, a commonly used NRTI drug in Nanjing, was only 1.1%, the most prevalent NRTI-related mutation sites in this region were M184V/I and K65R, which can reduce the susceptibility to 3TC. Therefore, NRTI-related mutations still need to be taken seriously. We detected few PI-related mutations, and the main mutation site identified was M46L/I, in agreement with survey results from Shanghai and Chongqing.[9,33] As PIs have a relatively high drug resistance barrier and are often used as second-line treatments in China,[34] the current drug resistance level is significantly lower for PIs than for NNRTIs or NRTIs. This suggests that non-NNRTI drugs, such as PI-based antiviral drugs, could be the preferred regimen when resistance to NNRTIs is experienced.

The drug-resistance mutation rate in this study reached as high as 18.5%, and the prevalence of potential low drug resistance caused by the V179D/E mutation was 10.3%. However, it was unclear whether this affected the efficacy of antiviral drugs. We are currently collecting follow-up information for a population with potential drug resistance who were enrolled during 2019–2021 and treated with antiviral therapy for 1 year, with the aim of evaluating the effect of potential drug resistance mutations on antiviral efficacy as well as observing changes in mutation sites during the course of treatment.

This study also analyzed the risk factors for TDR. A $CD4^+$ T lymphocyte count <50 /μL was a risk factor for TDR, suggesting that ART initiation in advanced HIV infection was more likely to be affected by drug resistance. Most studies have not detected a significant association between $CD4^+$ T cell count and TDR.[9,35,36] However, one investigation concluded that patients with HIV carrying a TDR mutation had a lower average $CD4^+$ T cell count than patients with non-drug resistant HIV (373/mm^3 vs 496/mm^3, P=0.013).[37] The mechanism underlying the association between low $CD4^+$ T cell count and TDR remains unestablished. However, one possibility is the crucial role of host immune in control of HIV-1[38] and treatment-naïve patients with lower $CD4^+$ T cell counts may have been infected with HIV for a longer period of time, allowing the virus to accumulate mutations as it rapidly replicated.[39] Further studies will be needed to characterize in detail the relationship between TDR and $CD4^+$ T cell count.

This study investigated the TDR prevalence rate and drug resistance characteristics of HIV-1 in Nanjing in recent years. Nanjing Second Hospital is the only designated hospital for AIDS treatment in Nanjing, which enabled us to include a comprehensive sample of patients with AIDS receiving treatment in this region, and this is an advantage of the present study. The prevalence of drug resistance in newly-treated patients can lead to failure of antiviral therapy and the accumulation of drug resistance in the population. In Nanjing, pre-treatment drug resistance testing of patients infected with HIV began to be formally promoted in 2018, and the drug resistance testing rates were 8.5%, 46.1%, 74.7% and 83.7%, respectively, during the four-year study period. However, the prevalence of TDR in the Nanjing area has not been

well elucidated recently, and this study has contributed data for recent years. Our study also has the following disadvantages. First, subtypes of patients were not included in this study, so it is not possible to understand their prevalence and association with TDR. Furthermore, drug resistance detection did not include INSTI-related drug resistance mutations, whereas the utilization rate of INSTI drugs has been increasing gradually in recent years, and the secondary mutation site E157Q/T97A, which confers INSTI resistance, has been detected in newly-treated patients in China.[40] Finally, cofounders such as level of education and occupation were not adjusted in this study, which would be addressed in future studies.

Conclusion

The prevalence of TDR in Nanjing during 2018–2021 was at a medium level and dominated by drug-resistance to NNRTIs. Continuous monitoring of TDR prevalence in ART-naive patients living with HIV is crucial to evaluate the efficacy of current major antiviral drugs and help formulate effective personalized treatments for patients living with HIV. However, more attention should be paid to drug resistance in patients with advanced disease and a low $CD4^+$ T lymphocyte count.

Abbreviations

3TC, lamivudine; ABC, abacavir; ATV/r, atazanavir/ ritonavir; AZT, zidovudine; CRF, circulating recombinant form; CI, confidence interval; DTG, dolutegravir; DOR, doravirine; DRV/r, denavir/ritonavir; EFV, efavirenz; ETR, etravirine; FTC, emtricitabine; IDU, intravenous drug use; LPV/r, lopinavir/ ritonavir; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NVP, nevirapine; OR, odds ratio; PCR, polymerase chain reaction; PI, protease inhibitor; RPV, rilpivirine; TDF, tenofovir disoproxil fumarate; TDR, transmitted drug resistance.

Data Sharing Statement

The datasets analyzed during the current study are available from the corresponding author Hongxia Wei upon reasonable requests.

Ethics Approval and Consent to Participate

The study protocol was approved by the Medical Ethics Committee of The Second Hospital of Nanjing (approval number: 2018-LY-kt027; approval date: 9 May, 2018), and all subjects provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that there are no competing interests associated with this study.

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