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Characteristics of molecular epidemiology and transmitted drug resistance among newly diagnosed HIV-1 infections in Lishui, China from 2020 to 2023

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Abstract

Background Transmitted drug resistance (TDR) is becoming an obstacle to the success of antiretroviral therapy (ART) as the HIV epidemic continues to spread. This study aimed to investigate the characteristics of TDR and the molecular epidemiology of ART-naive HIV-1 infections in Lishui.

Methods A total of 481 plasma samples were collected from ART-naive HIV-1 infections in Lishui between 2020 and 2023. The sequences acquired from infections were used to analyze the characteristics of genotype, TDR, and molecular transmission network.

Results This study discovered that the three most prevalent subtypes among the 455 sequences successfully obtained from infections in Lishui were CRF08_BC (35.8%), CRF07_BC (26.4%), and CRF01_AE (25.9%). The overall prevalence of TDR was 12.1%, and the K103N (2.4%) was the most frequent mutation. Multivariate analysis showed that CRF08_BC (OR = 5.401, $P < 0.001$) and $CD4^+$ cell concentration of 200–499 cells/μL (OR = 1.684, $P = 0.030$) were associated with a higher risk of entering the molecular transmission network and clustering, whereas the current address in other cities (OR = 0.328, $P = 0.004$), junior middle school (OR = 0.472, $P = 0.006$), and junior college or above (OR = 0.387, $P = 0.045$) were associated with a lower risk of clustering.

Conclusions This study revealed that the prevalence of TDR was at an intermediate level of drug resistance, and high levels of resistance were predominantly concentrated in efavirenz (EFV) and nevirapine (NVP) among the NNRTIs. Middle-aged and older infections represented a significant proportion of the molecular transmission network. This

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suggests that HIV surveillance and targeted prevention and treatment interventions are essential to reduce the risk of HIV transmission.

Keywords Molecular epidemiology, Transmitted drug resistance, HIV-1, Transmission network

Introduction

Human immunodeficiency virus type 1 (HIV-1), the causative agent of acquired immunodeficiency syndrome (AIDS), is characterized by high rates of recombination and mutation, and its genetic diversity increases during transmission in high-risk populations. AIDS continues to be a serious global public health issue, despite the tremendous efforts made worldwide to control the spread of HIV-1 [1]. According to UNAIDS, 39.9 million people worldwide were living with HIV, and 630,000 died from AIDS-related illnesses in 2023. Besides, 88.4 million people have been infected with HIV, and 42.3 million have died from AIDS-related diseases since the beginning of the epidemic globally [2]. HIV-1 strains have been classified into four groups (M, N, O, and P), and group M is the major group that has caused the global HIV-1 pandemic [3, 4]. Group M of HIV-1 is categorized based on mutation levels. It includes 11 subtypes (A–D, F–H, J, K, U), 6 sub-subtypes (A1, A2, A5, A6, F1, F2), 158 circulating recombinant forms (CRFs), and numerous unique recombinant forms (URFs) [5]. Antiretroviral therapy (ART) is a treatment that significantly reduces AIDS-related morbidity and mortality by suppressing viral replication [6–8]. China launched a national free ART program in 2003 [9].

However, new challenges have emerged with the widespread use of ART. The accumulation of HIV-1 drug resistance mutations allows the virus to reduce its susceptibility to antiretroviral drugs and develop drug resistance [10]. A World Health Organization (WHO) study concerning HIV drug resistance reveals that the number of countries exceeding the 10% threshold for pretreatment drug resistance (PDR) to non-nucleoside reverse transcriptase inhibitors (NNRTIs) is growing [11]. Other studies also show that the prevalence of PDR has exceeded 10% in some regions; in low-income regions, the prevalence of PDR has even surpassed 20% [12, 13]. In China, the overall prevalence of PDR remained at an intermediate level of 7.4% [14]. However, with the continuous spread of the HIV epidemic, understanding the prevalence and transmission patterns of transmitted drug resistance (TDR) in the environment over an extended period holds significant importance. A recent nationwide report in China showed that the overall prevalence of TDR stood at 4.6% during the period from 2001 to 2022; meanwhile, an alarming annual growth rate of 11.2% was observed from 2016 to 2022 [15]. Accumulating evidence indicates that geographic disparities may influence HIV transmission patterns [16]. Moreover, elucidating the dynamics of inter-regional epidemics through spatial epidemiology approaches could reduce the risk of HIV transmission, as demonstrated in a study [17]. Such a trend highlights the necessity of strengthening drug resistance surveillance and prevention efforts in Lishui, and the HIV molecular transmission network analysis method, which has been rapidly developing in recent years, is a technological approach to rapidly identify populations at risk of transmission and conduct precise interventions by constructing an HIV molecular transmission network to study the transmission characteristics based on the similarity of HIV gene sequences and correlate them with the epidemiological information [18, 19].

This study aimed to utilize the HIV molecular transmission network technique to characterize the molecular epidemiology and TDR of HIV-1 infections in Lishui from 2020 to 2023, in order to provide practical recommendations and a scientific basis for the formulation of precise prevention and treatment interventions.

Methods and materials

Study population and data collection

In this study, demographic, behavioral, clinical, and subtype resistance data were collected retrospectively from 481 individuals from 2020 to 2023 in Lishui, Zhejiang Province, China. The inclusion criteria were as follows: (1) newly diagnosed HIV-1 infections; (2) had never received ART before enrollment. The blood samples collected from these participants were stored in a freezer maintained at −80 °C. Written informed consent was obtained from all participants. Additionally, this study obtained approval from the Medical Ethics Committee of the Lishui Municipal Center for Disease Control and Prevention.

HIV-1 nucleic acid extraction, amplification, and sequencing

According to the manufacturer's instructions, HIV-1 RNA was extracted from the isolated plasma by means of Viral RNA Mini Kit (Tianlong, Suzhou, China) and supporting kits. The extracted RNA samples served as templates for amplification using an in-house nested polymerase chain reaction (PCR) and reverse transcription PCR protocol targeting the pol (1,316 bp, HXB2: 2147–3462) gene regions of HIV-1 [20]. The amplification products from the PCR-positive samples were identified by 1% agarose gel electrophoresis. Subsequently, they were sent to Hangzhou Qingke Biotechnology Co. Ltd. for purification and Sanger sequencing.

Sequence analysis

The calibration and sequence splicing of the obtained sequence fragments were conducted using Sequencer 5.4.6 (Gene Codes, Ann Arbor, Michigan, United States). Subsequently, all the obtained sequence fragments were manually aligned and edited using BioEdit 7.7.1 software (Gene Codes, Ann Arbor, Michigan, United States), and they were compared to the reference sequence from the HIV databases of the Los Alamos National Laboratory (http://hiv.lanl.gov). After that, the phylogenetic tree was constructed using the neighbor-joining method in MEGA 6.0 software. The Kimura 2-par-ameter model with 1000 bootstrap replicates was identified as the best fitting model among the models considered [21]. The CRFs of each sequence were initially analyzed based on clustering with international reference sequences and further reviewed with the assistance of the COMET online analysis tool (https://comet.lih.lu/index.php?cat=hiv1) [22]. Sequences that failed to be clustered were regarded as URFs and their recombination types were analyzed using the RIP online analysis tool (https://www.hiv.lanl.gov/content/sequence/RIP/RIP.html) and the jpHMM online analysis tool (http://jphmm.gobics.de/submission_hiv.html).

Drug resistance analysis

The aligned sample sequences were uploaded to the Stanford University HIV Drug Resistance Database (https://hivdb.stanford.edu/), where the HIVdb program (version 9.7) was used to identify and evaluate the level of HIV-1 drug resistance mutations (DRMs). The level of DRMs was classified according to the Stanford Penalty Score as low level (score of 15–29), intermediate level (score of 30–59), or high level (score of 60) with respect to the following drugs: eight protease inhibitors (PIs) [atazanavir/r (ATV/r), darunavir/r (DRV/r), fosamprenavir/r (FPV/r), indinavir/r (IDV/r), lopinavir/r (LPV/r), saquinavir/r (SQV/r), nelfinavir (NFV), and tipranavir/r (TPV/r)], five NNRTIs [doravirine (DOR), etravirine (ETR), efavirenz (EFV), rilpivirine (RPV) and nevirapine (NVP)], and seven nucleoside reverse transcriptase inhibitors (NRTIs) [abacavir (ABC), zidovudine (AZT), lamivudine (3TC), stavudine (D4T), didanosine (DDI), emtricitabine (FTC), and tenofovir (TDF)].

Genetic transmission network analysis

The aligned sample sequences were opened using MEGA 6.0 software, and the genetic distances between all sample sequences were estimated using the Tamura-Nei model. And then the optimal genetic distance (GD) threshold was found. The optimal GD threshold was defined as the distance that identifies the maximum number of TCs. The results showed that 0.009 was the optimal GD among the sequences. The HIV-1 molecular transmission network was visualized and analyzed using Cytoscape 3.9.1 [23]. In addition, the network entry rate indicates the percentage of sample sequences that have entered the transmission network among the total number of sample sequences. A node in the molecular network represents a sequence or case. The transmission correlation between two nodes is represented by a link or edge. Degree value refers to the number of node connections. The higher the degree value is, the higher the transmission risk of the node is, and a degree of 0 indicates non-clustered cases; ≥ 1, clustered cases; ≤ 3, low-risk transmission cases; ≥ 4, high-risk transmission cases [24].

Statistical analysis

EpiData 3.1 software was used to build a database. The demographic information and distribution of genotype were examined by chi-square tests. Fisher's exact test was used when the sample size was small (e.g., less than 40), or when the expected frequency was less than 5. Univariate and multivariate logistic regression models were used to identify factors that influenced the inclusion of variables into the HIV molecular network. All the analyses were conducted in SPSS Statistics version 26.0 software (IBM, Armonk, NY, United States) and R 3.5.1. Statistical significance was defined as a P value < 0.05.

Results

Demographic characteristics of the study population

Following amplification and sequencing of 481 HIV-infected patient samples collected between 2020 and 2023, 455 (94.6%) were successfully amplified and included in the analysis. Of the 455 sequenced infections, the majority were male (76.5%, 348/455) and of Han ethnicity (94.9%, 432/455). Among the other ethnic groups, She ethnicity comprised the highest proportion (43.5%, 10/23). More than half of the infections were 50 years old and above (52.3%, 238/455), with an average age of 48.7 years. The vast majority had Lishui household registration (90.3%, 411/455). In terms of marital status, 49.7% were married (226/455). Regarding the literacy level, it was mainly composed of those with an elementary school (37.6%, 171/455) and junior middle school (31.4%, 143/455) education. Moreover, the main route of infection was heterosexual transmission (74.5%, 339/455) (Table 1).

Subtype analysis

After genotyping the 455 successfully amplified samples from infections, a total of 13 subtypes were obtained, including 9 CRFs and 4 URFs. The CRFs comprised 163 cases of CRF08_BC (35.8%, 163/455), 120 cases of CRF07_BC (26.4%, 120/455), 118 cases of CRF01_AE (25.9%, 118/455), 21 cases of subtype B (4.6%, 21/455), 14 cases of CRF55_01B (3.1%, 14/455), 4 cases of CRF85_BC

Table 1 Characteristics of newly diagnosed HIV-1 infections in Lishui from 2020 to 2023

Characteristics	Total *n* (%)	HIV-1 subtypes				χ^2	*P*-value
		CRF07_BC *n* (%)	CRF08_BC *n* (%)	CRF01_AE *n* (%)	others *n* (%)		
Total	455	120	163	118	54		
Gender						7.180	0.066
Male	348(76.5)	101(84.2)	115(70.6)	90(76.3)	42(77.8)		
Female	107(23.5)	19(15.8)	48(29.4)	28(23.7)	12(22.2)		
Ethnicity						1.502[a]	0.693
Han	432(94.9)	112(93.3)	157(96.3)	112(94.9)	51(94.4)		
Others	23(5.1)	8(6.7)	6(3.7)	6(5.1)	3(5.6)		
Age (years)						76.471	< 0.001
< 30	67(14.7)	22(18.3)	4(2.5)	32(27.1)	9 (16.7)		
30–39	63(13.8)	27(22.5)	8(4.9)	16(13.6)	12(22.2)		
40–49	87(19.1)	27(22.5)	29(17.8)	24(20.3)	7(13.0)		
≥ 50	238(52.3)	44(36.7)	122(74.8)	46(39.0)	26(48.1)		
Current address						5.960	0.114
Lishui	411(90.3)	102(85.0)	151(92.6)	107(90.7)	51(94.4)		
Other cities	44(9.7)	18(15.0)	12(7.4)	11(9.3)	3(5.6)		
Marital status						34.266	< 0.001
Unmarried	122(26.8)	38(31.7)	20(12.3)	46(39.0)	18(33.3)		
Married	226(49.7)	55(45.8)	91(55.8)	57(48.3)	23(42.6)		
Divorced or widowed	107(23.5)	27(22.5)	52(31.9)	15(12.7)	13(24.1)		
Level of education						51.032	< 0.001
Primary school or below	209(45.9)	39(32.5)	100(61.3)	48(40.7)	22(40.7)		
Junior middle school	143(31.4)	38(31.7)	50(30.7)	35(29.7)	20(37.0)		
Senior high school	45(9.9)	17(14.2)	11(6.7)	10(8.5)	7(13.0)		
Junior college or above	58(12.7)	26(21.7)	2(1.2)	25(21.2)	5(9.3)		
Route of transmission						77.605	< 0.001
MSM	116(25.5)	52(43.3)	3(1.8)	40(33.9)	21(38.9)		
Heterosexual transmission	339(74.5)	68(56.7)	160(98.2)	78(66.1)	33(61.1)		
Transmitted drug resistance						9.906	0.019
Sensitive	400(87.9)	97(80.8)	143(87.7)	110(93.2)	50(92.6)		
Resistance	55(12.1)	23(19.2)	20(12.3)	8(6.8)	4(7.4)		
$CD4^+$cell count (cells/μL)						29.097	0.001
< 200	151(33.2)	30(25.0)	56(34.4)	43(36.4)	22(40.7)		
200–499	255(56.0)	73(60.8)	98(60.1)	62(52.5)	22(40.7)		
≥ 500	45(9.9)	17(14.2)	9(5.5)	12(10.2)	7(13.0)		
Unknow	4(0.9)	0(0.0)	0(0.0)	1(0.8)	3(5.6)		

[a]The group was examined by Fisher's exact test

(0.9%, 4/455), 2 cases of subtype A1 (0.4%, 2/455), 2 cases of subtype A6 (0.4%, 2/455), 1 case of subtype C (0.2%, 1/455). The URFs contained URF (CRF07_BC/CRF01_AE) in 7 cases (1.5%, 7/455), URF (B/C) in 1 case (0.2%, 1/455), URF (CRF01_AE/B) in 1 case (0.2%, 1/455), URF (CRF65_cpx/ C/CRF07_BC) in 1 case (0.2%, 1/455) (Fig. 1). χ^2 test/Fisher's exact test analysis showed that the distribution of subtypes of HIV infections was significantly correlated with age ($P < 0.001$), marital status ($P < 0.001$), level of education ($P < 0.001$), route of transmission ($P < 0.001$), transmitted drug resistance ($P = 0.019$) and $CD4^+$ cell count ($P = 0.001$) (Table 1).

Drug resistance analysis

The overall prevalence of TDR from 2020 to 2023 was 12.1% (55/455) with NNRTI resistance being the most prevalent (5.9%, 27/455), followed by PI resistance (4.6%, 21/455) and NRTI resistance (2.4%, 11/455). Moreover, four infections exhibited dual-class resistance (2 NNRTI + NRTI, 1 NNRTI + PI, and 1 NRTI + PI). In total, 11.2% (51/455) showed single-class resistance and 0.9% (4/455) dual-class resistance. TDR had been continuously increasing from 6.8% in 2021 to 13.3% in 2023, and NNRTI resistance also showed an increasing trend (from 2.5% in 2021 to 19.5% in 2023). In contrast, PI resistance showed a decreasing trend (from 8.3% in 2020 to 1.9% in 2023). Additionally, the percentage of NNRTI resistance

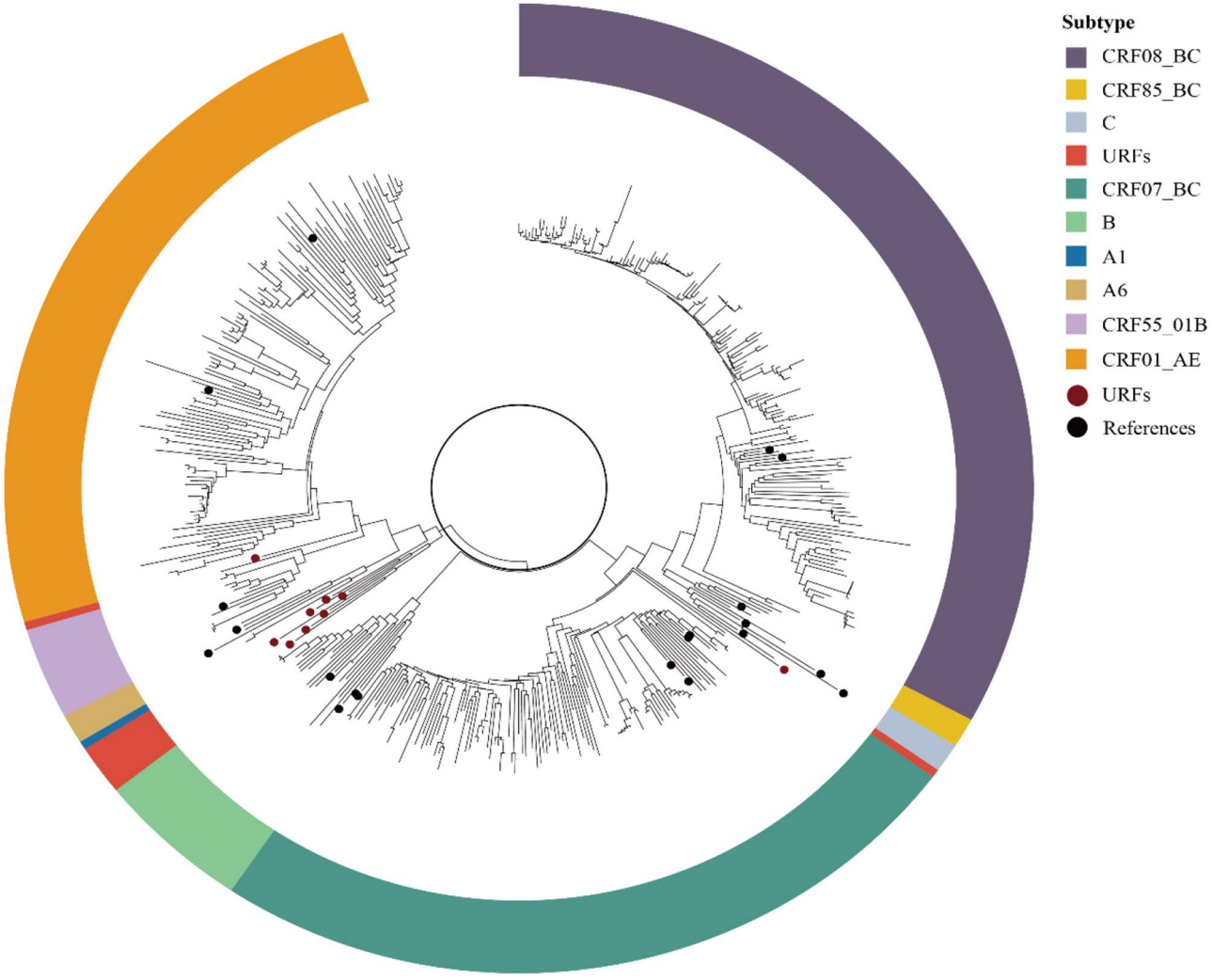


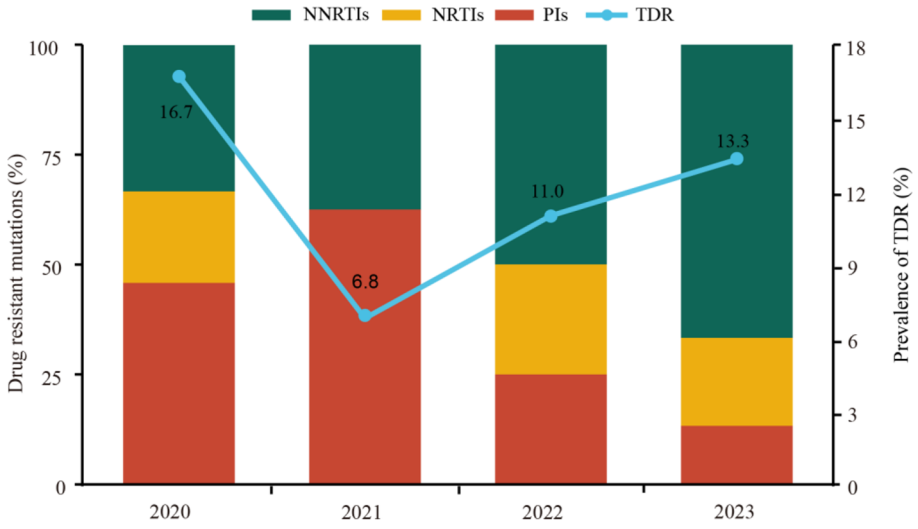
Fig. 1 Phylogenetic tree of 455 newly diagnosed HIV-1 infections in Lishui, 2020–2023

in TDR showed an increasing trend (from 33.3% in 2020 to 66.7% in 2023). The percentage of PI resistance in TDR showed a decreasing trend (from 62.5% in 2021 to 13.3% in 2023) (Fig. 2). Among the NNRTI resistance mutations, K103N (2.4%, 11/455) showed the highest mutation frequency, followed by E138A/G (1.5%, 7/455), and for NRTI resistance, the most common mutations were M41L/ML (1.1%, 5/455) and M184V/MV (0.9%, 4/455), and for PI resistance, M46I/L/MI/MV (2.0%, 9/455) and Q58E (2.0%, 9/455) were the most frequent mutations (Fig. 3).

According to the HIVdb Stanford database algorithm, we found that TDR to single-class drugs was highest for NVP (4.0%, 18/455), EFV (3.1%, 14/455), NFV (2.6%, 12/455), RPV (2.6%, 12/455), and TPV/r (2.2%, 10/455), while the lowest rates were observed for TDF (0.2%, 1/455), DRV/r (0.2%, 1/455), FPV/r (0.2%, 1/455), IDV/r (0.2%, 1/455), and LPV/r (0.2%, 1/455). Among the drug classes, NNRTI resistance showed the highest proportion of high-level resistance (11.0%, 50/455), followed by NRTI resistance (6.8%, 31/455) and PI resistance (6.8%, 31/455). Among NNRTI agents, both NVP and EFV showed the highest proportion of high-level resistance (2.9%, 13/455). As for NRTI agents, both 3TC and FTC showed the highest proportion of high-level resistance (0.9%, 4/455). Among PI agents, both NFV and ATV/r showed the highest proportion of high-level resistance (0.4%, 2/455) (Fig. 4).

Molecular transmission network analysis

As shown in Fig. 5, a total of 55.8% (254/455) infections were contained in the HIV molecular transmission network when the GD threshold was 0.9%, which formed 49 transmission clusters. The median cluster size was 2 (IQR 2–4), with the largest size of 91 (1 cluster) and the smallest size of 2 (25 clusters). Among those included in the molecular transmission network, 75.6% (192/254) were male and 24.4% (62/254) were female. In addition,



	Total n (%)	Year			
		2020 n (%)	2021 n (%)	2022 n (%)	2023 n (%)
Total	455	132	118	100	105
Any DRMs	55(12.1)	22(16.7)	8(6.8)	11(11.0)	14(13.3)
NNRTI resistance	27(5.9)	8(6.1)	3(2.5)	6(6.0)	10(9.5)
NRTI resistance	11(2.4)	5(3.8)	0(0.0)	3(3.0)	3(2.9)
PI resistance	21(4.6)	11(8.3)	5(4.2)	3(3.0)	2(1.9)
Single-class resistance	51(11.2)	20(15.2)	8(6.8)	10(10.0)	13(12.4)
Dual-class resistance	4(0.9)	2(1.5)	0(0.0)	1(1.0)	1(1.0)

Fig. 2 Trends in drug-resistant mutations and TDR in ART-naive infections

the subtype with the highest percentage was CRF08_BC, accounting for 52.0% (132/254), followed by CRF07_BC (22.4%, 57/254), CRF01_AE (19.3%, 49/254), CRF55_01B (3.5%, 9/254), and subtype B (2.0%, 5/254). Meanwhile, subtype A1 had the lowest number of cases, accounting for 0.8% (2/254). Regarding the route of transmission, 83.1% (211/254) were infected through heterosexual transmission, while 16.9% (43/254) were infected through MSM. Within the network of CRF55_01B sequences, 88.9% (8/9) were infected through MSM. The primary drug resistance mutations within the network were K103N (22.6%, 7/31), followed by M41L (9.7%, 3/31), M46L (9.7%, 3/31), M46I (9.7%, 3/31), and Q58E (9.7%, 3/31). Regardless of subtype, TDR was exclusively identified in the heterosexual population, and heterosexual population showed 35.5% (11/31) of TDR among CRF07_BC sequences, followed by 11.6% (5/43) among CRF01_AE sequences and 11.5% (15/130) among CRF08_BC sequences. Notably, the number of infections with high-transmission-risk was 140 cases, accounting for 55.1% (140/254), distributed in 10 transmission clusters of three subtypes, including CRF08_BC, CRF07_BC, and CRF01_AE, and the CRF08_BC Contained the maximum number of infections, 102 cases, accounting for 72.9% (102/140).

Univariate analysis showed that, when comparing the infections included with those not included in the network, there were significant differences in the following aspects: for age groups, the age of 40–49 (OR = 2.050, 95% CI = 1.063–3.952, $P = 0.032$) and ≥ 50 (OR = 3.924, 95% CI = 2.214–6.955, $P < 0.001$); for current address, other cities (OR = 0.372, 95% CI = 0.194–0.715, $P = 0.003$); for level of education, junior middle school (OR = 0.462, 95% CI = 0.299–0.716, $P = 0.001$) and junior college or above (OR = 0.235, 95% CI = 0.126–0.437, $P < 0.001$); for subtype, CRF08_BC (OR = 6.614, 95% CI = 3.869–11.305, $P < 0.001$); for transmission route, heterosexual transmission (OR = 2.799, 95% CI = 1.809–4.328, $P < 0.001$); and for $CD4^+$ cell count, ≥ 500 cells/μL (OR = 0.444, 95% CI = 0.221–0.891, $P = 0.022$) (Fig. 6). After adjusting for

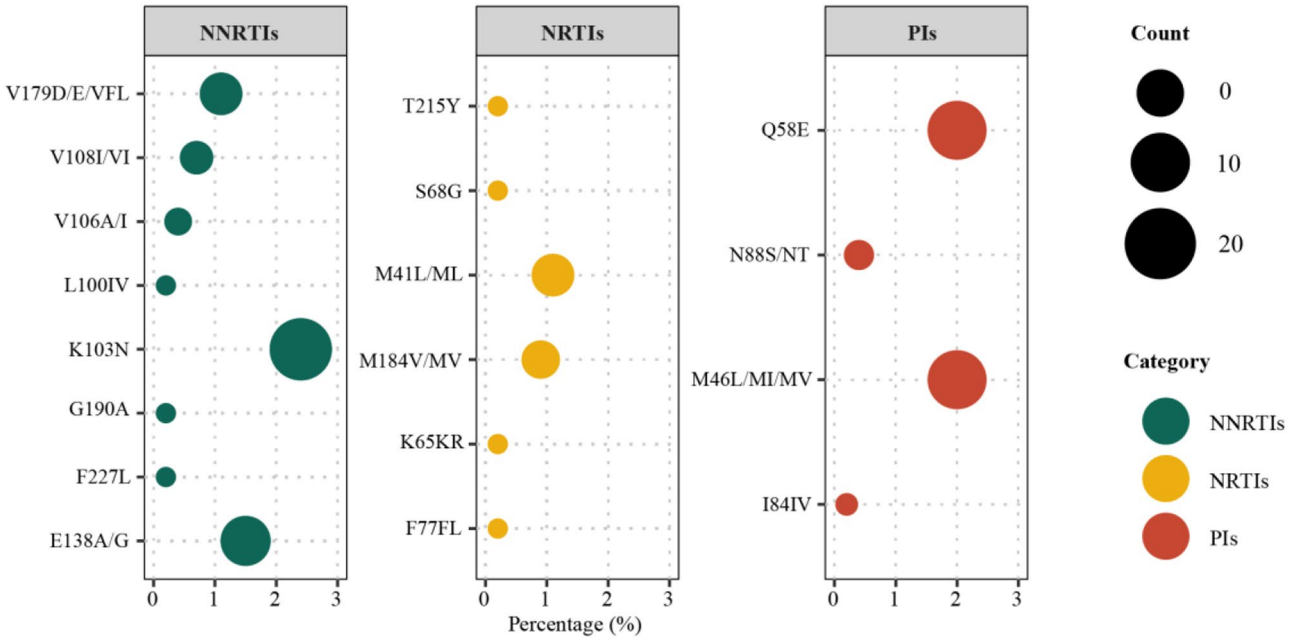


Fig. 3 Percentage of drug resistance to NNRTIs, NRTIs, and PIs

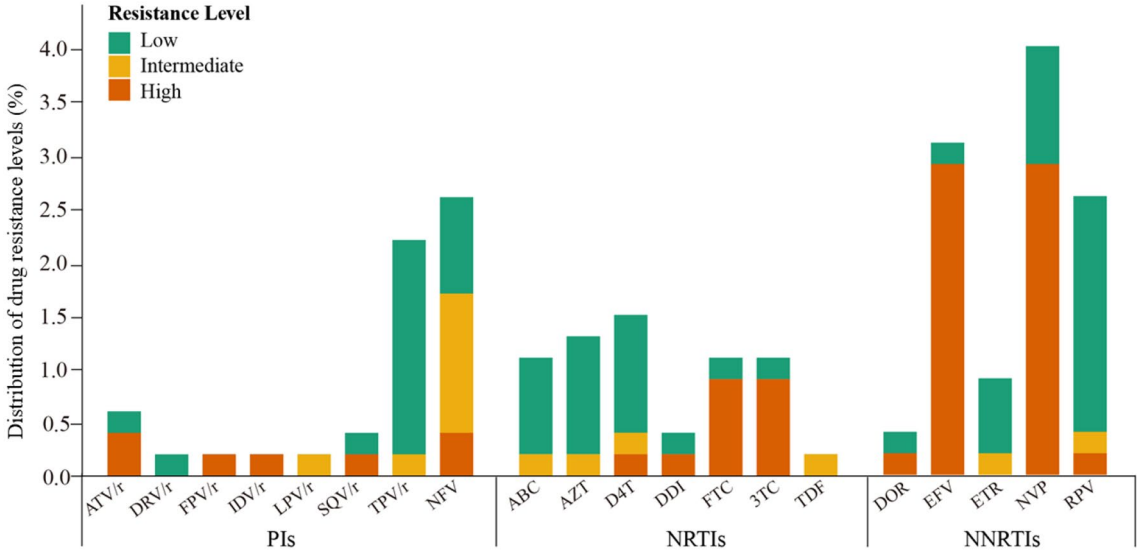


Fig. 4 Distribution of drug resistance levels in newly diagnosed HIV-1 infections

significant baseline covariates ($P<0.05$), multivariate analysis showed that, compared with the reference group, infections with current address in other cities had an OR of 0.328 (95% CI = 0.152–0.705, $P=0.004$), indicating a 67.2% lower risk of clustering; those with a junior middle school education had an OR of 0.472 (95% CI = 0.275–0.809, $P=0.006$), and those with a junior college education or above had an OR of 0.387 (95% CI = 0.153–0.980, $P=0.045$), corresponding to 0.472 and 0.387 times the risk of clustering, respectively; those with CRF08_BC had an OR of 5.401 (95% CI = 2.936–9.937, $P<0.001$), suggesting a 5.401-fold higher risk of clustering; those with $CD4^+$ cell count of 200–499 cells/μL had an OR of 1.684 (95% CI = 1.051–2.697, $P=0.030$), meaning a 1.684-fold higher risk of clustering (Fig. 7).

Discussion

In this study, we investigated the characteristics of demographics, subtype distribution, TDR, and molecular transmission networks of HIV-1 infections who were newly diagnosed with HIV and had never obtained ART from 2020 to 2023 in Lishui.

Our study identified that the predominant subtypes among HIV-1 infections in Lishui were as

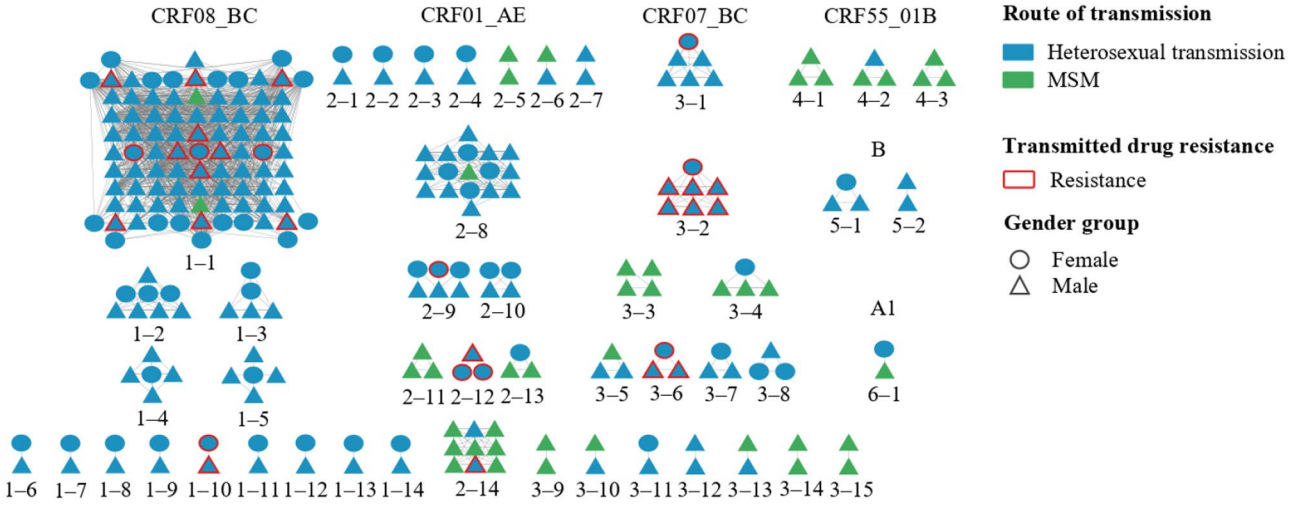


Fig. 5 Molecular transmission network among newly diagnosed HIV-1 infections in Lishui

follows: CRF08_BC (35.8%, 163/455), CRF07_BC (26.4%, 120/455), CRF01_AE (25.9%, 118/455), and subtype B (4.6%, 21/455). These findings were different from the results shown by the 4th National HIV Molecular Epidemiology Survey conducted in 2015 [25]. It indicated that the distribution of subtypes among HIV-1 infections in Lishui showed certain regional characteristics, which was consistent with the results of significant spatial heterogeneity of HIV transmission revealed by an analysis of genomic and spatial epidemiology in Sichuan Province [26]. Other studies has shown that floating populations tend to influence the distribution of HIV-1 subtypes [27]. We hypothesize that this may be due to the location in a mountainous area, which limits communication with the outside world, fosters more stable demographic characteristics, and contributes to localized epidemics.

By 2020, Lishui's resident population was 2,508,000, which accounted for only 3.9% of the total resident population in Zhejiang Province (64,680,000), according to the Lishui Municipal Bureau of Statistics [28]. Moreover, Lishui had a floating population of 975,700, accounting for 3.49% (975,700/279,197,000) of the Zhejiang Province's floating population, which was only higher than Quzhou's 2.11% (588,600/279,197,000) and Zhoushan's 1.31% (366,500/279,197,000), according to the Zhejiang Provincial Bureau of Statistics [29]. These statistics suggest that the limited resident and floating populations may restrict the prevalence of HIV subtypes.

Although subtype B was ranked fourth in this study, ethnicity has been demonstrated to be a major determinant of AIDS progression, and subtype B may progress more rapidly in the Han Chinese population than in Western ethnic groups [30]. Furthermore, some research indicates that subtype B is undergoing natural selection, potentially leading to increased virulence [31]. Recent studies have shown that the prevalence of TDR in CRF07_BC is lower than in subtype B and CRF01_AE [32, 33]. Chinese patients infected with CRF07_BC showed better correlation with immune recovery after ART compared to those infected with CRF01_AE [34, 35]. Notably, while subtype B was associated with virulence enhancement, similar phenomenon was also observed in other CRFs in regions with high recombination rates [36]. This suggests that virulence enhancement may be a broader evolutionary adaptation rather than a subtype-specific trait, underscoring the need for longitudinal studies to track viral dynamics across different genetic backgrounds. In addition, although the prevalence of the three main dominant subtypes, CRF07_BC, CRF08_BC, and CRF01_AE, varied in Lishui City, their combined prevalence remained relatively stable throughout the study period, with an average combined prevalence of 88.3%. This suggests that these subtypes may indicate similarly stable transmission rates of HIV infection in Lishui. In recent years, it is worth noting that CRF55_01B has shown a trend of expanding prevalence and has become the fifth major prevalent dominant subtype in China [37, 38]. This consistency aligns with our findings. The circulating recombinant form virus of CRF55_01B was first discovered and reported in 2012 [39], and subsequently spread rapidly across the country [40]. Although some research has found that CRF55_01B can be transmitted among heterosexuals [41], the MSM route still plays a dominant role in the spread of this subtype. In this study, it was evidenced by the result that the majority of CRF55_01B infections (78.6%, 11/14) were transmitted through MSM.

Due to the lack of pre-ART drug resistance testing among HIV infections, the prevalence of TDR has increased rapidly in recent years [42]. In this study, drug resistance was defined by detecting any single major mutation associated with antiretroviral failure.

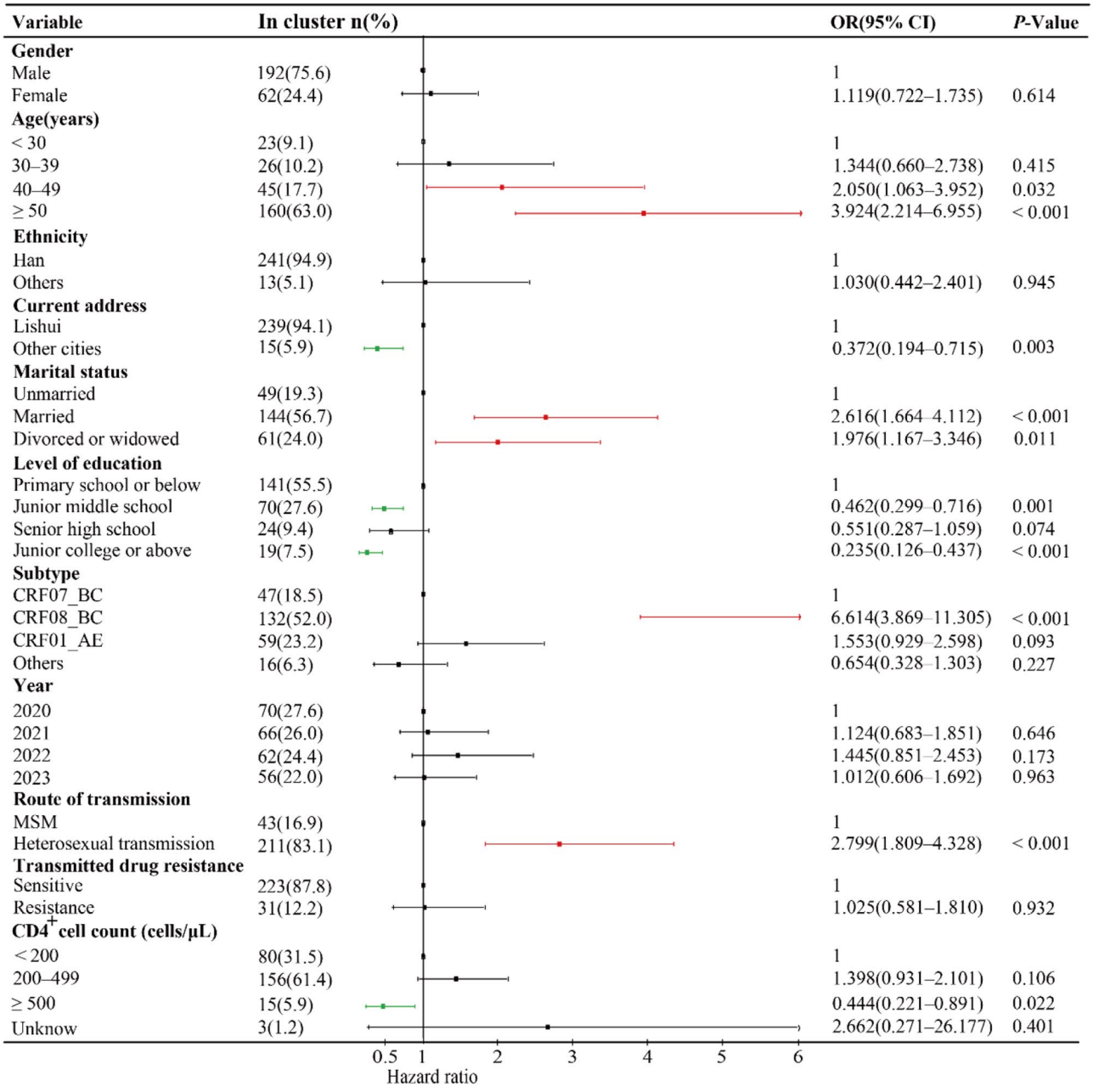


Fig. 6 Univariate analysis of factors associated with infections within clusters. Red lines indicate factors with a higher risk of clustering and green lines indicate factors with a lower risk of clustering (Reference groups: OR = 1)

The prevalence of TDR among HIV infections in Lishui was 12.1% (55/455), which is classified as an intermediate level of resistance (5-15%) under the WHO criteria based on single-mutation surveillance [43]. This prevalence exceeds the national average of 7.4% reported in China, where resistance is similarly defined by individual key mutations [14]. The K103N mutation, a single amino acid substitution conferring high-level resistance to EFV and NVP, was identified as the most prevalent in this study. Recent regional studies in Wenzhou, Chongqing, and Ningbo also reported K103N as a dominant single-mutation driver of resistance, particularly in CRF07_BC subtypes [44–46]. Additionally, this study observed high-level resistance against NVP and EFV (2.9%, 13/455) and 3TC and FTC (0.9%, 4/455), each linked to specific single mutations. Given that NVP, EFV, and 3TC are first-line ART drugs in China [47], targeted monitoring of these single-mutation hotspots is critical to mitigating TDR transmission.

The HIV molecular transmission network is essential for developing precision intervention and treatment measures as well as enhancing the efficiency of public

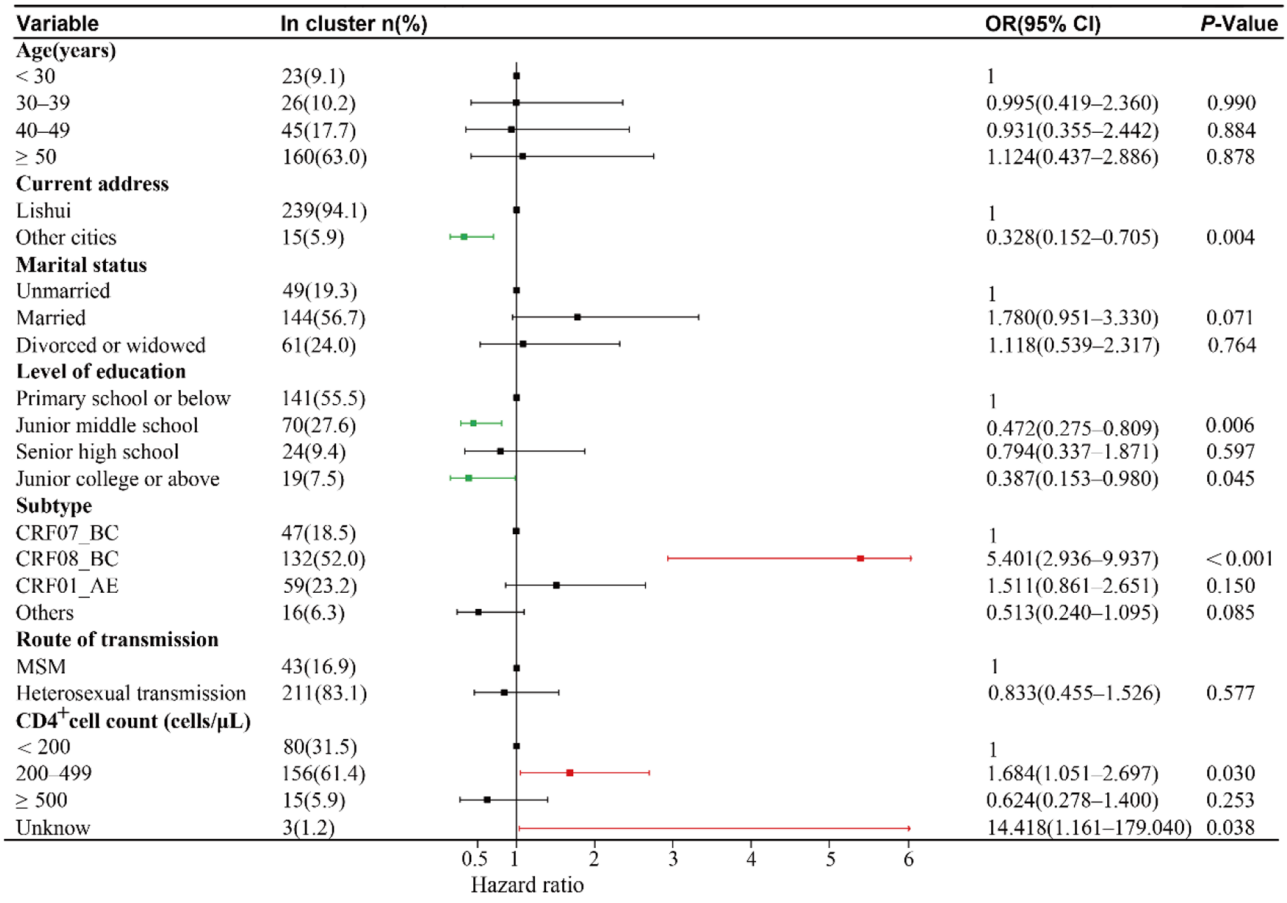


Fig. 7 Multivariate analysis of factors associated with clustered HIV-1 infections. Red lines indicate factors with a higher risk of clustering and green lines indicate factors with a lower risk of clustering (Reference groups: OR = 1)

health based on the characterization of HIV-1 transmission clusters and associated TDR [48]. Unlike other densely populated cities, such as Hangzhou and Shenzhen [49, 50], HIV infections in the Lishui molecular transmission network were predominantly transmitted through heterosexual routes (83.1%, 211/254). Multivariate analysis demonstrated that compared with infections with education level of elementary school and below, infected persons with education level of junior middle school (OR = 0.472, 95% CI = 0.275–0.809, $P = 0.006$) and those with a junior college education or above (OR = 0.387, 95% CI = 0.153–0.980, $P = 0.045$) had a lower risk of entering the transmission, suggesting that an increased education level helps to reduce the risk of entering the HIV molecular transmission network into clusters to some extent. Compared with CRF07_BC, CRF08_BC (OR = 5.401, 95% CI = 2.936–9.937, $P < 0.001$) had a higher risk of entering the transmission network, suggesting that CRF08_BC is more likely to cluster. Meanwhile, we found a tremendous CRF08_BC cluster in the molecular transmission network, containing 91 sequences, and heterosexual transmission was the predominant route, which partly explains the highest proportion of CRF08_BC observed in this study. In addition, the majority of the drug-resistant infections in the network were middle-aged and elderly (71.0%, 22/31), with all transmission routes being heterosexual. This finding indicates that heterosexual transmission among the middle-aged and elderly populations is an important driver of the sexual transmission within the Lishui TDR clusters.

This study has several limitations. First, although it encompasses 455 newly confirmed HIV-1 infections diagnosed from 2020 to 2023 in Lishui, the voluntary nature of HIV testing may introduce selection bias, potentially compromising sample representativeness. Furthermore, the current dataset does not allow us to accurately calculate infection duration or assess potential confounding factors (e.g., coinfections, comorbidities, medications, or environmental exposures) due to limited follow-up data. Additionally, the acute HIV infection (AHI) could not be systematically diagnosed in this study due to limited availability of HIV RNA viral load testing and seroconversion markers. Finally, the amplified region included only the pol gene sequence, which focused on protease and reverse transcriptase mutations; thus, it did not include resistance mutations associated with INSTIs.

Conclusion

This study revealed that the prevalence of TDR was at an intermediate level of drug resistance, and resistance to NNRTIs was the most prevalent, with high levels of resistance concentrated mainly in EFV and NVP. Middle-aged and older infections represented a significant proportion of the molecular transmission network. These findings suggest that HIV surveillance and targeted prevention and treatment interventions are essential to reduce the risk of HIV transmission.

Abbreviations

HIV-1	Human immunodeficiency virus type 1
AIDS	Acquired immunodeficiency syndrome
CRFs	Circulating recombinant forms
URFs	Uunique recombinant forms
ART	Antiretroviral therapy
WHO	World Health Organization
PDR	Pretreatment drug resistance
NNRTIs	Non-nucleoside reverse transcriptase inhibitors
TDR	Transmitted drug resistance
PCR	Polymerase chain reaction
DRMs	Drug resistance mutations
PIs	Protease inhibitors
ATV/r	Atazanavir/r
DRV/r	Darunavir/r
FPV/r	Fosamprenavir/r
IDV/r	Indinavir/r
LPV/r	Lopinavir/r
SQV/r	Saquinavir/r
NFV	Nelfinavir
TPV/r	Tipranavir/r
DOR	Doravirine
ETR	Etravirine
EFV	Efavirenz
RPV	Rilpivirine
NVP	Nevirapine
NRTIs	Nucleoside reverse transcriptase inhibitors
ABC	Abacavir
AZT	Zidovudine
3TC	Lamivudine
D4T	Stavudine
DDI	Didanosine
FTC	Emtricitabine
TDF	Tenofovir
GD	Genetic distance

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Author contributions

LJK designed the study; CXY and CDQ coordinated the study; CXL and ZJL collected the data; ZDY and YJJ did the primary data analysis; The experiments were conducted by MJH and YJ; LJK drafted the article.

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Data availability

All datasets used in this study are reasonably available from the corresponding authors.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Chinese Center for Disease Control and Prevention (X140617334). All participants provided a statement on informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. De Cock KM, Jaffe HW, Curran JW. Reflections on 40 years of AIDS. Emerg Infect Dis. 2021;27:1553–60. https://doi.org/10.3201/eid2706.210284.
2. UNAIDS, Global HIV & AIDS Statistics — Fact Sheet. 2024. https://www.unaids.org/sites/default/files/media_asset/UNAIDS_FactSheet_en.pdf
3. Plantier JC, Leoz M, Dickerson JE, De Oliveira F, Cordonnier F, Lemée V, et al. A new human immunodeficiency virus derived from gorillas. Nat Med. 2009;15:871–2. https://doi.org/10.1038/nm.2016.
4. Sharp PM, Bailes E, Chaudhuri RR, Rodenburg CM, Santiago MO, Hahn BH. The origins of acquired immune deficiency syndrome viruses: where and when? Philos Trans R Soc Lond B Biol Sci. 2001;356:867–76. https://doi.org/10.1098/rstb.20-01.0863.
5. Los Alamos National Laboratory. HIV Circulating Recombinant Forms. 2024. https://www.hiv.lanl.gov/components/sequence/HIV/crfdb/crfs.comp. Accessed 27 Sept 2024.
6. Olson AD, Walker AS, Suthar AB, Sabin C, Bucher HC, Jarrin I, et al. Limiting Cumulative HIV viremia Copy-Years by early treatment reduces risk of AIDS and death. J Acquir Immune Defic Syndr. 2016;73:100–8. https://doi.org/10.1097/QAI.0000000000001029
7. TEMPRANO ANRS 12136 Study Group, Danel C, Moh R, Gabillard D, Badje A, Le Carrou J, et al. A trial of early antiretrovirals and Isoniazid preventive therapy in Africa. N Engl J Med. 2015;373:808–22. https://doi.org/10.1056/NEJMoa1507198.
8. INSIGHT START Study Group, Lundgren JD, Babiker AG, Gordin F, Emery S, Grund B, et al. Initiation of antiretroviral therapy in early asymptomatic HIV Infection. N Engl J Med. 2015;373:795–807. https://doi.org/10.1056/NEJMoa1506816
9. Zhang F, Haberer JE, Wang Y, Zhao Y, Ma Y, Zhao D, et al. The Chinese free antiretroviral treatment program: challenges and responses. AIDS. 2007;21(Suppl 8):S143–8. https://doi.org/10.1097/01.aids.0000304710.10036.2b.
10. Clutter DS, Jordan MR, Bertagnolio S, Shafer RW. HIV-1 drug resistance and resistance testing. Infect Genet Evol. 2016;46:292–307. https://doi.org/10.1016/j.meegid.2016.08.031
11. WHO. WHO releases HIV drug resistance report 2021. https://www.who.int/news/item/24-11-2021-who-releases-hiv-drug-resistance-report-2021. Accessed 27 Sept 2024.
12. Gupta RK, Gregson J, Parkin N, Haile-Selassie H, Tanuri A, Andrade Forero L, et al. HIV-1 drug resistance before initiation or re-initiation of first-line antiretroviral therapy in low-income and middle-income countries: a systematic review and meta-regression analysis. Lancet Infect Dis. 2018;18:346–55. https://doi.org/10.1016/S1473-3099(17)30749-1.
13. Macdonald V, Mbuagbaw L, Jordan MR, Mathers B, Jay S, Baggaley R, et al. Prevalence of pretreatment HIV drug resistance in key populations: a systematic review and meta-analysis. J Int AIDS Soc. 2020;23:e25656. https://doi.org/10.1002/jia2.25656
14. Chen H, Hao J, Hu J, Song C, Zhou Y, Li M, et al. Pretreatment HIV drug resistance and the molecular transmission network among HIV-Positive individuals in China in 2022: multicenter observational study. JMIR Public Health Surveill. 2023;9:e50894. https://doi.org/10.2196/50894

15. Liu X, Hu J, Song C, Wang D, Hao J, Liao L, et al. Joinpoint regression analysis of the trend of transmitted drug resistance prevalence among ART-naïve HIV infected patients in China from 2004 to 2022. Int J Virol. 2023;30:441–6. https://doi.org/10.3760/cma.j.issn.1673-4092.2023.06.001
16. Gibney KB, Cheng AC, Hall R, Leder K. Sociodemographic and geographical inequalities in notifiable infectious diseases in Australia: a retrospective analysis of 21 years of National disease surveillance data. Lancet Infect Dis. 2017;17:86–97. https://doi.org/10.1016/S1473-3099(16)30309-7.
17. Dowdy DW, Golub JE, Chaisson RE, Saraceni V. Heterogeneity in tuberculosis transmission and the role of geographic hotspots in propagating epidemics. Proc Natl Acad Sci U S A. 2012;109:9557–62. https://doi.org/10.1073/pnas.1203517109
18. Wertheim JO, Leigh Brown AJ, Hepler NL, Mehta SR, Richman DD, Smith DM, et al. The global transmission network of HIV-1. J Infect Dis. 2014;209:304–13. https://doi.org/10.1093/infdis/jit524.
19. Zhong P. Can molecular epidemiology help nip HIV transmission in the bud? China Trop Med. 2016;16:1052–6. https://doi.org/10.13604/j.cnki.46-1064/r.2016.11.02. 1099.
20. Zhang J, Guo Z, Yang J, Pan X, Jiang J, Ding X, et al. Genetic diversity of HIV-1 and transmitted drug resistance among newly diagnosed individuals with HIV infection in Hangzhou, China. J Med Virol. 2015;87:1668–76. https://doi.org/10.1002/jmv.24223
21. Zhang J, Guo Z, Pan X, Yang J, Yao Y, Chen L, et al. Study on mutation of drug resistant gene in HIV infected patients with antiretroviral therapy in the major regions in Zhejiang Province. Chin J Health Lab Technology (CJHLT). 2010;20:2391–4.
22. Struck D, Lawyer G, Ternes AM, Schmit JC, Bercoff DP. COMET: adaptive context-based modeling for ultrafast HIV-l subtype identification. Nucleic Acids Res. 2014;42:e144. https://doi.org/10.1093/nar/gku739.
23. Zhang Z, Dai L, Jiang Y, Feng K, Liu L, Xia W, et al. Transmission network characteristics based on Env and gag sequence from MSM during acute HIV-1 infection in Beijing, China. Arch Virol. 2017;162:3329–38. https://doi.org/10.1007/s00705-017-3485-z
24. Oster AM, Wertheim JO, Hernandez AL, Ocfemia MC, Saduvala N, Hall HI. Using molecular HIV surveillance data to understand transmission between Subpopulations in the united States. J Acquir Immune Defic Syndr. 2015;70:444–51. https://doi.org/10.1097/QAI.0000000000000809
25. Zhong P. Progress in research and practice of molecular epidemiology of HIV-1. Electron J Emerg Infect Dis. 2019;4:137–44. https://doi.org/10.3877/j.issn.2096-2738.2019.03.003.
26. Yuan D, Yu B, Liang S, Fei T, Tang H, Kang R, et al. HIV-1 genetic transmission networks among people living with HIV/AIDS in Sichuan, China: a genomic and Spatial epidemiological analysis. Lancet Reg Health West Pac. 2021;18:100318. https://doi.org/10.1016/j.lanwpc.2021.100318.
27. Zhang L, Chow EPF, Jahn HJ, Kraemer A, Wilson DP. High HIV prevalence and risk of infection among rural–to-urban migrants in various migration stages in China: a systematic review and meta-analysis. Sex Transm Dis. 2013;40:136–47. https://doi.org/10.1097/OLQ.0b013e318281134f.
28. Lishui Bureau of Statistics. Lishui statistical yearbook 2023. 2023. http://tjj.lishui.gov.cn/art/2023/11/22/art_1229215928_58847301.html. Accessed 27 Sept 2024.
29. Zhejiang Bureau of Statistics. Series analysis of the seventh population census in Zhejiang Province: Floating population. 2022.Available from: https://tjj.zj.gov.cn/art/2022/7/22/art_1229129214_4956222.html. Accessed 27 Sept 2024.
30. Klein MB, Young J, Dunn D, Ledergerber B, Sabin C, Cozzi-Lepri A, et al. The effects of HIV-1 subtype and ethnicity on the rate of CD4 cell count decline in patients Naive to antiretroviral therapy: a Canadian-European collaborative retrospective cohort study. CMAJ Open. 2014;2:E318–29. https://doi.org/10.9778/cmajo.20140017
31. Wertheim JO, Oster AM, Switzer WM, Zhang C, Panneer N, Campbell E, et al. Natural selection favoring more transmissible HIV detected in united States molecular transmission network. Nat Commun. 2019;10:5788. https://doi.org/10.1038/s41467-019-13723-z
32. Ye J, Hao M, Xing H, Zhang F, Wu H, Lv W, et al. Transmitted HIV drug resistance among individuals with newly diagnosed HIV infection: A multicenter observational study. AIDS. 2020;34:609–19. https://doi.org/10.1097/QAD.0000000000002468
33. Ye J, Hao M, Xing H, Wang Y, Wang J, Feng Y, et al. Characterization of subtypes and transmitted drug resistance strains of HIV among Beijing residents between 2001–2016. PLoS ONE. 2020;15:e0230779. https://doi.org/10.1371/journal.pone.0230779.
34. Ge Z, Feng Y, Li K, Lv B, Zaongo SD, Sun J, et al. CRF01_AE and CRF01_AE cluster 4 are associated with poor immune recovery in Chinese patients under combination antiretroviral therapy. Clin Infect Dis. 2021;72:1799–809. https://doi.org/10.1093/cid/ciaa380.
35. Cao Z, Li J, Chen H, Song C, Shen Z, Zhou X, et al. Effects of HIV-1 genotype on baseline CD4 + cell count and mortality before and after antiretroviral therapy. Sci Rep. 2020;10:15875. https://doi.org/10.1038/s41598-020-72701-4.
36. Wirden M, Tombette F, Lambert-Niclot S, Chaix M, Marque-Juillet S, Bouvier-Alias M, et al. Benefits of HIV-1 transmission cluster surveillance: a French retrospective observational study of the molecular and epidemiological co-evolution of recent Circulating Recombinant forms 94 and 132. J Int AIDS Soc. 2025;28:e26416. https://doi.org/10.1002/jia2.26416.
37. Vrancken B, Zhao B, Li X, Han X, Liu H, Zhao J, et al. Comparative circulation dynamics of the five main HIV types in China. J Virol. 2020;94:e00683–20. https://doi.org/10.1128/JVI.00683-20.
38. Liang S, Xin R. Research progress on the epidemic characteristics of HIV-1 CRF55_01B. Practical Prev Med. 2022;29:766–9.
39. Han X, An M, Zhang W, Cai W, Chen X, Takebe Y, et al. Genome sequences of a novel HIV-1 Circulating Recombinant form, CRF55_01B, identified in China. Genome Announc. 2013;1:e00050–12. https://doi.org/10.1128/genomeA.00050-12.
40. Han X, Takebe Y, Zhang W, An M, Zhao B, Hu Q, et al. A Large-scale survey of CRF55_01B from Men-Who-Have-Sex-with-Men in China: implying the Evolutionary history and public health impact. Sci Rep. 2015;5:18147. https://doi.org/10.1038/srep18147
41. Gan M, Zheng S, Hao J, Ruan Y, Liao L, Shao Y, et al. The prevalence of CRF55_01B among HIV-1 strain and its connection with traffic development in China. Emerg Microbes Infect. 2021;10:256–65. https://doi.org/10.1038/srep18147.
42. Zuo L, Liu K, Liu H, Hu Y, Zhang Z, Qin J, et al. Trend of HIV-1 drug resistance in China: A systematic review and meta-analysis of data accumulated over 17 years (2001–2017). EClinicalMedicine. 2020;18:100238. https://doi.org/10.1016/j.eclinm.2019.100238
43. WHO. HIV drug resistance report 2019. 2019. https://iris.who.int/handle/10665/325891. Accessed 27 Sept 2024.
44. Zhang T, Dou H, Ye H, Tang H, Wang W, Hu W, et al. Transmitted drug resistance and molecular transmission network among treatment-naive HIV-1 patients in Wenzhou, China, 2020–2023. Virol J. 2024;21:257. https://doi.org/10.1186/s12985-024-02528-2.
45. Tan T, Bai C, Lu R, Chen F, Li L, Zhou C, et al. HIV-1 molecular transmission network and drug resistance in Chongqing, China, among men who have sex with men (2018–2021). Virol J. 2023;20:147. https://doi.org/10.1186/s12985-023-02112-0
46. Hong H, Tang C, Liu Y, Jiang H, Fang T, Xu G. HIV-1 drug resistance and genetic transmission network among newly diagnosed people living with HIV/AIDS in Ningbo, China between 2018 and 2021. Virol J. 2023;20:233. https://doi.org/10.1186/s12985-023-02193-x.
47. Zhou F, Kominski GF, Qian HZ, Wang J, Duan S, Guo Z, et al. Expenditures for the care of HIV-infected patients in rural areas in China's antiretroviral therapy programs. BMC Med. 2011;9:6. https://doi.org/10.1186/1741-7015-9-6.
48. Zhang Z, Dai L, Jiang Y, Feng K, Liu L, Xia W, et al. Transmission network characteristics based on Env and gag sequences from MSM during acute HIV-1 infection in Beijing, China. Arch Virol. 2017;162:3329–38. https://doi.org/10.1007/s00705-017-3485-z
49. Zhu M, Sun Z, Zhang X, Luo W, Wu S, Ye L, et al. Epidemiological dynamics and molecular characterization of HIV drug resistance in Eastern China from 2020 to 2023. Front Microbiol. 2024;15:1475548. https://doi.org/10.3389/fmicb.2024.1475548.
50. Li M, Zhou J, Zhang K, Yuan Y, Zhao J, Cui M, et al. Characteristics of genotype, drug resistance, and molecular transmission network among newly diagnosed HIV-1 infections in Shenzhen, China. J Med Virol. 2023;95:e28973. https://doi.org/10.1002/jmv.28973.

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