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Phylogenetic analysis and transmission networks highlight the role of older adults in the transmission of HIV-1 in northern Zhejiang, China

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Abstract

Background In China, human immunodeficiency virus (HIV) poses a significant challenge to older heterosexual adults. This study explored the transmission dynamics of HIV infection among older adults in northern Zhejiang Province, China using phylogenetic analysis and transmission networks.

Methods HIV *pol* sequences without any antiretroviral therapy were obtained from newly diagnosed HIV-positive patients in Huzhou between 2017 and 2022. Pairwise genetic distances between sequences were calculated using HIV Trace based on the Tamura-Nei 93 method. The transmission network was constructed using Cytoscape v3.9.1. The effective reproductive number (*Re*) of each large cluster was estimated using the birth-death skyline model in BEAST v2.4.2. Multivariate logistic regression was performed to identify factors associated with clustering using R v4.4.3 software.

Results A total of 931 HIV *pol* sequences were successfully obtained, of which CRF07_BC (51.7%, 481/931) and CRF01_AE (27.5%, 256/931) were the predominant subtypes. The proportion of CRF07_BC increased from 43.5% in 2017 to 59.8% in 2022, whereas that of CRF01_AE decreased from 33.3% in 2017 to 19.5% in 2022. In total, 448 individuals formed 110 putative transmission networks with a clustering rate of 48.1%, ranging from 2 to 83 sequences per network. Four large clusters were identified, with a higher proportion of individuals aged ≥ 50 years (49.0%) compared to that in small/ medium clusters (35.5%) and non-clustered cases (26.1%). Multivariable logistic regression showed that clustering was associated with age ≥ 50 years (adjusted odds ratio [aOR] = 2.125, 95% confidence interval [CI]: 1.251–3.632), registered households in Huzhou (aOR: 1.677, 95% CI: 1.252–2.249), and CRF07_BC subtype (aOR: 2.119, 95% CI: 1.542–2.924). Only one of the four large transmission clusters had a *Re* > 1, with a high proportion (63.0%, 29/46) of male older adults exposed through commercial sexual contact.

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Conclusions The subtype of CRF07_BC was the predominant subtype locally, showing an increasing trend over time. Molecular transmission network analysis and multivariate logistic regression revealed that older adults play a key role in local HIV-1 transmission. Public health services should target this key population to curb the spread of HIV-1.

Keywords Human immunodeficiency virus (HIV), Older adults, Phylogenetic analysis, Transmission networks

Introduction

The human immunodeficiency virus (HIV) epidemic remains a significant global public health issue, with an estimated 39 million people living with the virus in 2022 and 1.3 million new infections reported in that year according to the data from The Joint United Nations Programme on HIV/AIDS (UNAIDS) [1]. Although UNAIDS has proposed a 95-95-95 target by 2025 [2], achieving this goal poses considerable challenges. As of the most recent data, 86% of people living with HIV are aware of their status, 89% of those aware are receiving treatment, and 93% of those on treatment have achieved viral suppression [1]. In China (excluding Hong Kong, Macao and Taiwan), there were 1.223 million people living with HIV by the end of 2022, with 107,000 newly reported cases in that year. Notably, 97.6% of transmission occurred via sexual contact [3], with heterosexual transmission accounting for 72.0% of infections in 2022. This figure is even higher among adults aged 50 and older, where 90.9% of infections were linked to heterosexual transmission [3, 4], significantly higher than in other countries, such as 39.3% in the United States in 2018 [5], 37.8% in Australia in 2021 [6]. Consequently, HIV presents a significant challenge to older heterosexual adults in China.

Older adults have not traditionally been considered a key population for HIV prevention and control [1]. However, studies have shown that older adults remain sexually active and engaged in a variety of sexual behaviors, including having multiple sexual partners, engaging in commercial sex and having unprotected sex with non-spousal partners [7–10]. Several factors contribute to the increased risk of HIV infection in this age group, such as limited knowledge about HIV, low awareness of self-protection methods, and the presence of chronic diseases [11]. The rising number of HIV infections among older adults is a growing concern in China. The annual number of newly diagnosed HIV infections in this population increased from 32,850 in 2015 to 51,856 in 2022, representing 48.1% of all newly reported cases in 2022 [4, 12, 13]. This trend has also been observed in Zhejiang and other provinces [14–16].

Phylogenetic analysis and transmission networks are increasingly used to monitor HIV transmission dynamics. When combined with epidemiological data, these methods can effectively inform the development of targeted intervention strategies [17–19]. It also allows inference of the effective reproductive number (*Re*) directly from genetic data [20], instead of the clinical surveillance data [21, 22]. *Re* represents the average number of secondary infections attributed to an infected individual when only part of the population is susceptible, being used to assess real-time changes in transmission and the effects of interventions [21–24]. These approaches have been applied to various key populations for HIV prevention and control, including men who have sex with men, commercial sex workers, injecting drug users, and, more recently, older adults [25–29]. However, studies focusing on older adults have often been limited by small sample sizes or short observation periods, despite revealing that this group plays a crucial role in local HIV transmission [29–31]. Few studies have explored the changing molecular profile and transmission efficacy among older adults under long time series.

This study aimed to better understand the transmission dynamics of HIV infection in northern Zhejiang Province, China, using phylogenetic analysis and transmission networks from 2017 to 2022, and to clarify the role of older adults in HIV transmission.

Materials and methods

Study population and sample collection

In total, 1196 HIV-positive cases were newly diagnosed between 2017 and 2022 in Huzhou, Zhejiang Province. Of which, 393 (32.9%) older cases were reported, showing an increasing trend during the period ($z = 3.723$). Whole blood samples, collected using ethylenediaminetetraacetic acid as an anticoagulant, were obtained at the first follow-up from January 2017 to December 2022. All patients were newly confirmed as HIV-1-positive and without any antiretroviral therapy at the time of sampling. Sociodemographic characteristics, transmission routes, and other HIV-related information were collected by local Center for Disease Control and Prevention staff using standardized forms and face-to-face interviews conducted in a private room. All data used in this study were anonymized to protect participants' privacy.

Sequence data

Viral RNA was extracted from plasma isolated from blood samples using the QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. Reverse transcription polymerase chain reaction (RT-PCR) and nested PCR were used to amplify the partial HIV-1 *pol* coding region, which encodes the protease and the first 300 codons of the reverse transcriptase gene. Amplification and sequencing were performed as described previously [32]. The PCR products were sent to Hangzhou TsingKe Bio-Tech Co. for purification and sequencing using five overlapping primers.

Subtypes identification

Sequencher version 5.0 (Gene Codes, Ann Arbor, MI, USA) was used to trim, assemble sequences and interpret ambiguous bases. All sequences were submitted to the HIV-1 Sequence Quality Control Tool (https://www.hiv.lanl.gov/content/sequence/QC/index.html) to confirm sequence quality. Assembled sequences were aligned using MAFFT v7.520 [33] and trimmed to identical lengths (≥ 1,000 bp, HXB2: 2253–3257 nt). Reference sequences were obtained from the Los Alamos National Laboratory HIV Sequence Database (https://www.hiv.lanl.gov), covering the major HIV-1 subtypes and Circulating Recombinant Forms (CRFs). Context-based Modeling for Expeditious Typing (COMET) v2.4.0 [34], HIV Basic Local Alignment Search Tool (BLAST) analysis, and phylogenetic analyses were used to identify HIV-1 subtypes. Unclassified sequences were designated as unique recombinant forms (URFs), and the recombination pattern was confirmed using a jumping profile hidden Markov model (jpHMM) and Simplot v3.5.1 [35]. Phylogenetic analyses were conducted using MEGA v6.0 software to generate a phylogenetic tree by the neighbor-joining method, based on the Kimura two-parameter model with 1000 bootstrap replicates.

Construction of the transmission network

Pairwise genetic distances between sequences were calculated using HIV Trace, based on the Tamura-Nei 93 (TN93) method. The transmission network was constructed in Cytoscape v3.9.1. Genetic distances ranging from 0.001 to 0.020 substitutions per site were evaluated to determine the optimal genetic distance threshold for forming the maximum number of clusters. In the network, each node represents a *pol* sequence, and the edges (links) between nodes indicate potential transmission. Clusters were defined as groups consisting of at least two nodes within the molecular network. Large clusters were defined as those containing 10 or more nodes, and small/medium clusters were defined as having between two and nine nodes.

Estimating the effective reproductive number (*Re*) of large clusters

For each large cluster, the *Re* was estimated with the birth-death skyline model in BEAST v2.4.2 [36]. The best-fitting model was confirmed via jModelTest v2.0.1 based on the Akaike information criterion and Bayesian information criterion [37]. The selected best-fitting model incorporated gamma distribution rate changes, invariant site proportions, and uncorrelated log-normal relaxation molecular clocks while considering the date of diagnosis for each individual. The become uninfectious rate (δ) was calibrated based on the average duration of infection for HIV-1, assuming a constant δ across all large clusters. We used a greater number of dimensions to estimate the mean effective reproductive number for each large cluster. Conducting Markov chain Monte Carlo (MCMC) simulations for 100 million generations ensured the attainment of stationarity, with the initial 10% discarded as burn-in factors. Convergence was assessed using Tracer v1.7, ensuring an effective sample size (ESS) greater than 200 for all the parameters [38]. The analyses were implemented with literature informed set of parameters [39]. This value is used to describe the temporal dynamics of an epidemic, with $Re > 1$ indicating epidemic growth, and $Re < 1$ signifying a decline.

Statistical analyses

The Cochran-Armitage trend test was used to assess the trend characteristics over time. The demographics of subjects in clusters were described as frequencies. Uni- and multivariate logistic regression analyses were performed to explore associations with clustering. Crude and adjusted odds ratios (aORs) and their 95% confidence intervals (CIs) were calculated. All factors in the univariate analysis were included in the multivariate regression model. The final multivariate logistic model was constructed using backward elimination. All analyses were conducted using R v4.4.3 software.

Result

Study population characteristics

In total, 931 HIV *pol* sequences (77.8%, 931/1196) were successfully obtained from newly diagnosed HIV-positive patients in Huzhou between 2017 and 2022. The demographic characteristics of the study subjects are presented in Table 1. The median age was 41.0 years (IQR: 29–54 years), with the majority being men (85.1%). Nearly half of all individuals were divorced/with the spouse passed away (49.4%), and most were of Han ethnicity (94.2%). A significant portion had an education level of junior high school or below (65.8%). Heterosexual transmission (58.8%) was the primary route of infection, and 57.3% of individuals had registered households in Huzhou. Additionally, 57.8% had a first cluster of differentiation 4 (CD4) count of 200–499 after HIV confirmation, and 17.3% had a history of sexually transmitted diseases (Table 1).

Table 1 Factors associated with clustering among HIV infections in northern Zhejiang, Province

Variables	Total (%)	Clustered (%)	no Clustered (%)	cOR (95% CI)	*p* value	aOR (95% CI)	*p* value
Gender							
Female	139 (14.9)	69 (15.4)	70 (14.5)	1			
Male	792 (85.1)	379 (84.6)	413 (85.6)	0.931 (0.649–1.336)	0.697		
Age group							
≤29	241 (25.9)	97 (21.7)	144 (29.8)	1		1	
30–39	201 (21.6)	88 (19.6)	113 (23.4)	1.156 (0.791–1.690)	0.454	1.379 (0.864–2.209)	0.178
40–49	183 (19.7)	83 (18.5)	100 (20.7)	1.232 (0.835–1.818)	0.292	1.487 (0.869–2.557)	0.149
≥50	306 (32.9)	180 (40.2)	126 (26.1)	2.121 (1.506–2.997)	0.000	2.125 (1.251–3.632)	0.005
Marital status							
Single	289 (31.07)	124 (27.7)	165 (34.2)	1		1	
Married	182 (19.5)	77 (17.2)	105 (21.7)	0.976 (0.670–1.419)	0.898	0.613 (0.363–1.029)	0.065
Divorced/with the spouse passed away	460 (49.4)	247 (55.1)	213 (44.1)	1.543 (1.148–2.078)	0.004	0.989 (0.627–1.555)	0.961
Ethnicity							
Han	877 (94.2)	431 (96.2)	446 (92.3)	1			
Other	54 (5.8)	17 (3.8)	37 (7.7)	0.475 (0.258–0.844)	0.013		
Education							
College or above	148 (15.9)	58 (12.9)	90 (18.6)	1			
Senior high school	170 (18.3)	83 (18.5)	87 (18.0)	1.480 (0.949–2.319)	0.085		
Junior/primary school or illiteracy	613 (65.8)	307 (68.5)	306 (63.4)	1.557 (1.082–2.253)	0.018		
Occupation							
Farmers	330 (35.5)	184 (41.1)	146 (30.2)	1			
Domestic workers and unemployed individuals	113 (12.1)	50 (11.2)	63 (13.0)	0.579 (0.373–0.892)	0.014		
Workers	280 (30.1)	141 (31.5)	139 (28.8)	0.845 (0.614–1.162)	0.300		
Cadres, teachers and doctors	27 (2.9)	17 (3.8)	10 (2.1)	1.320 (0.600-3.007)	0.495		
Commercial service workers	96 (10.3)	47 (10.5)	49 (10.1)	0.621 (0.390–0.982)	0.043		
Others	85 (9.1)	43 (9.6)	42 (8.7)	0.846 (0.524–1.363)	0.491		
Registered household							
Other	398 (42.7)	153 (34.2)	245 (50.7)	1		1	
Huzhou	533 (57.3)	295 (65.8)	238 (49.3)	1.985 (1.525–2.589)	0.000	1.677 (1.252–2.249)	0.001
Transmission route							
MSM/other	384 (41.2)	181 (40.4)	203 (42.0)	1			
Heterosexual behavior	547 (58.8)	267 (59.6)	280 (58.0)	1.069 (0.824–1.389)	0.614		
Sexually transmitted diseases history							
No	770 (82.7)	366 (81.7)	404 (83.6)	1			
Yes	161 (17.3)	82 (18.3)	79 (16.4)	1.146 (0.816–1.610)	0.433		
First clusters of differentiation 4 level after HIV confirmation							
<200	297 (32.1)	143 (32.1)	154 (32.2)	1			
200–499	535 (57.8)	266 (59.6)	269 (56.2)	1.065 (0.802–1.415)	0.664		
≥500	93 (10.1)	37 (8.3)	56 (11.7)	0.712 (0.441–1.139)	0.159		
Subtype							
CRF01_AE	256 (27.5)	100 (22.3)	156 (32.3)	1		1	
CRF07_BC	481 (51.7)	285 (63.6)	196 (40.6)	2.268 (1.666-3.100)	0.000	2.119 (1.542–2.924)	0.000
Others	194 (20.8)	63 (14.1)	131 (27.1)	0.750 (0.506–1.108)	0.150	0.683 (0.453–1.024)	0.067

Distribution of HIV-1 subtypes

Overall, CRF07_BC (51.7%, 481/931) and CRF01_AE (27.5%, 256/931) were the predominant subtypes, followed by CRF08_BC (8.6%, 80/931), B (2.7%, 25/931), CRF55_01B (1.8%, 17/931), CRF67_01B (1.2%, 11/931), CRF85_BC (1.3%, 12/931), CRF59_01B (0.4%, 4/931), A1 (0.4%, 4/931), CRF02_AG (0.3%, 3/931), C (0.3%, 3/931), CRF68_01B (0.2%, 2/931), BC (0.1%, 1/931), CRF52_01B (0.1%, 1/931), CRF65_cpx (0.1%, 1/931), CRF79_0107 (0.1%, 1/931) (Fig. S1). Additionally, 29 sequences were classified as unique recombinant forms (URFs) (3.1%, 29/931), with 28 sequences displaying a recombinant genomic structure of CRF01_AE and CRF07_BC, and the remaining sequence consisting of CRF01_AE and B. The proportion of CRF07_BC increased across years from 43.5% in 2017 to 59.8% in 2022 ($z = 3.266$, $P = 0.001$), and that of CRF01_AE decreased from 33.3% in 2017 to 19.5% in 2022 ($z = 3.761$, $P < 0.001$) (Fig. 1). In contrast, the proportion of individuals infected through homosexual transmission did not show a significant change over time ($z = 0.351$, $p = 0.726$). However, the proportion of older individuals increased over time ($z = 3.251$, $P = 0.001$), with the highest proportion observed among those with the subtype of CRF07_BC (Fig. 1).

Molecular transmission network analysis

Molecular transmission networks were analyzed based on *pol* sequences using an optimal genetic distance threshold of 0.01 substitutions per site. In total, 448 individuals formed 110 putative transmission networks, with sizes ranging from 2 to 83 sequences, encompassing eight subtypes. The subtype of CRF07 BC formed 50 transmission clusters, including the largest cluster of 83 cases (range: 2–83, median: 2). The subtype of CRF01 AE formed 34 transmission clusters, with the largest containing 14 cases (range: 2–14, median: 2). Other subtypes accounted for 26 transmission clusters, with the largest comprising 6 cases (range: 2–6, median: 2) (Fig. 2).

Overall, 448 of the 931 individuals belonged to transmission networks, yielding a clustering rate of 48.1%. Among different age groups, the clustering rate for individuals younger than 30 years showed a significant downward trend, decreasing from 46.8% in 2017 to 17.5% in 2022 ($z = 3.183$, $p = 0.001$). However, no significant changes were observed in the clustering rates for the 30–39, 40–49, and ≥50 age groups ($P > 0.05$). The contribution of individuals aged ≥ 50 years to transmission networks across clusters of varying sizes was also evaluated. Individuals aged ≥ 50 years constituted the highest proportion in large clusters (49.0%), compared to those in small/ medium clusters (35.5%) and non-clustered cases (26.1%) ($\chi^2 = 29.336$, $P < 0.001$, Fig. 3).

We performed multivariate logistic regression to assess the associations between clustering in a putative transmission network and various demographic or behavioral characteristics. The odds of clustering were 2.125 (95% CI: 1.251–3.632) times higher in individuals aged ≥ 50 years compared to those under 30 years of age. Additionally, individuals with registered households in Huzhou had significantly higher odds of clustering (aOR = 1.677, 95% CI: 1.252–2.249) compared to those from other areas. Furthermore, individuals with the subtype of

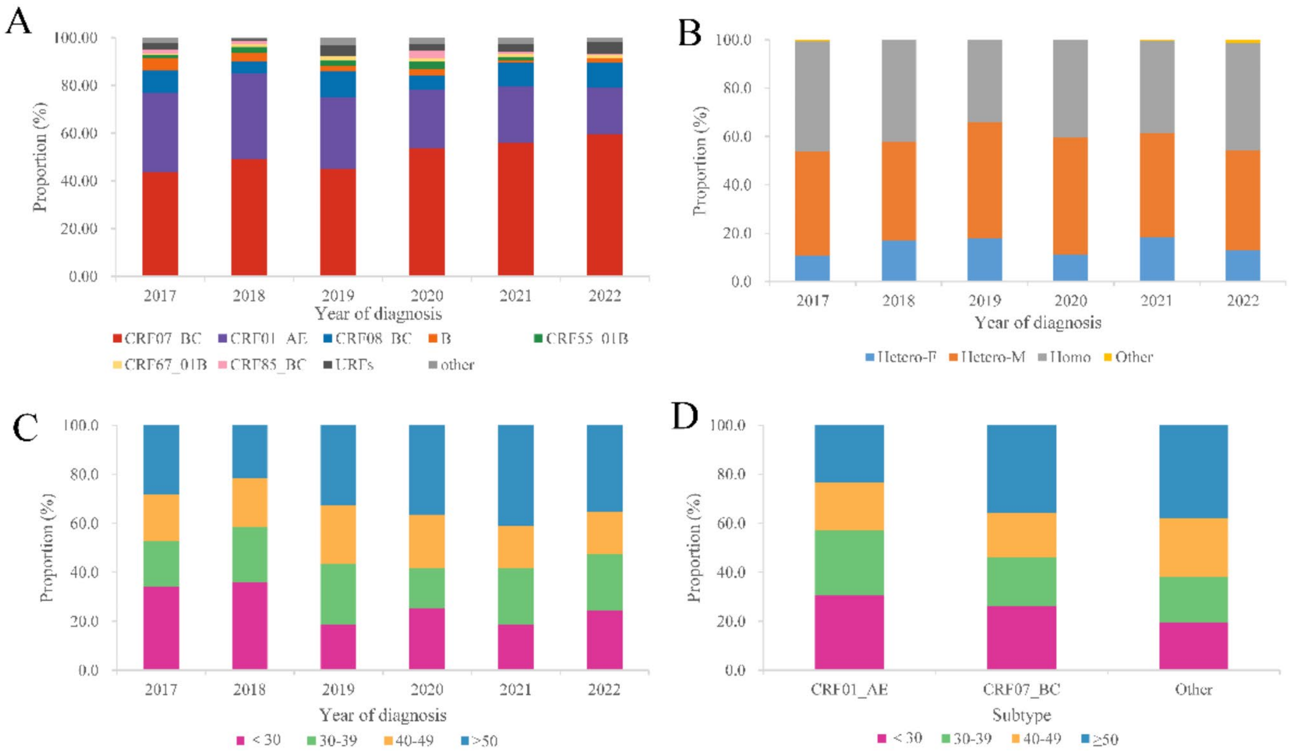

Fig. 1 Subtype compositions and characteristics of HIV infections identified in Huzhou from 2017–2022. (**A**) The subtype distribution of the 931 newly diagnosed cases from 2017–2022. (**B**) Transmission route of the 931 newly diagnosed cases from 2017–2022. (**C**) Age distribution of the 931 newly diagnosed cases from 2017–2022. (**D**) The age distribution among subtypes

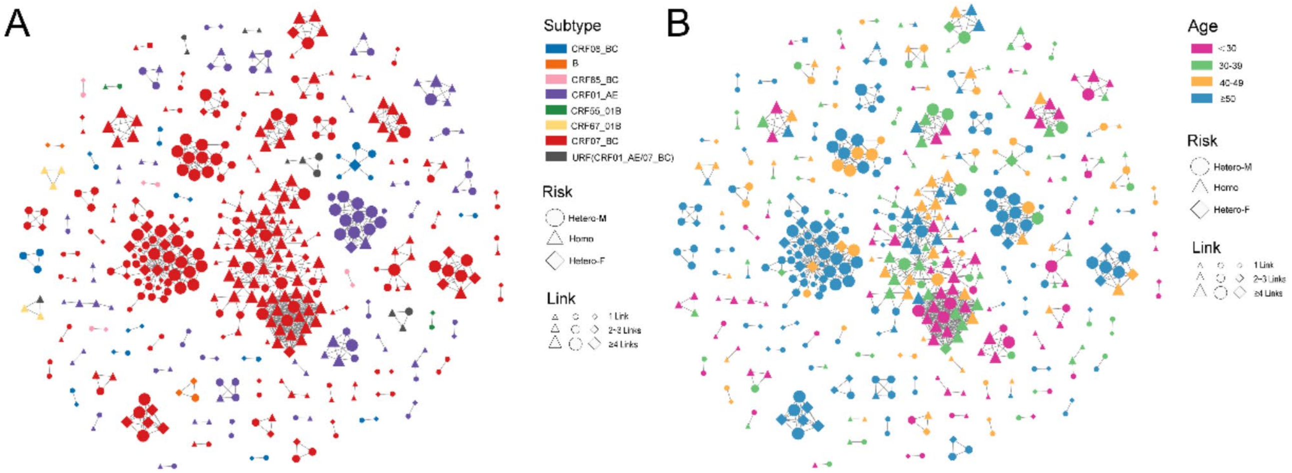

Fig. 2 The molecular transmission networks among newly diagnosed individuals. All edges represent a genetic distance between nodes of less than 0.01 substitutions/site, and the colour of the node indicates (**A**) the different HIV-1 subtypes and (**B**) the different age groups. Different shapes represent risk, and the node size indicates the number of associated links

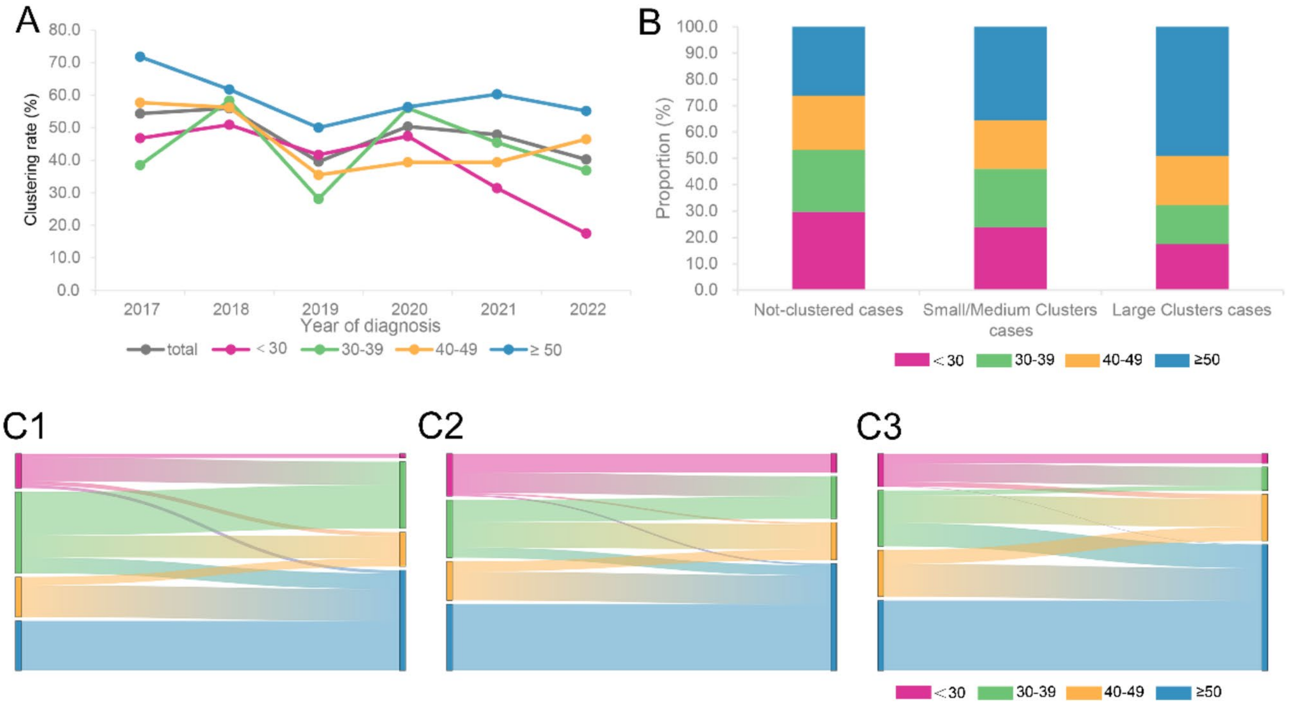

Fig. 3 The clustering rate, proportion and linkages analysis in different age groups. (**A**) Clustering rate in molecular networks of different age groups. (**B**) The proportion of different age group among not-clustered cases, small/medium clusters and large clusters. (**C**) Linkages analysis of different age groups with the main HIV-1 subtypes in the network. The colour indicates the different age groups. (**C1**) The Sankey diagram of CRF01_AE, (**C2**) The Sankey diagram of CRF07_BC, (**C3**) The Sankey diagram of other HIV-1 subtypes

CRF07_BC were more likely to cluster than those with the subtype of CRF01_AE (aOR = 2.119, 95% CI: 1.542–2.924) (Table 1).

Age groups according to HIV-1 subtypes in the network

Sankey diagrams were created to illustrate the relationships among the four age groups and different HIV-1 subtypes within the transmission networks, based on individual linkages (Fig. 3). The strongest correlations were observed within the same age group for individuals aged ≥ 50 years in the CRF07_BC, CRF01_AE, and other subtypes, accounting for 24.1% (163/676), 32.2% (39/121), and 34.1% (15/44), respectively. Among cross-age group interactions, the highest correlations were between individuals aged ≥ 50 years and those aged 40–49 years, accounting for 15.5% (105/676) in CRF07_BC,

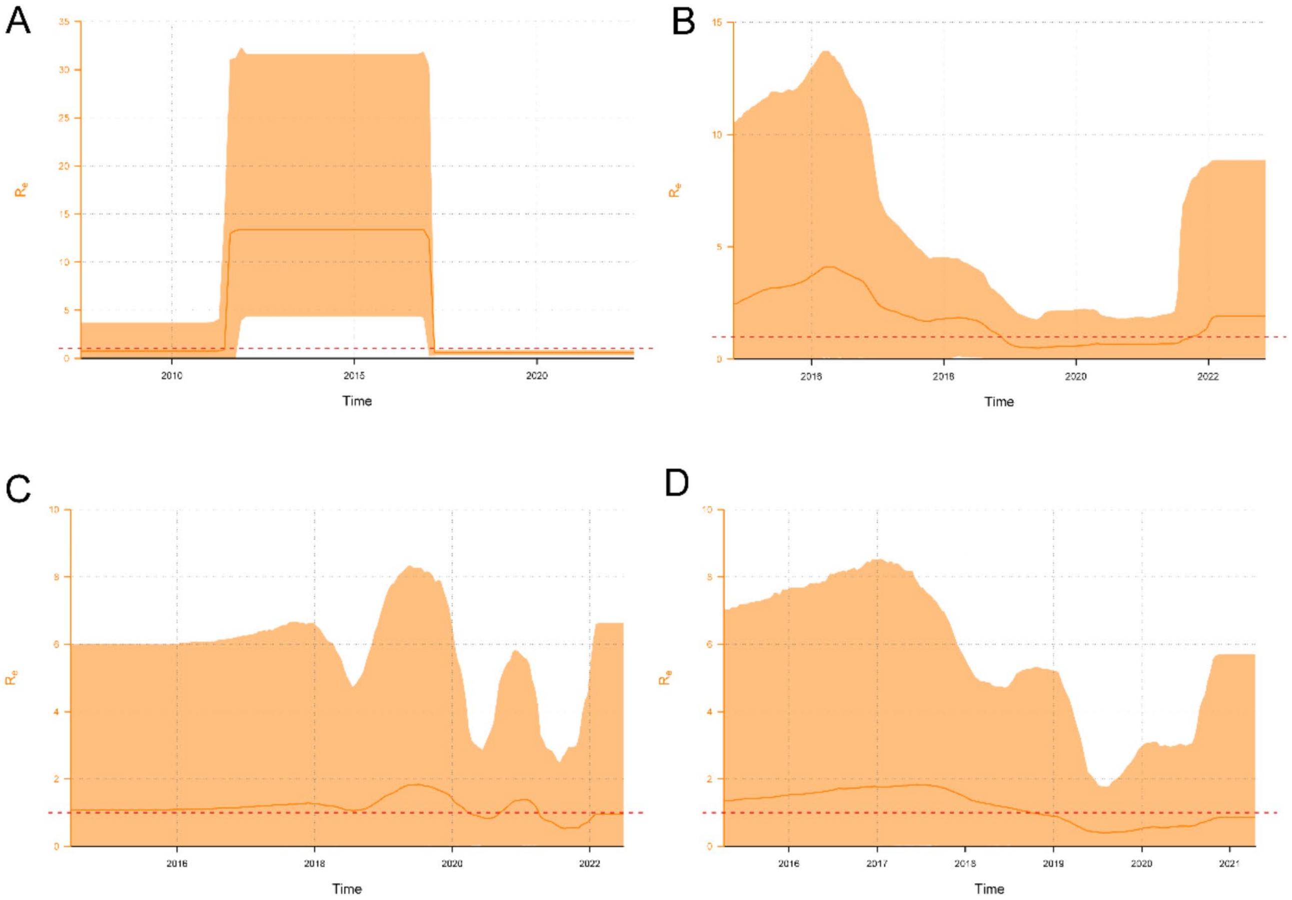

Fig. 4 Estimating effective reproductive number of large clusters

(14.0%, 17/121) in CRF01_AE and 15.9% (7/44) in other subtypes.

Characteristics and *Re* of large clusters

Four clusters comprised 10 or more nodes, with three belonging to the CRF07_BC, and one to CRF01_AE. The largest cluster was predominantly linked to homosexual contact (73.5%, 61/83), whereas the remaining three clusters were primarily driven by heterosexual contact among males, accounting for 76.1% (35/46), 85.7% (12/14), and 100.0% (12/12), respectively. In terms of age distribution across the large clusters, the largest cluster was dominated by individuals under 30 years of age (32.5%, 27/83), whereas the other three clusters were dominated by individuals aged ≥ 50 years, representing 87.0% (40/46), 78.6% (111/14) and 58.3% (7/12), respectively.

The peak *Re* for CRF07_BC-Cluster 1 occurred between 2013 and 2017, after which it declined and remained below 1 from 2017 onward (Fig. 4A). For CRF07_BC-Cluster 3, the *Re* peaked during 2019–2020, then fluctuated and fell below 1 by 2022 (Fig. 4C). CRF01_AE-Cluster 4 reached its peak *Re* between 2017 and 2018, then declined and increased gradually below 1 by 2021 (Fig. 4D). In contrast, the *Re* of CRF07_BC-Cluster 2 peaked in 2017, but declined and fluctuated, reaching a *Re* of 2.2 after 2022 (Fig. 4B).

In CRF07_BC-Cluster 2, where $Re > 1$, 73.9% (34/46) of individuals reported a history of commercial sexual contact (comprising 32 males [29 aged ≥ 50, 3 aged < 50] and 2 females). Additionally, 13.0% (6/46) indicated having extramarital non-commercial sexual contact, and another 13.0% (6/46) stated that their spouse was their only sexual partners. One female, identified as HIV positive by public health authorities while working as a female sex worker, had the highest degree of 37 in the cluster. In total, five sero-concordant couples were reported within this cluster.

Discussion

We conducted a high-density sampling of 931 newly diagnosed patients in a developed city in northern Zhejiang from 2017 to 2022 to perform a phylogenetic analysis of HIV-1 epidemic strains. The subtype of CRF07_BC was predominant locally and exhibited an upward trend over time. Specifically, we generated molecular transmission networks and conducted multiple logistic analyses to illustrate the role of older adults in transmission. Our findings clearly indicate that older adults played a crucial role in the transmission dynamics observed in this study.

The study revealed that HIV-1 subtypes were diverse, identifying 16 distinct subtypes along with some URFs, with CRF07_BC being the predominant subtype, followed by CRF01_AE. CRF07_BC showed an increasing trend from 2017 to 2022, whereas CRF01_AE decreased during the same period. Previous studies indicated that CRF07_BC and CRF01_AE were the predominant subtypes in China in 2015 and 2018 [40], as well as in Huzhou in 2015 [41] and Shenzhen from September 2019 to December 2020 [42]. The upward trend of CRF07_BC and the downward trend of CRF01_AE were also observed in other studies conducted in China and Shenzhen [40, 42]. Some research has suggested that the upward trend of CRF07_BC is attributable to the population of men who have sex with men [43, 44]; however, our study emphasizes the significant influence of the older population instead. Alongside the increasing trend of CRF07_BC, the diversity of HIV-1 subtypes and URFs across years, highlights the complexity of HIV epidemiology and underscores the importance of ongoing genetic surveillance of the virus.

In our study, multivariate logistic regression indicated that individuals aged ≥ 50 years and those with registered households in Huzhou were more likely to be clustered within the transmission networks. Notably, the proportion of individuals aged ≥ 50 years was highest in large clusters, suggesting that older individuals from local registered residences contribute significantly to local HIV transmission and should be prioritized for intervention. This finding contrasts with many other studies that identify floating populations as a contributing factor to the local HIV epidemic [19, 45]. Currently, HIV infections among older adults aged ≥ 50 years represent a considerable burden in China [4, 13]. The neglect of the sexual needs of the older population by both partners and society has led to high-risk sexual behaviors. Epidemiological surveys and molecular network analyses have shown that HIV infections among the elderly primarily result from commercial heterosexual behavior, highlighting the transmission links between this demographic and female sex workers [31, 46]. Furthermore, the substantial reliance on lower-tier female sex workers has facilitated an increase in the prevalence of HIV among older clients [47].

There was a tendency towards increased popularity of transmission network analyses based on HIV-1 sequence data, of which distance-based methodologies were often used to define [48]. The threshold selection is the foundation for network construction. Some studies selected 1.5% as the optimal threshold based on subtype B threshold [49–51]. Here, we used optimal thresholds for subtypes according to the principle outlined in previous studies with the maximum number of clusters [52–54]. In our study, four large clusters were identified, with the largest consisting of 83 individuals, who tended to be younger and exhibited a higher proportion of homosexual contact. The *Re* remained high from 2013 to 2017 but then declined rapidly to below one, indicating decline in HIV transmission during the study period. This decline may be linked to public health interventions, particularly the expansion of highly active antiretroviral therapy in China since the end of 2016 [55]. Notably, only one of the remaining three large transmission clusters had a *Re* greater than 1; this cluster tended to include older individuals and had a higher proportion of commercial heterosexual contact. Although a key individual identified as a female sex worker was present in this cluster, the estimated *Re* remained above 1, suggesting epidemic growth in HIV transmission during this period. To effectively control transmission, active HIV testing targeting local older individuals, along with additional specific interventions (e.g., partner services), should be implemented.

Our results should be interpreted with the following limitations in mind. First, the data do not allow for an assessment of the directionality of transmission, such that we cannot distinguish between transmitters and recipients within our clusters. However, we found that older individuals are more likely to be involved in HIV transmission clusters, underscoring the importance of directing public health services toward this key population. Second, the incomplete coverage of sequences may subject our results to sampling bias. We anticipate that improving the completeness of molecular surveillance data in future will enable us to draw even stronger inferences about the characteristics of the transmission network. Third, the threshold genetic distance of 0.01 substitutions/site applied to construct the transmission network, which was selected based on experience and calculation, may not have been the optimal threshold for our analysis.

Conclusions

Our study applied phylogenetic analysis and transmission networks to reveal the epidemic patterns of HIV-1 epidemic strains and the role of older adults played in transmission in northern Zhejiang, China. The subtype of CRF07_BC was predominant locally and exhibited an increasing trend over time. Our findings highlight the crucial role of older adults played in local HIV-1 transmission, with results of older adults being more likely to be clustered, being the highest proportion in large clusters and having a transmission cluster acquired *Re* greater than 1. Therefore, public health services should target this key population to curb the spread of HIV-1.

Abbreviations

HIV	Human immunodeficiency virus
Re	The effective reproductive number
aOR	Adjusted odds ratio
cOR	Crude odds ratio
CI	Confidence interval
UNAIDS	The Joint United Nations Programme on HIV/AIDS
RT-PCR	Reverse transcription polymerase chain reaction
CRF	Circulating Recombinant Form
CD4	Cluster of differentiation 4
URF	Unique recombinant form

Supplementary information

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Supplementary Material 1

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Author contributions

WJC analyzed and interpreted the data, drafted and revised the manuscript. FQ participated in the phylogenetic analysis and transmission networks process. WC, JFZ, LH, JJ analyzed the data, XQL conducted the epidemiological investigation. XJZ participated in acquiring viral sequences. HX, YF and PZ provided important technical guidance. CLC and XHP designed the research, supervised the study, and revised the manuscript. All authors have read and approved the final manuscript.

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Data availability

The datasets used and analyzed are available from the corresponding author (Chengliang Chai, Email: chlchai@cdc.zj.cn) upon reasonable request.

Declarations

Ethics approval and consent to participate

This study was approved by the ethics committee of the National Center for AIDS/STD Control and Prevention, Chinese Center for Disease Control and Prevention (number: X231028769). Participants provided written informed consent. The study was conducted in accordance with the «Helsinki Declaration».

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. UNAIDS. Fact sheet - Latest global and regional statistics on the status of the AIDS epidemic. In. 2023.
2. UNAIDS. Political Declaration on HIV and AIDS: Ending Inequalities and Getting on Track to End AIDS by 2030. In. 2021.
3. Han MJ. Analysis of the situation of the AIDS epidemic and prospects for prevention and treatment in China. Chin J AIDS STD. 2023;29(03):247–50.
4. Tang Houlin J, Yichen. Lv fan: HIV/AIDS epidemic in the elderly and prevention and control challenges in China. Chin J Epidemiol. 2023;44(11):1669–72.
5. HIV Surveillance Report. 2018 (Preliminary). In., vol. 30: Centers for Disease Control and Prevention; Published November 2019.
6. Kirby Institute, The University of New South Wales. 2022. Access: https://www.kirby.unsw.edu.au/research/reports/australian-hiv-public-access-dataset-2021
7. Lindau ST, Schumm LP, Laumann EO, Levinson W, O'Muircheartaigh CA, Waite LJ. A study of sexuality and health among older adults in the united States. N Engl J Med. 2007;357(8):762–74.
8. Wang B, Peng X, Liang B, Fu L, Lu Z, Li X, et al. Sexual activity, sexual satisfaction and their correlates among older adults in China: findings from the sexual well-being (SWELL) study. Lancet Reg Health West Pac. 2023;39:100825.
9. Field N, Mercer CH, Sonnenberg P, Tanton C, Clifton S, Mitchell KR, et al. Associations between health and sexual lifestyles in Britain: findings from the third National survey of sexual attitudes and lifestyles (Natsal-3). Lancet. 2013;382(9907):1830–44.
10. Sun X, Feng C, Feng L, Xiao T, Zhao X, Liu H, et al. An exploratory transmission mode of HIV/AIDS among older people based on data from multiple sources in China. Sci Rep. 2022;12(1):16077.
11. Zou HC, He N. Sexual health among older adults. Chin J Epidemiol. 2023;44(12):1864–8.
12. Xing J, Li YG, Tang W, Guo W, Ding Z, Ding G, et al. HIV/AIDS epidemic among older adults in China during 2005–2012: results from trend and Spatial analysis. Clin Infect Dis. 2014;59(2):e53–60.
13. Zhang Y, Cai C, Wang X, Li Y, Tang H, Ma J. Disproportionate increase of new diagnosis of HIV/AIDS infection by sex and Age - China, 2007–2018. China CDC Wkly. 2020;2(5):69–74.
14. Huang G, Cheng W, Xu Y, Yang J, Jiang J, Pan X, et al. Spatiotemporal pattern and its determinants for newly reported HIV/AIDS among older adults in Eastern China from 2004 to 2021: retrospective analysis study. JMIR Public Health Surveill. 2024;10:e51172.
15. Ge XM, Yang WM, Zhu QY, Wu XL, Shen ZY, Zhu JH, et al. Epidemiological characteristics of HIV/AIDS in Guangxi Zhuang autonomous region, 2010–2017. Chin J Epidemiol. 2019;40(3):315–21.
16. Zhang D, Dai S, Jin L, Shen Y, Liu A, Wu J, et al. Characterizations of newly reported HIV/AIDS patients aged 50 years and above in Anhui Province, 2016–2021. Chin J AIDS STD. 2023;29(04):466–7.
17. Ragonnet-Cronin M, Hu YW, Morris SR, Sheng Z, Poortinga K, Wertheim JO. HIV transmission networks among transgender women in Los Angeles County, CA, USA: a phylogenetic analysis of surveillance data. Lancet HIV. 2019;6(3):e164–72.
18. Novitsky V, Steingrimsson J, Howison M, Dunn C, Gillani FS, Manne A, et al. Longitudinal typing of molecular HIV clusters in a statewide epidemic. Aids. 2021;35(11):1711–22.
19. Zhang J, Xu K, Jiang J, Fan Q, Ding X, Zhong P, et al. Combining molecular network analysis and field epidemiology to quantify local HIV transmission and highlight ongoing epidemics. Int J Infect Dis. 2023;128:187–93.
20. Stadler T, Kühnert D, Bonhoeffer S, Drummond AJ. Birth-death skyline plot reveals Temporal changes of epidemic spread in HIV and hepatitis C virus (HCV). Proc Natl Acad Sci U S A. 2013;110(1):228–33.
21. Lima VD, Zhu J, Barrios R, Toy J, Joy JB, Williams BG, et al. Longitudinal evolution of the HIV effective reproduction number following sequential expansion of treatment as prevention and pre-exposure prophylaxis in British Columbia, Canada: a population-level programme evaluation. Lancet HIV. 2024;11(7):e461–9.

22. Khailaie S, Mitra T, Bandyopadhyay A, Schips M, Mascheroni P, Vanella P, et al. Development of the reproduction number from coronavirus SARS-CoV-2 case data in Germany and implications for political measures. BMC Med. 2021;19(1):32.
23. Bsat R, Chemaitelly H, Coyle P, Tang P, Hasan MR, Kanaani ZA, et al. Characterizing the effective reproduction number during the COVID-19 pandemic: insights from Qatar's experience. J Glob Health. 2022;12:05004.
24. Chen YH, Farnham PG, Hicks KA, Sansom SL. Estimating the HIV effective reproduction number in the united States and evaluating HIV elimination strategies. J Public Health Manag Pract. 2022;28(2):152–61.
25. Bachmann N, Kusejko K, Nguyen H, Chaudron SE, Kadelka C, Turk T, et al. Phylogenetic cluster analysis identifies virological and behavioral drivers of human immunodeficiency virus transmission in men who have sex with men. Clin Infect Dis. 2021;72(12):2175–83.
26. Springfield O, Brouwer KC, Avila-Rios S, Morales-Miranda S, Mehta SR. Molecular epidemiology of HIV-1 among adult female sex workers at the Guatemala-Mexico border. Glob Public Health. 2023;18(1):2278873.
27. Golden MR, Lechtenberg R, Glick SN, Dombrowski J, Duchin J, Reuer JR et al. Outbreak of Human Immunodeficiency Virus Infection Among Heterosexual Persons Who Are Living Homeless and Inject Drugs - Seattle, Washington, 2018. MMWR Morb Mortal Wkly Rep. 2019;68(15):344–349.
28. Peters PJ, Pontones P, Hoover KW, Patel MR, Galang RR, Shields J, et al. HIV infection linked to injection use of oxymorphone in Indiana, 2014–2015. N Engl J Med. 2016;375(3):229–39.
29. Zhang F, Yang Y, Liang N, Liang H, Chen Y, Lin Z, et al. Transmission network and phylogenetic analysis reveal older male-centered transmission of CRF01_AE and CRF07_BC in Guangxi, China. Emerg Microbes Infect. 2023;12(1):2147023.
30. Wu J, Zhang Y, Shen Y, Wang X, Xing H, Yang X, et al. Phylogenetic analysis highlights the role of older people in the transmission of HIV-1 in Fuyang, Anhui Province, China. BMC Infect Dis. 2019;19(1):562.
31. Jiang J, Fan Q, Zhang J, Luo M, Ding X, Pan X, et al. A geographic hotspot and emerging transmission cluster of the HIV-1 epidemic among older adults in a rural area of Eastern China. AIDS Res Hum Retroviruses. 2020;36(9):712–20.
32. 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(10):1668–76.
33. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 2013;30(4):772–80.
34. Struck D, Lawyer G, Ternes AM, Schmit JC, Bercoff DP. COMET: adaptive context-based modeling for ultrafast HIV-1 subtype identification. Nucleic Acids Res. 2014;42(18):e144.
35. Schultz AK, Zhang M, Bulla I, Leitner T, Korber B, Morgenstern B, et al. JpHMM: improving the reliability of recombination prediction in HIV-1. Nucleic Acids Res. 2009;37:W647–651. (Web Server issue).
36. Bouckaert R, Vaughan TG, Barido-Sottani J, Duchêne S, Fourment M, Gavryushkina A, et al. BEAST 2.5: an advanced software platform for bayesian evolutionary analysis. PLoS Comput Biol. 2019;15(4):e1006650.
37. Darriba D, Taboada GL, Doallo R, Posada D. jModelTest 2: more models, new heuristics and parallel computing. Nat Methods. 2012;9(8):772.
38. Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. Posterior summarization in bayesian phylogenetics using tracer 1.7. Syst Biol. 2018;67(5):901–4.
39. Fan Q, Zhang J, Pan X, Ding X, Xing H, Feng Y et al. Insights into the molecular network characteristics of major HIV-1 subtypes in developed Eastern China: a study based on comprehensive molecular surveillance data. Infection 2024.
40. Hao J, Zheng S, Gan M, Dong A, Kang R, Li M, et al. Changing proportions of HIV-1 subtypes and transmitted drug resistance among newly diagnosed HIV/AIDS Individuals - China, 2015 and 2018. China CDC Wkly. 2021;3(53):1133–8.
41. Wu X, Zha B, Ji L, Jin M, Dong Z, Yang Z. Gene subtype analysis of HIV - 1 strains isolated from different population groups in Huzhou. Chin J Health Lab Tec. 2015;25(04):538–40.
42. 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(7):e28973.
43. Zhang D, Zheng C, Li H, Li H, Liu Y, Wang X, et al. Molecular surveillance of HIV-1 newly diagnosed infections in Shenzhen, China from 2011 to 2018. J Infect. 2021;83(1):76–83.
44. Zhang W, Chen J, Pan X, Zhang J, Guo Z, Luo Y, et al. Trends of HIV-1 subtypes among young people in Hangzhou, China. AIDS Res Hum Retroviruses. 2017;33(3):219–27.
45. Okano JT, Sharp K, Valdano E, Palk L, Blower S. HIV transmission and source-sink dynamics in sub-Saharan Africa. Lancet HIV. 2020;7(3):e209–14.
46. Chen X, Qin C, Chen R, Huang Y, Xu Y, Tang Q, et al. Epidemiological profile and molecular genetic characterization of HIV-1 among female sex workers and elderly male clients in Guangxi, China. Emerg Microbes Infect. 2021;10(1):384–95.
47. Zhang L, Chow EP, Su S, Yiu WL, Zhang X, Iu KI, et al. A systematic review and meta-analysis of the prevalence, trends, and geographical distribution of HIV among Chinese female sex workers (2000–2011): implications for preventing sexually transmitted HIV. Int J Infect Dis. 2015;39:76–86.
48. Hassan AS, Pybus OG, Sanders EJ, Albert J, Esbjörnsson J. Defining HIV-1 transmission clusters based on sequence data. Aids. 2017;31(9):1211–22.
49. Lebedev A, Kuznetsova A, Kim K, Ozhmegova E, Antonova A, Kazennova E et al. Identifying HIV-1 transmission clusters in Uzbekistan through analysis of molecular surveillance data. Viruses 2022;14(8).
50. Stecher M, Chaillon A, Eberle J, Behrens GMN, Eis-Hübinger AM, Lehmann C, et al. Molecular epidemiology of the HIV epidemic in three German metropolitan Regions - Cologne/Bonn, Munich and Hannover, 1999–2016. Sci Rep. 2018;8(1):6799.
51. Rich SN, Cook RL, Mavian CN, Garrett K, Spencer EC, Salemi M, et al. Network typologies predict future molecular linkages in the network of HIV transmission. Aids. 2023;37(11):1739–46.
52. Liu M, Han X, Zhao B, An M, He W, Wang Z, et al. Dynamics of HIV-1 molecular networks reveal effective control of large transmission clusters in an area affected by an epidemic of multiple HIV subtypes. Front Microbiol. 2020;11:604993.
53. Wertheim JO, Kosakovsky Pond SL, Forgione LA, Mehta SR, Murrell B, Shah S, et al. Social and genetic networks of HIV-1 transmission in new York City. PLoS Pathog. 2017;13(1):e1006000.
54. Cao R, Lei S, Chen H, Ma Y, Dai J, Dong L, et al. Using molecular network analysis to understand current HIV-1 transmission characteristics in an inland area of Yunnan, China. Epidemiol Infect. 2023;151:e124.
55. Zhang F, Zhao Y, Ma Y, Zhao D, Dou Z, Wu Y, et al. Progress and achievements of free HIV antiretroviral treatment in China. Chin J AIDS STD. 2022;28(01):6–9.

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