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Increase in HIV-1 transmitted drug resistance among untreated 16~25-y-old youths in the China-Myanmar border areas during 2009~2017

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Abstract

Background Transmitted drug resistance (TDR) can affect antiretroviral therapy (ART) efficacy. Surveillance drug resistance mutations in untreated youths newly reported with HIV-1 are highly representative of local TDR. We investigated HIV-1 TDR, TDR transmission based on molecular networks, and the effect of TDR mutations (TDRMs) on the CD4 count among youths in the China-Myanmar border area near the "Golden Triangle" to better understand TDR and guide ART.

Methods From 2009 to 2017, 573 ART-naïve youths (16~25 y) newly reported with HIV-1 were enrolled. CD4 counts were obtained from whole blood. HIV *pol* gene sequences were amplified from RNA extracted from plasma. The Stanford REGA program and phylogenetic trees were used to determine genotypes. TDRMs were analyzed using the Stanford Calibrated Population Resistance tool. TDR transmission was evaluated from molecular networks of HIV-1 *pol* genes.

Results The average prevalence of TDR was 6.3%, and the resistance to NNRTIs, NRTIs, and PIs was 3.49%, 2.62%, and 0.52%, respectively. TDR prevalence increased significantly during the period 2009~2017 (3.92%~9.48%, $p<0.05$). The mean CD4 count was significantly lower among individuals with TDRMs (373/mm^3 vs. 496/mm^3, $p=0.013$). The rate of network entry of youths harboring TDRMs (63.89%) was significantly higher than that of youths without TDRMs (44.9%).

Conclusions The HIV-1 TDR increase and low CD4 count of patients with TDRMs in Dehong at the China-Myanmar border suggest the need for early ART and completion of resistance testing before initiating ART in HIV hotspots. Youths with TDRMs are likely to have links to others, necessitating intervention in onward transmission.

Introduction

Worldwide, 37.9 million people live with HIV, and 24.5 million people were receiving antiretroviral therapy (ART) at the end of 2018.[1] However, with the increasing use of ART, the problem of drug resistance (DR) has also been a focus. DR, transmitted HIV drug resistance (TDR) and acquired drug resistance (ADR) are caused by one or more mutations in the viral genetic structure that affect the efficacy of ART. ADR develops during viral replication in the presence of ART drugs. TDR is found in ART–naive populations and occurs when uninfected individuals are infected with virus that carries DR mutations.[2] TDR surveys can effectively guide future first- and second-line ART regimens, help prevent mother-to-child transmission and aid pre-/postexposure prophylactic therapy.[3]

TDR has been detected in ART-naïve populations in the United States, Switzerland, Peru, Argentina, Brazil, and Colombia, and the estimate of the number of people infected with virus carrying TDR mutations has increased with time.[3-5] In these populations, demographic and clinical characteristics usually cannot predict TDR. Interestingly, some studies reported higher CD4 counts in people with TDR mutations (TDRMs) than in individuals without TDRMs,[6, 7] but others came to the opposite conclusion.[8, 9] Additional evidence is needed to verify an association between TDR and decreased CD4 count. In

addition, molecular networks analysis can identify genetically similar sequences; viruses with close genetic similarity indicate the spread between individuals. These network-forming clusters increase the efficiency of HIV spread in a population.[10] Molecular network analysis can play a crucial role in revealing potential transmission relationships and in evaluating the relationship of TDR within the context of a cohort.[9, 11]

Free ART has been widely provided in China since 2006. At the end of 2019, 0.958 million people in China were living with HIV, and 0.83 million people (86.6%) were accessing ART.[12] Yunnan Province is in the southwestern part of China, bordering Myanmar, Vietnam, and Laos. By the end of 2016, the number of people living with HIV/AIDS in Yunnan (91,986) was the second highest of all provinces in China; of these, 70,577 (76.7%) were receiving ART. Dehong city, as a hotspot of HIV recombination and transmission,[13] is a major city for trading in the Yunnan-Myanmar border area. Dehong shares an international border with Kachin and with Shan state, Myanmar, two of the major states of the "Golden Triangle" (Fig 1). The "Golden Triangle" is one of the world's largest drug production centers,[14] and there is a severe HIV transmission problem around this area. In China, the first HIV spread in people who inject drugs (PWID) was found in Dehong.[15] According to the WHO HIV drug resistance (HIVDR) threshold survey method, untreated youths (<25 y) are more likely to have recent and incident infections.[2, 16, 17] To better understand TDR and guide ART therapy, we aimed to investigate TDRMs in youths over a 9-year period. We examined the effect of TDRMs on CD4 counts and the relationships of HIV youths in molecular networks in Dehong city in the China-Myanmar border area near the "Golden Triangle".

Methods

Study population and ethical review

The ethical review of this study was approved by the Medical Ethics Certification Committee of the Chinese Center for Disease Control and Prevention (approval no. X190111549).

From 2009 to 2017, a total of 10832 people were newly reported with HIV-1 infection at the Dehong border of China. Among these individuals, 2210 are youths (<25 y). We attempted to amplify the HIV-1 *pol* gene in virus isolated from 666 of these youths who were chosen according to the following criteria: 1) 16~25 y, newly diagnosed with HIV within 3-6 months, and not infected as a result of mother-to-infant transmission; 2) never received ART; 3) agreed to provide written informed consent and to allow plasma samples to be collected and stored for follow-up studies; 4) agreed to provide epidemiological information. Finally, 573 (86%, 573/666) youths from whom the viral *pol* gene was successfully amplified were used in the analysis. The sampling percentage for this study, by year, was 13.58% in 2009~2011, 22.65% in 2012~2013, 26.90% in 2014~2015, and 52.25% in 2016~2017 (Table 1).

Table 1 Age distribution of newly reported HIV infections in Dehong city, during 2009~2017

	2009~2011	2012~2013	2014~2015	2016~2017	Total
:al of newly reported individuals(n)	4120	2480	2143	2089	10832
Total of <25y youths(n)	847	499	420	444	2210
nples which according to the sampling andard (n)	140	138	148	240	666
genes were amplified and TDR analysis as performed successfully	115	113	113	232	573

CD4 count and *pol* gene amplification

The CD4 counts of 461 (80%) of the youths in the study were recorded. RNA was extracted from 140 μL of plasma samples using the QIAamp RNA Blood Mini Kit (Qiagen, Hilden, Germany). The partial *pol* gene, including the protease and reverse transcriptase (PR and RT) coding regions, was amplified by nested polymerase chain reaction (PCR) using an “in-house” method[18] by one-step RT-PCR using the primers F1a (TGAARGAITGYACTGARAGRCAGGCTAAT), F1b (ACTGARAGRCAGGCTAATTTTTTAG), and RT-R1 (ATCCCTGCATAAATCTGACTTGC). The primers used in nested PCR were PRT-F2 (CTTTARCTTCCCTCARATCACTCT) and RT-R2 (CTTCTGTATGTCATTGACAGTCC). PR/RT covered a fragment of 1056 bp corresponding to nucleotides 2259 to 3314 relative to the HXB2 genome. The positive PCR amplicons were purified using the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA) and sequenced. Amplicons were purified using the Illustra GFXR PCR DNA and Gel Band Purification Kit (GE Healthcare, United Kingdom) according to the manufacturer’s recommendations. The purified DNA was sequenced using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit v.3.1 (Applied Biosystems, CA, United States) with processing on an automated ABI 3130xl sequencer (Applied Biosystems) using Sanger’s method. All sequence data were spliced and cleaned using Sequencher v5.1 (Gene Codes Corporation).

Genotype identification and analysis

Sequences were edited in AliView software and then aligned with HXB2 reference sequences by the Los Alamos HIV Align tool. A phylogenetic tree was approximated using the maximum likelihood method with general time-reversible (GTR) modeling with RAxML (version 8) software[19] and Figtree v1.4.3. Identification of HIV-1 genotypes was conducted using the Stanford REGA HIV-1 Subtyping Tool 3.0.[20] If the result of the REGA tool contained ‘Recombination’, ‘potential-Recombination’ or ‘check the report’, the jpHMM recombination prediction tool[21] was used for confirmation. Discordant subtyping results between the two tools were analyzed by using the phylogenetic tree.

Drug resistance mutations and analysis

TDRMs were defined as the proportion of surveillance drug resistance mutations (SDRMs), identified by the Stanford Calibrated Population Resistance (CPR) tool 8.0 (last updated on 2019-07-01), according to the WHO-2009 SDRM list.[22] The Stanford HIVDB Program 8.9.1 (last updated on 2019-10-25, https://hivdb.stanford.edu/hivdb/) was used to score resistance to protease inhibitors (PIs), nucleoside

reverse transcriptase inhibitors (NRTIs) and nonnucleoside reverse transcriptase inhibitors (NNRTIs).[23] Sequences were determined to be either susceptible (<15, including potential resistance) or resistant (15≤low<30, 30≤medium<60, or high>60) based on their scores.

HIV molecular network analysis of TDR

Pairwise genetic distances were calculated by HyPhy 2.2.4 under the TN93 model. Cytoscape_v3.7.1 was used to visualize and analyze the molecular transmission network. We tested distance thresholds from 0.25% to 2% and inferred 1.25% as the genetic distance threshold, since 1.25% identified the maximum number of clusters.[24] We defined a node as one youth and an edge as a single link between two nodes. Edges linked nodes to each other when their genetic distance was =<1.25%. Nodes were classified as belonging to a network if they were linked to at least one other node.

Statistical analysis

Statistical analyses were conducted using R 3.6.2 and R-Studio 1.1.463. The Chi-square test or Fisher's exact test was used to verify differences in the distribution of demographic and clinical characteristics and genotypes. The Cochran-Armitage trend test was used to test trends such as TDR trend and the proportion of genotypes changed. Univariate logistic analysis was used to report proportions of TDRMs or network inclusion by participant characteristics (for example, nationality, educational status, etc.). Multivariate logistic analysis was performed on variables that showed statistically significant differences in the univariate logistic analysis. When appropriate, $p<0.05$ was defined as statistically significant.

Results

Demographic and clinical characteristics

Overall, the *pol* gene was amplified successfully from 573 untreated youths with HIV-1; of these, 351 (61%) were male, and 222 (39%) were female. The most common infection route was sex among heterosexuals (70.51%), followed by PWID (19.20%) and men who have sex with men (MSM) (8.9%). The proportion of the MSM population is increasing annually ($p<0.001$). More than half of the youths were single (58.81%), and the rest were married/cohabiting (37.35%) or divorced (3.84%). The subjects were Chinese (55.67%) or Burmese (44.33%). The proportion of PWID was higher among Burmese than among Chinese subjects (29.13% vs. 11.29%, $p<0.001$). The educational status of the subjects was mainly primary education (28.27%) and junior high school education (27.23%) (Table 2). The Chinese subjects had a higher rate of junior high school education (34.84%) than the others, while the Burmese subjects had a higher illiteracy rate (38.58%).

Table 2 Clinical characterizes of untreated 16~25y youths infected HIV during 2009~2017

	2009 ~ 2011 n=115	2012 ~ 2013 n=113	2014 ~ 2015 n=113	2016 ~ 2017 n=232	Total N=573 (100%)
Gender					
Male	53	54	71	173	351(61%)
Female	62	59	42	59	222(39%)
Nationality					
China	90	64	47	118	319(56%)
Myanmar	25	49	66	114	254(44%)
Route of transmission					
Heterosexual	88	80	78	158	404(71%)
IDU	19	26	25	40	110(19%)
MSM	1	7	9	34	51(9%)
Unknown	7	0	1	0	8(1%)
Marriage status					
Single	51	53	72	161	337(59%)
Married	56	57	37	64	214(37%)
Divorce\bereavement	8	3	4	7	22(4%)
Ethnicity					
Han	42	47	30	41	160(28%)
Minorities	73	66	83	191	413(72%)
Education					
Illiteracy	24	29	22	40	115(20%)
Primary school	46	29	28	59	162(28%)
Junior school	38	39	38	41	156(27%)
senior school	4	2	7	20	33(6%)
College and Above	3	4	3	5	15(3%)
Unknown	0	10	15	67	92(16%)
Job					
Jobless	11	12	14	21	58(10%)
Farmer	84	76	53	107	320(56%)
Business services	13	4	3	8	28(5%)
Other	7	9	27	25	68(12%)
Unknown	0	12	16	71	99(17%)

High genotype diversity among ART-naïve HIV-infected youths

The Stanford REGA online tool[20] was used for classification of genotypes, and the classification was then rechecked in the phylogenetic tree and using the jpHMM recombination prediction tool.[21] The distribution of HIV genotypes included C (31.59%), unique recombinant form (URF) (31.59%), URF_BC (25.48%), URF01BC (4.19%), URF01B (0.7%), URF01C (1.22%), CRF01_AE (21.12%), CRF07_BC (5.41%), CRF08_BC (4.54%), B’ (4.01%), CRF5501B (0.7%), CRF57BC (0.17%), and CRF64BC (0.87%) (Fig 1b). The prevalence of genotypes changed over time; the prevalence of B and C decreased from 2009~2017, while that of CRF01AE, CRF07BC and URFs continued to increase (Fig 1c). According to the route of infection, the proportion of URF and C in PWID was higher than that observed via sexual transmission (heterosexuals and MSM), but the proportions of CRF01_AE and CRF_08BC in cases of sexual transmission were high (p<0.001) (Fig 1d). The distribution of infection routes differed between China and Myanmar; the distribution of genotypes also differed. URF was higher in Burmese subjects than in

Chinese subjects (31.89% vs. 21.63%, $p<0.05$), and CRF07_BC was higher in Chinese subjects than in Burmese subjects (8.46% vs. 1.57%, $p<0.01$) (Fig 1e).

The prevalence of TDR increased significantly from 2009 to 2017

A total of 36 (6.3%) youths with TDRMs were identified by the CPR program. The prevalence of TDR increased significantly from 2009 to 2017 (3.48% to 9.48%, $p<0.05$, Cochran-Armitage trend test). Among these youths, the TDR prevalence in Chinese subjects increased from 3.33% to 5.93% ($p=0.37$, Cochran-Armitage trend test), and the highest prevalence appeared in 2014~2015 (6.38%). The TDR prevalence in Burmese subjects significantly increased from 4.00% to 13.16% ($p<0.05$, Cochran-Armitage trend). The prevalence of TDR to NNRTIs (3.9% to 4.31%, $p=0.57$) and PIs (0% to 0.86%, $p=0.20$) increased from 2009 to 2017. Moreover, the resistance to NRTIs increased significantly (0.87% to 5.17%, $p=0.004$, Cochran-Armitage trend test) (Fig 2a).

Overall, the average TDR to NNRTIs, NRTIs, and PIs was 3.49%, 2.62%, and 0.52%, respectively. Among these cases of TDR, 94.4% (n=34) had one class of TDRMs, and 5.6% (n=2) had two classes. Y181I/C and K103N were the most common NNRTI-related mutations and were found in 7 and 9 infected individuals, respectively. The distributions of NRTI- and PI-related mutations were scattered (Table 3). We used the Stanford HIVdb tool to assess the clinical impact of these mutations (excluding other polymorphism sites). Among NNRTIs, resistance to doravirine (DOR), etravirine (ETR), and rilpivirine (RPV) occurred mainly at a moderate level (50%, 63%, and 78%, respectively), and efavirenz (EFV) and nevirapine (NVP) had the highest composition ratio of high-level resistance (55% and 95%). Among NRTIs, excepting M184I (1), L74I (1), D67N (1), and T215I (1), the remaining mutations (75%, 12/16) only conferred potential resistance (score<15) to NRTIs. Although only three youths carried TDRMs associated with PI resistance, two I54M sites caused universal resistance to PI drugs (Fig 2b).

Table 3 Prevalence of HIV Transmitted Drug Resistance Mutations among untreated 16~25y youths in Dehong during 2009~2017

Type	With TDRM (%)	Heterosexual n=404	IDU n=110	MSM n=51	2009-2011 n=115	2012-2013 n=113	2014-2015 n=113	2016-2017 n=232
Total TDRM	38*	24(5.94%)*	10(9.1%)	4(6.25%)	4(3.48%)	5(4.42%)	5(4.42%)	24(10.34%)*
NRTI	15(2.62%)							
D67E	4(0.70%)	2	1	1				4
D67N	1(0.17%)	1						1
F77L	3(0.52%)	3						3
K219N	3(0.52%)	1	2					3
L74I	1(0.17%)	1						1
M184I	1(0.17%)	1			1			
T215I	1(0.17%)	1					1	
T69D	1(0.17%)	1					1	
NNRTI	20(3.49%)							
G190A	1(0.17%)	1			1			
K101E	1(0.17%)			1				1
K103N	9(1.57%)	5	4		1	3	2	3
V106M	2(0.35%)	1		1				2
Y181C	6(1.05%)	3	2	1	1	1		4
Y181I	1(0.17%)		1			1		
PI	3(0.52%)							
I54M	2(0.35%)	2						2
M46I	1(0.17%)	1					1	

* “Heterosexual” and “2016-2017” have two infectors who have two classes of TDRMs.

Correlates of TDR

The characteristics of individuals with and without TDR were comparable with respect to nationality, year of diagnosis, infection route, gender, ethnicity, marital status, educational level and CD4 cell count (Table 4). Only the mean CD4 count was significantly lower among youths with TDRMs than among those without TDRMs (373/mm^3 vs. 496/mm^3, p=0.013). The average CD4 count was 483/mm^3 (2009~2011: 529/mm^3, 2012~2013: 523/mm^3, 2014~2015: 451/mm^3, and 2016~2017: 450/mm^3).

Table 4 Demography and clinical characteristics of untreated 16~25y youths infected HIV with or without TDRMs

	Total	Without TDR	TDR	*p*	OR	OR 95% C.I.	
CD4 count (mean)		496	373	**0.013**	0.998	0.996~0.999	
Sex (n)							
Male	351	324(92%)	27(8%)		Reference		
Female	222	213(96%)	9(4%)	0.085	0.507	0.234	1.099
Year of diagnosis (n)							
2009-2011	115	111(96.5%)	4(3.5%)		Reference		
2012-2013	113	108(96%)	5(4%)	0.714	1.285	0.336	4.912
2014-2015	113	108(96%)	5(4%)	0.714	1.285	0.336	4.912
2016-2017	232	210(91%)	22(9%)	0.055	2.907	0.978	8.646
Marriage status (n)							
single	337	312(93%)	25(7%)		Reference		
Marriage	214	204(95%)	10(5%)	0.202	0.612	0.288	1.301
Divorce	22	21(96%)	1(4%)	0.618	0.594	0.077	4.603
Route (n)							
Heterosexual	404	382(95%)	22(5%)		Reference		
IDU	110	100(91%)	10(9%)	0.165	1.736	0.797	3.785
MSM	51	47(92%)	4(8%)	0.490	1.478	0.488	4.473
Unknown	8	8(100%)	0(0%)	0.999	0	0	
HIV-1 genotype (n)							
CRF01AE	121	110(91%)	11(9%)		Reference		
CRF07BC	31	29(94%)	2(6%)	0.641	0.690	0.145	3.286
CRF08BC	26	25(96%)	1(4%)	0.391	0.400	0.049	3.243
B	23	22(96%)	1(4%)	0.461	0.455	0.056	3.703
C	181	170(94%)	11(6%)	0.326	0.647	0.271	1.544
URF	181	171(94%)	10(6%)	0.237	0.585	0.240	1.423
Other	10	10(100%)	0(0%)	0.999	0	0	
Nationality (n)							
Chinese	227	303(95%)	16(5%)		Reference		
Myanmar	254	234(92%)	20(8%)	0.165	1.619	0.821	3.192

Youths with TDRMs were more likely to be linked to others based on the molecular transmission network

Molecular transmission networks were established to show transmission relationships among the youths with HIV-1. Of the 573 sequences, 264 (46.1%) were linked to at least one other sequence, forming 87 networks (nodes>=2). A total of 23 (63.89%) youths with TDRMs entered networks, and 15 networks contained at least one TDR node (Fig 3). The network entry rate of sequences containing TDRMs was significantly higher than that of sequences without TDRMs (63.89% vs. 44.9%, p=0.037, Chi-square test). The result also shows the different frequencies of TDRMs in in-network versus nonnetwork youths (8.7% vs. 4.2%). We determined that people who have a risk factor, i.e., those with TDRMs and those with CRF07_BC or C genotypes, were likely to have a link to others (Table S1). Y181C, D67E, V106M, and K103N were cases of shared DRMs with recent transmission (gene distance <0.5%) (Fig 3).

Discussion

The city of Dehong is located in the China-Myanmar border area near the “Golden Triangle” and is a hotspot of HIV transmission and recombination, having a strong impact on the HIV-1 epidemic in China.[25, 26] We determined and statistically analyzed the age distribution of newly reported HIV infections in Dehong city (Table 1). TDR can better guide future ART regimens, prevent mother-to-child transmission and aid pre-/post-exposure prophylactic therapy. Untreated youths (<25 y) are more likely to have recent and incident infections.[2, 16, 17] Therefore, we analyzed the TDR of untreated youths (16~25 y) newly diagnosed with HIV-1 over a relatively long period (from 2009 to 2017) in Dehong.

The distribution of HIV-1 genotypes in China is mainly CRF01AE, CRF07BC, CRF08BC, and B, while C, URF, and other circulating recombinant forms (CRFs) account for only a small proportion of cases.[27] However, the distribution of genotypes differs in Dehong, which has a high prevalence of URF and C subtypes.[28, 29] Similar to previous studies, the distribution of HIV genotypes in this study was diverse and complex. Interestingly, the prevalence of B and C has decreased annually, while that of CRF01AE, CRF07BC and URFs continues to increase. We also found that the proportion of URFs in Burmese and PWID populations was significantly higher than that in other populations. The result may indicate that due to the influence of drug injection in the "Golden Triangle", the presence of HIV-1 recombination networks occurred early among PWID in Dehong.[30-32] This has had a long-term impact on the HIV-1 epidemic in this area, making Dehong a hotspot for HIV recombination.

Frequent recombination is more effective than mutation in spreading drug resistance mutations.[33, 34] Frequent communication around the China-Myanmar border has increased the frequency of recombination[28, 35] and the probability of TDRM transmission. Overall, the average prevalence of TDR was 5.4%, a value that exceeds the 5% moderate prevalence level. It is worth noting that during 2016~2017, the prevalence of TDR was 9.48%, significantly higher than the average TDR prevalence in China[36] and Myanmar.[3] The prevalence of TDR in this study does not represent the average resistance level in China and Myanmar but indicates the increase in TDR among youths in hotspots of HIV transmission and recombination. In this study, no significant difference was found in TDR prevalence between Burmese and Chinese subjects. Furthermore, whereas the prevalence of TDR in Chinese subjects increased from 2009 to 2017 (from 3.92% to 5.93%), the prevalence of TDR in Burmese migrants increased significantly from 2010 to 2017 (from 4.00% to 13.16%). Burmese migrants are a key population for HIV prevention in this region.

Resistance to NNRTIs (2.92%) was the most frequently observed TDRM. Among these mutations, K103N (n=9) and Y181C/I (n=7) were the most common DRMs. These mutations caused a high level of drug resistance to first-line treatment drugs (EFV and NVP). Among NRTI-related TDRMs (2.34%), most (75%, 12/16) exhibited only potential resistance. Azidothymidine (AZT), lamivudine (3TC), and tenofovir (TDF), as first-line NRTI drugs in China, have meager rates of transmission resistance (0.3%, 0.15%, and 0%, respectively). However, unlike the findings reported in other studies,[4, 5, 36-40] NRTI resistance showed the most significant increase (from 0% to 5.17%) from 2009 to 2017 in this study. Although the prevalence of TDR to PIs (0.44%) was significantly lower than the prevalence of TDR to NNRTIs/NRTIs,

the I54M mutation caused universal resistance to PI drugs. These results suggest that, in the future, resistance testing before initiating ART is essential.

Previous studies suggested that DRMs impair viral fitness, resulting in increased CD4 count.[6, 7] However, some DRMs have a low impact on viral fitness and even improve it, which may lead to a more rapid decline in CD4 count.[9, 41] In the latest research,[8] no association was found between DRMs and decreased CD4 count. In this study, HIV-1-infected youths with TDRMs had low CD4 counts; this provides some evidence for a relationship between TDRMs and decreased CD4 count.

We analyzed TDR transmission based on the molecular transmission network. The rate of entry into the network (46.1%) of youths in our study was significantly higher than that reported in other studies.[42, 43] Moreover, youths with TDR (63.89%) were more likely to enter the network. These results indicate that youths in Dehong are in an active period of HIV transmission. Youths who had TDRMs were more likely to be linked to others in the HIV-1 transmission network. Moreover, the Y181C, D67E, and V106M mutations have recent transmissions (gene distance <0.5%) and were shared TDRMs among 8 youths during 2016~2017. The result indicates that the rapid increase in TDR during 2016~2017 is related to the recent spread of TDRMs such as Y181C, D67E, and V106M. The result also indicates the need for monitoring and intervention in the spread of HIV through molecular transmission network analysis of youths.

Our research has some limitations. Since the first years of ART scale-up, TDR strains of HIV are likely to be limited, and all youths were ART-naïve; in addition, the total number of TDRMs is small (n=36), possibly resulting in statistical bias. In 2016-2017, we increased the sample capacity and observed a significant increase in TDR. This suggests that TDR may be increasing rapidly among young people in this border region. In addition, we did not investigate TDR to integrase inhibitor (INSTI); because these sequences were previously amplified and stored by our laboratory, the primers did not include the INSTI region. We will continue to increase the sample capacity and to monitor TDR (including INSTI) in the China-Myanmar border region.

Conclusion

TDR increased by 200% from 2009 to 2017 among untreated youths in Dehong at the China-Myanmar border. The prevalence of TDR exceeded the 5% moderate prevalence level. The HIV-1 TDR increase and low CD4 count of patients with TDRMs suggest the need for early ART and completion of resistance testing before initiating ART in the future. The youths in Dehong are in an active period of HIV transmission. Furthermore, molecular network analysis shows that youths with TDRMs are more likely to have links to others, suggesting the need to intervene to prevent onward transmission.

Abbreviations

HIV: Human immunodeficiency virus

PDR: Pretreatment drug resistance

PLWHA: People living with HIV or AIDS

WHO: World Health Organization

ART: Antiretroviral therapy

NNRTI: Non-nucleoside reverse-transcriptase inhibitor

NRTI: Nucleoside reverse-transcriptase inhibitor

PI: Protease inhibitor

PWID: People who injected drug

PrEP: Pre-exposure prophylaxis

EFV: Efavirenz

NVP: Nevirapine

FTC: Emtricitabine

3TC: Lamivudine

ABC: Abacavir

DDI: Didanosine

D4T: Stavudine

TDF: Tenofovir

AZT: Azidothymidine

DRV/r: Darunavir

LPV/r : Lopinavir

ATV/r: Atazanavir

DRM: Drug resistance mutation

HIVDR: HIV Drug Resistance Database

TN93: Tamura–Nei 93

OR : Odds ratio

CI : Confidence interval

Declarations

Ethical Approval: The ethical review of this study was approved by the Medical Ethics Certification Committee of the Chinese Center for Disease Control and Prevention (approval no. X190111549).

Consent for publication: Not applicable.

Conflicts of Interest: The authors report no conflicts of interest in this work.

Availability of data and materials: The datasets are available from the corresponding author on reasonable request.

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Author Contributions: L.M. M.H. conceived and designed the experiments; M.C., J.B., Y.Y., Y.M. collected samples. Y.D. M.C. Y.Z., L.W., Q.L. performed the experiments; Y.D., Y.F., H.X., S.D. analyzed the data; Y.D., L.M. M.H. wrote the paper; all authors discussed the results and contributed to the final manuscript.

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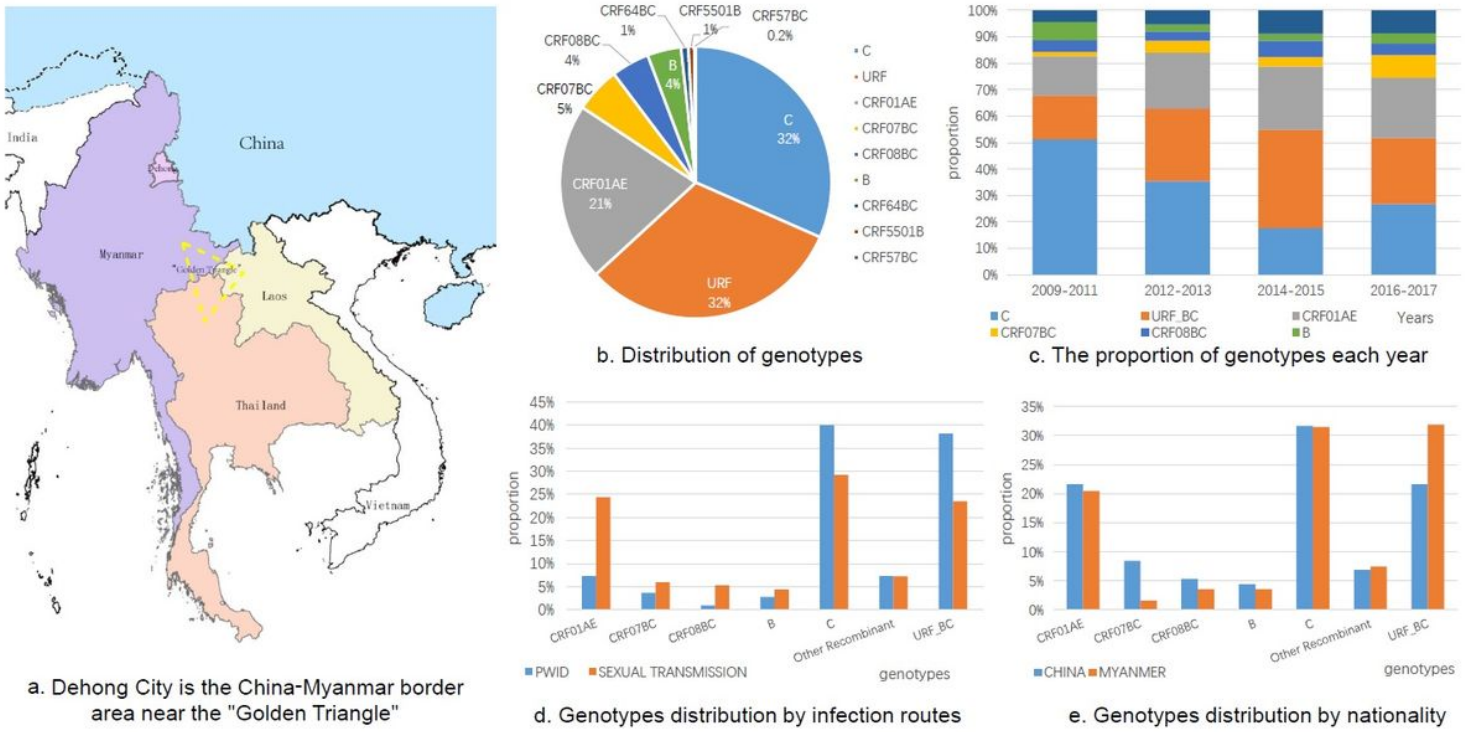
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Supplemental Table 1

Table S1 Network analysis of demography and clinical characterizes among untreated 16~25y youths infected HIV

	network n(%)	Univariate			Multivariate		
		p	OR	95% C.I.	p	OR	95% C.I.
Gender							
Male	179(51%)			Reference			
Female	85(38%)	**0.003**	0.60	0.423~0.839	0.078	0.686	0.451~1.043
Marriage status							
Single	172(51%)			Reference			
Married	83(39%)	**0.005**	0.608	0.429~0.861	0.107	0.725	0.490~1.072
Divorce	9(41%)	0.360	0.664	0.276~1.595	0.765	0.869	0.347~2.178
Ethnicity							
Han	76(48%)		Reference				
minorities	188(46%)	0.670	0.924	0.641~1.331			
Infected Routes							
Heterosexual	177(44%)			Reference			
PWID	52(47%)	0.518	1.150	0.753~1.755	0.557	0.861	0.522~1.419
MSM	32(63%)	**0.012**	2.160	1.185~3.939	0.140	1.663	0.846~3.272
Unknown	3(38%)	0.722	0.769	0.181~3.263	0.765	0.798	0.182~3.506
Genotypes							
CRF01AE	49(41%)			Reference			
CRF07BC	25(81%)	**0.001**	6.122	2.339~16.02	**0.001**	5.179	1.933~13.87
CRF08BC	9(35%)	0.578	0.778	0.321~1.886	0.718	0.876	0.351~2.182
B	8(35%)	0.608	0.784	0.309~1.990	0.748	0.942	0.365~2.433
C	110(55%)	**0.012**	1.796	1.137~2.838	**0.008**	2.151	1.320~3.506
URFBC	60(37%)	0.554	0.864	0.533~1.401	0.704	0.986	0.593~1.640
OTHER	3(30%)	0.517	0.630	0.155~2.555	0.523	0.631	0.152~2.616
Nationality							
China	160(50%)			Reference			
Myanmar	104(41%)	**0.028**	0.689	0.494~0.961	.144	.761	0.528~1.098
TDR							
Without-TDR	241(45%)			Reference			
TDR	23(64%)	**0.037**		1.058~4.227	**0.042**	2.152	1.029~4.499

Figures



a. Dehong City is the China-Myanmar border area near the "Golden Triangle"

b. Distribution of genotypes

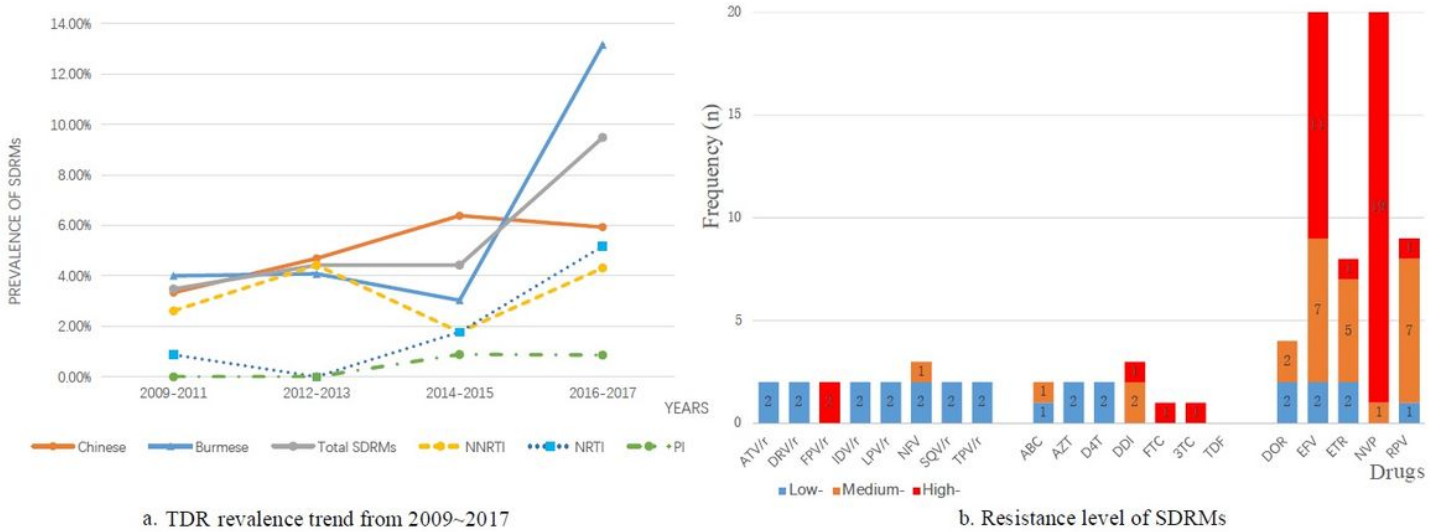
c. The proportion of genotypes each year

d. Genotypes distribution by infection routes

e. Genotypes distribution by nationality

Figure 1

Genotypes analysis of HIV-1 pol genes. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.



a. TDR revalence trend from 2009~2017

b. Resistance level of SDRMs

Figure 2

Resistance level and TDR prevalence trend

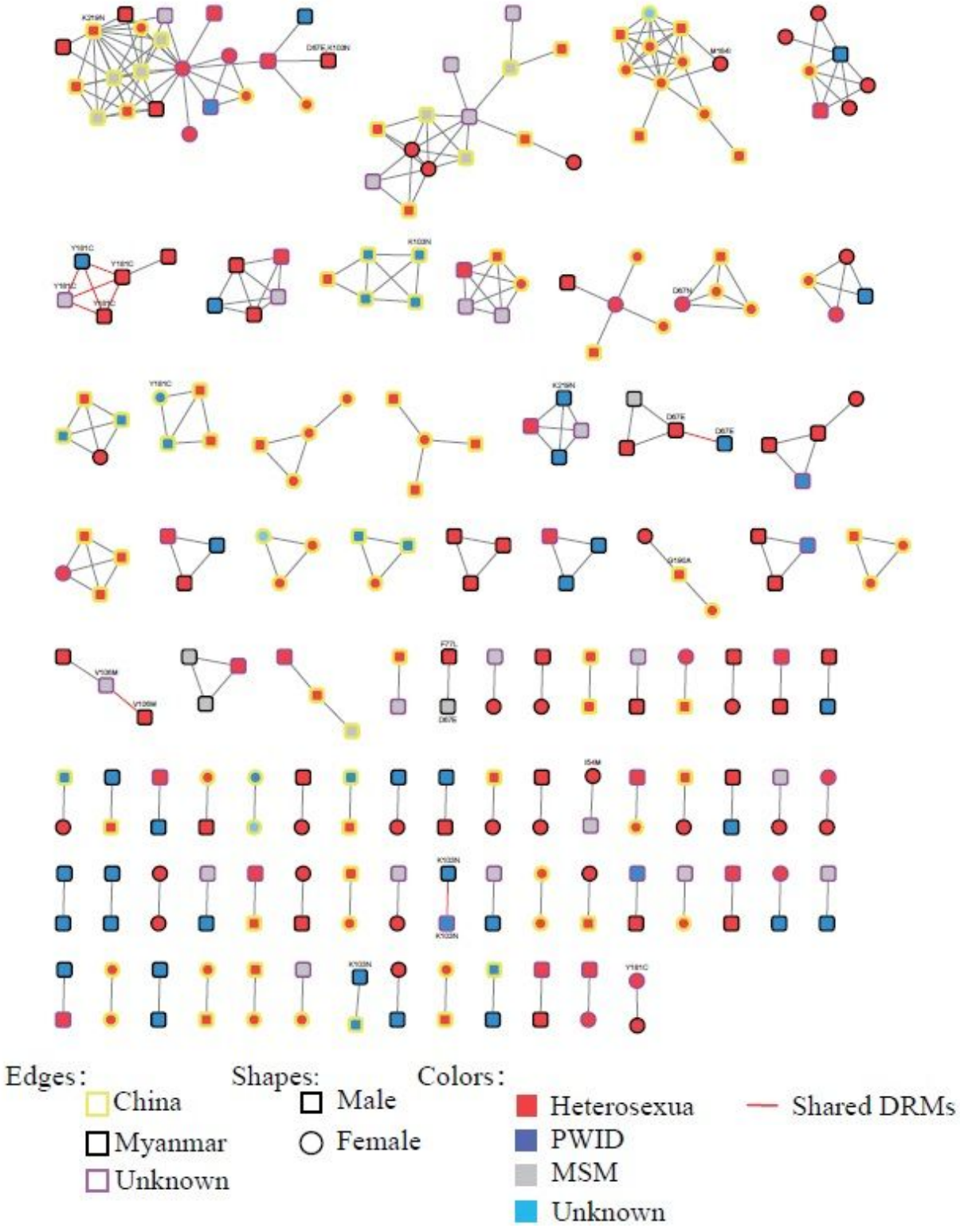


Figure 3

Network of youths newly reported with HIV from 2009 to 2017