RESEARCH ARTICLE

Dynamic Characteristics and HIV Infection of Men who have Sex with Men from 2011 to 2017 in Yinchuan, Ningxia, China

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Abstract: ***Objectives***: Ningxia Hui Autonomous Region, an important area for ethnic Hui settlement in Northwest China, is a low HIV prevalence region. However, HIV infection rates among men who have sex with men (MSM) in Ningxia have increased to an alarming level, despite scale-up of control measures in recent years. This study aimed to understand the demographical and sexual behavior dynamics of MSM and to explore the factors associated with HIV infection.

Methods: Annual cross-sectional surveys were carried out among MSM during 2011~2017 in Yinchuan, the capital city of Ningxia. Information regarding social demographics, sexual behavior and HIV prevention knowledge was collected. Blood samples were taken for HIV, HCV serological and genetic analysis, and syphilis serological analysis. The dynamic trend was analyzed with trend χ2 test and factors associated with HIV infection were identified by multivariate logistic regression analysis.

Results: The study found a decreasing trend for mean age of the MSM population over the study period. MSMs with a college education or higher increased significantly, while the proportions that were in a marriage significantly decreased over the study period. The rate of HIV positive among MSM increased during the study period (p<0.05), however, the rate of recently diagnosed infections decreased from 2012 (p<0.05). Overall, a very high proportion (98%) of MSM had basic knowledge of HIV prevention, however, only approximately 40% of them used condoms consistently during anal sex with male partners. Unprotected anal sex was identified as a risk factor associated with HIV infection, as was syphilis infection. Local residency status and MSM who received intervention and detection services were the factors that decreased HIV infection risk. Sequence analysis identified the HIV-1 CRF55_01B subtype from MSM for the first time in Yinchuan.

Conclusion: The reduction of recent HIV diagnoses is an encouraging sign of successful HIV control measures in MSM in Ningxia. The finding that a high proportion of MSM had knowledge of HIV prevention but still conducted unprotected sex highlights the need for further control measures to change unsafe sexual practices among MSM.

1. INTRODUCTION

Acquired immune deficiency syndrome (AIDS) arising from human immunodeficiency virus (HIV) infection has recently become the leading cause of death among all infectious diseases in China [1, 2]. The people most at risk of HIV infection has transitioned over time from injection drug users and plasma donors to heterosexual transmission *via* commercial sex workers, and currently to homosexual transmission among men who have sex with men (MSM). HIV is now spreading at an alarming rate between MSM throughout China, and the proportion of reported HIV cases among MSM increased from 1.77% in 2000 to 8.0% in 2015 [3]. A meta-analysis showed that 5.3% of MSM were infected with HIV in China in 2009 [4], indicating the importance of controlling HIV transmission between MSM [5-9]. HIV prevalence remains low in China overall, however, this varies in different areas of China as does the major transmission routes and HIV subtypes, and these features have been changing with time [10-13]. Ningxia Hui Autonomous Region (Ningxia), located in Northwest China, is known for its ethnic diversity with 64.58% Han, 35.42% Hui (Muslims) and less than 1% otherminorities[14-16]. There are 54 ethnic

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groups in China and the dominant ethnic group of Han makes up 90% of the entire Chinese population. Ningxia is one of the most important settlement areas for Hui ethnic people, which descended from the Arabic and Persian merchants who came to China during the 7th century [17]. Compared with other provinces in China, Ningxia has a low HIV prevalence of less than 0.5%, however, nearly 30% of the total reported HIV cases in Ningxia is among MSM [12].

In this study, we conducted consecutive annual cross-sectional studies between 2011 and 2017 to monitor the HIV infection rates in MSM and their demographics and sexual behaviors in Yinchuan, the capital city of Ningxia. The results revealed several important trends in MSM demographics and behavioral characteristics and changes in HIV prevalence and recent infection rates. The analysis also identified factors associated with HIV infection.

2. METHODS

2.1. Study Sample and Data Collection

Men who have sex with men (MSM), were defined as males, aged 18 or older, who were engaged in oral or/and anal sex with other men during the prior 12 months. The cross-sectional studies were conducted from April to June every year from 2011 to 2017 by the local Center for Disease Control and Prevention (CDC) staff in Yinchuan city, Ningxia. The target number of MSM was 400 each year. The participants for the study were recruited by contacting various MSM community groups (*e.g.* bars, clubs, websites and online chat groups) to find participants. The initial respondents helped to recruit further participants from their personal network for snowball sampling to reach 400 participants each year. Eligibility criteria of participants include being at least 18 years of age, conducting same-gender sex with a male in the past 12 months, and have not participated in the study previously. Written informed consent was obtained from all participants. The participants were interviewed face-to-face by trained local CDC staff to collect information including age, ethnicity, occupation, education, drug usage, sexual behavior and HIV prevention knowledge. The blood samples were collected for HIV, HCV and syphilis detection. The history of receiving intervention and detection services was also investigated. These services are provided by health professionals or staff from non-government organizations (NGO) and include training for knowledge of HIV transmission and prevention, as well as blood testing and pre- and post-test counselling.

2.2. HIV, Syphilis and HCV Test

HIV antibody testing was conducted using enzyme-linked immunosorbent assay (ELISA) with HIV-1 ELISA kit (WanTai, Beijing, China), and the positive samples were further confirmed by Western-Blot (Western Blot HIV1/2 BLOT 2.2, MP Bio-medicals, Singapore). The RPR Card Test was used for primary screening of Syphilis and the positive samples were confirmed by Syphilis Treponema Palladium (TPA) IgG ELISA (WanTai, Beijing, China). HCV infection was determined by anti-HCV IgG detection (Wan-Tai, Beijing, China).

2.3. HIV Recent Infection Test

Confirmed HIV positive people were further tested by BED HIV-1 capture enzyme immunoassay (BED-CEIA) to determine if the infection was recently acquired. Recent HIV infection was diagnosed using either documented evidence that seroconversion occurred in the preceding 12 months (BED-CEIA assay) or when the acute retroviral syndrome was documented, and based on a compatible pattern of viral load, CD4 cell count, and clinical history [18, 19]. All the HIV positive subjects were referred to the local CDC and clinics for HIV primary care and follow-ups.

2.4. HIV-1 Pol Gene Sequencing and Subtype Analysis

Viral RNA was extracted from plasma samples using QIAamp Viral RNA Mini kit (QIAGEN), and RNA was reverse transcribed and amplified by one-step RT-PCR (Trans Gene Biotech, Beijing, China) using a set of 4 pan-HIV-1 primers set designed for the amplification of HIV-1 genomes of all groups and subtypes[20, 21]. The RT-PCR conditions were 45℃ 30 min, 94℃ 2 min, followed by 35 cycles of 94℃ 30 s, 50℃ 30 s, 72℃ 90 s, and then 72℃ 10 min. The second round of PCR was performed from 5 μl of the first round of PCR product using *pol*-specific internal primers[22] and Promega Taq PCR Master mix kit (Promega, USA). The final PCR product was analyzed by agarose gel electrophoresis and sequenced by Sanger sequencing. The acquired sequences were compared with the international subtype references. The phylogenetic trees were constructed to determine the subtype of the samples.

2.5. Statistical Analysis

Statistical analyses were performed using SPSS 17.0 software. Chi-square tests for trends were used for categorical variables. A stepwise multivariate logistic regression was constructed to select the variables that were independently associated with HIV infection. A p-value <0.05 was considered to be statistically significant.

3. RESULTS

3.1. Demographic Characteristics of MSM

3.1.1. The Mean Age of MSM Decreased between 2011 and 2017

The mean age of the 400 MSM was 32.4 ± 8.2 (mean ± SD) in 2011, which decreased to 28.3 ± 7.0 in 2017 (Fig. **1**). This trend is statistically significant (p<0.01), indicating that the MSM population is getting younger (Fig. **1** and Table **1**).

3.1.2. MSM had Increasing Education Levels and Increasingly Stayed in Unmarried Statue

The proportion of MSM who had an education at a college level or above increased from 43.3% in 2011 to 70.6% in 2017, and a significant increasing trend was noted ($\chi2$=65.21, P<0.001) (Table **1**). Another significant trend in the demographics of the MSM population was that more MSM stayed as a single status rather than in a marriage or cohabitation with a female ($\chi2$=25.62, P<0.001). In 2011, 56% MSM was single and the figure increased to 70.3% in 2017. There was an exception in 2016 in which only 34%
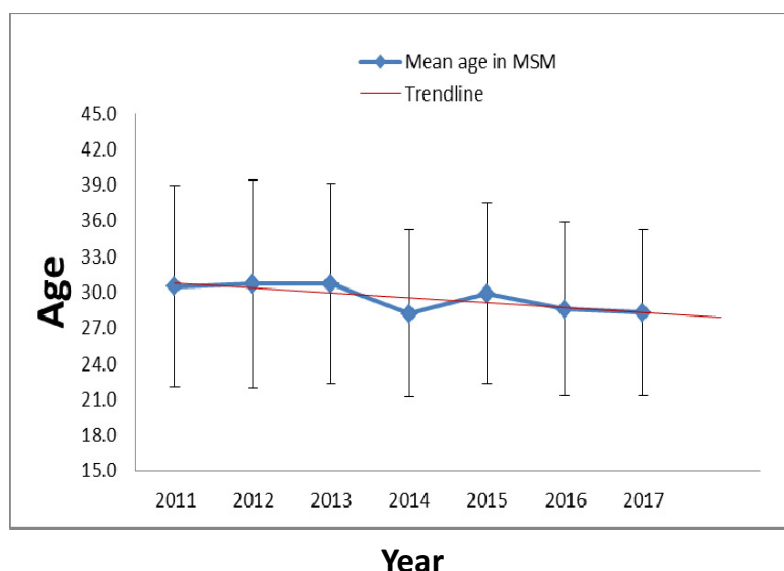
Fig. (1). The mean age of MSM tended to drop during 2011-2017 in Yinchuan, Ningxia. The blue line shows means of age ±SD among MSM, The red line is the trend of mean.

Table 1.   Demographic characteristics of MSM population in 2011 ~ 2017 in Yinchuan.

Investiga-tion Item	Total N=2800	Year							χ2 for trend	P
		2011	2012	2013	2014	2015	2016	2017		
Age									16.403	<0.001
~20		32 (8)	32 (8)	30 (7.5)	42 (10.5)	35 (8.8)	34 (8.5)	37 (9.3)		
~30		185 (46.3)	195 (48.8)	210 (52.5)	231 (57.8)	216 (54.0)	218 (54.5)	221 (55.3)		
~40		124 (31.0)	109 (27.3)	104 (26.0)	104 (26.0)	110 (27.5)	109 (27.3)	108 (27.0)		
~50		51 (12.8)	58 (14.5)	52 (13.0)	17 (4.3)	35 (8.8)	34 (8.5)	28 (7.0)		
~70		8 (2.0)	6 (1.5)	4 (1.0)	6 (1.5)	4 (1.0)	5 (1.3)	6 (1.5)		
Education									65.21	<0.001
Under college		227(56.8)*	192 (48.0)	119 (29.8)	130 (32.5)	134 (33.5)	60(15.0)	95 (23.8)		
College and above		173 (43.3)	208 (52.0)	281 (70.3)	270 (67.5)	266 (66.5)	340(85.0)	305 (76.2)		
Marriage									25.62	<0.001
Sin-gle/divorced/widowed		224 (56.0)	239 (59.8)	256 (64.0)	293 (73.3)	287 (71.8)	135(33.8)	281 (70.3)		
Married/cohabitation		176 (44.0)	161 (40.3)	144 (36.0)	107 (26.8)	113 (28.3)	265(66.3)	119 (29.7)		
Ethnics									9.84	0.002
Han		306 (76.5)	303 (75.8)	356 (89.0)	343 (85.8)	347 (86.8)	341(85.3)	327 (81.7)		
Hui		89 (22.3)	90 (22.5)	38 (9.5)	44 (11.0)	47 (11.8)	55(13.7)	70 (17.5)		
Others		5 (1.3)	7 (1.8)	6 (1.5)	13 (3.3)	6 (1.5)	4(1.0)	3 (0.8)		
Residence									0.12	0.743
Local		314 (78.5)	327 (81.8)	354 (88.5)	327 (81.8)	319 (79.8)	326(81.5)	312 (78.0)		
Non-local		86 (21.5)	73(18.3)	46 (11.5)	73 (18.3)	81 (20.3)	74(18.5)	88 (22.0)		

*The sample number outside the bracket and the proportion (%) in the bracket.

Table 2.   Sexual behaviours, venereal disease detection and received intervention service among MSM 2011~2017, Yinchuan.

Investigation Item	Total N=2800	Year							χ^2 for trend	P
		2011	2012	2013	2014	2015	2016	2017		
Conducted anal sex with any male partners in the past six months										
	2460(87.9)	364(91.0)	342 (85.5)	361 (90.3)	364 (91.0)	395(98.8)	323(81.2)	311(77.8)	22.22	<0.001
Condom consistently used in anal sex in the past six months										
	1069(38.2)	163(40.8)	217 (54.3)	204 (51.0)	208 (52.0)	46 (11.5)	77(19.3)	154(38.5)	81.76	<0.001
Had commercial sex with males in the last six month										
Yes	314(11.2)	64 (16.0)	50 (12.6)	53 (13.3)	28 (7.0)	60 (15.0)	17(4.3)	42(10.5)	14.007	<0.001
No	2486 (88.8)	336(84.0)	350(87.4)	347(86.7)	372(93.0)	340(85.0)	383(95.7)	358(89.5)		
Condom consistently used in the commercial sex with males in the past six month										
Yes	266(84.7)	60(93.7)	40(80.0)	50(94.3)	24(85.7)	40(66.7)	14(82.3)	38(90.5)	1.916	0.166
No	48(15.3)	4(6.3)	10(20.0)	3(5.7)	4(14.3)	20(33.3)	3(17.7)	4(9.5)		
Condom used in the last commercial sex with male										
	2537(90.6)	375 (93.8)	356 (88.9)	343 (85.7)	343 (85.7)	373 (93.3)	387(96.7)	360(90.0)	2.317	0.128
Had sex with woman in the past six months										
Yes	814(29.0)	166 (41.5)	134 (33.5)	117 (29.3)	48 (12.0)	97 (24.3)	118(29.5)	134(33.5)	9.481	0.002
No	1986(71.0)	234(58.5)	266(66.5)	283(70.7)	352 (88.0)	303(75.7)	282(70.5)	266(66.5)		
Condom consistently used when sex with woman in the past six month										
Yes	372(45.7)	74 (44.7)	66 (49.2)	56 (47.9)	23 (47.9)	31 (31.9)	59(50.0)	63(47.0)	9.435	0.151
No	442(54.3)	92(55.3)	68(50.8)	61(52.1)	25(52.1)	66(68.1)	59(50.0)	71(53.0)		
Used condom in the last sex with woman										
	996(35.6)	178 (44.6)	198 (49.6)	195 (48.7)	196 (48.9)	128 (32.0)	60(15.1)	41(10.3)	221.4	<0.001
Syphilis test positive										
	198(7.1)	28(7.0)	25(6.3)	22(5.5)	26(6.5)	20(5.0)	34(8.5)	43(10.8)	5.05	0.025
Drug usage among MSM										
	13(0.5)	3 (0.8)	1(0.3)	0 (0.0)	0 (0.0)	0 (0.0)	1(0.3)	8 (2.0)	2.18	0.140
Venereal disease										
	152(5.4)	22(5.5)	33(8.3)	8(2.0)	8(2.0)	46(11.5)	11(2.8)	24(6.0)	57.06	<0.001
Received intervention services										
	2701(96.5)	359(89.8)	396(99.0)	400(100.0)	398(99.5)	400(100.0)	400(100.0)	348(87.0)	1.64	0.201
Rate of knowledge about AIDS										
	2757(98.5)	390 (97.5)	393(98.2)	397 (99.3)	394 (98.5)	395 (98.7)	393(98.2)	395 (98.7)	2.57	0.109

Table 3.   HIV-1 infection among MSM and the recent infection in HIV-1 positive MSM in 2011 ~ 2017.

Year	Sample for HIV-1 Tested	HIV-1 Positive	Rate of HIV-1 Positive	BED Tested	BED Positive	BED Positive Rate in HIV-1 Positive	The Recent Infection Rate for MSM Population
2011	400	15*	3.80%	13	5	38.46%	2.88%
2012	400	29*	7.30%	26	10	38.46%	5.70%
2013	400	22	5.50%	22	10	45.45%	5.19%
2014	400	24	6.00%	24	11	45.83%	5.73%
2015	400	33*	8.30%	31	10	32.26%	5.28%
2016	400	36	9.00%	36	9	25.00%	4.67%
2017	400	25	6.25%	25	6	24.00%	2.68%
Total	2800	184	6.57%	177	61	34.46%	2.18%
P**			0.036			0.01	0.155

*There were 7 pre-existent HIV-infection (2 cases in 2011, 3 cases in 2012 and 2cases in 2015) and were not conducted for BED-Test.
** Trend test.

were single, while 66% were married or cohabitated with a female. This outlier did not change the trend of increasingly unmarried MSM.

3.1.3. The Proportion of MSM with Hui Ethnicity Tended to Decrease, while the Proportion of Han Increased from 2011 to 2017

The proportion of ethnic Han in MSM population was around 76% in 2011~ 2012, and increased to over 81% since 2013, while the proportion of ethnic Hui was around 22% in 2011~ 2012, and dropped under 18% thereafter (Table **1**). The trend of the proportion of ethnic Han increasing and Hui decreasing overall was statistically significant ($\chi2=9.84$, P=0.002).

3.1.4. The Proportion of Local and Non-local Residents in MSM Population Remained Stable

During the period of 2011 to 2017, the proportion of local and non-local residents remained stable, in which local residents accounted for around 80% of MSM population, while around 20% MSM were non-local residents (Table **1**).

3.2. Sexual Behavior Characteristics and Intervention Services

Overall, very few MSM reported using drugs over the study period (0.5% of total). There was an increased number of MSM (2%) in 2017 who used drugs, but no trend was formed (Table **2**).

Overall 87.9% MSM conducted anal sex with male partners in the prior six months at the time of the interview, and only 38.2% consistently used condoms in anal sex. There was a decreasing trend of consistent condom use during this period (trend test $\chi2=81.76$, P<0.0001). The result is consistent with a trend of increasing syphilis infection (trend test $\chi2=5.05$, P=0.025), which is an indicator of unprotected sex. The results suggest that unprotected anal sex is a very common sexual practice among MSM.

Overall 11.2% MSM conducted commercial sex with a male in the prior 6 months and there was a decreasing trend in the behavior (trend test $\chi2=14.01$, P<0.001). Compared to anal sex with male partners, there was a higher rate (~ 85%) of MSM who consistently used condoms during commercial sex, and the figures remained at a high level during the study period (Table **2**). Overall, 29% MSM also conducted sex with women in the prior 6 months and 45.7% of them consistently used condoms. This condom usage rate is slightly higher than that of anal sex with male partners (38.2%), but lower than that in commercial sex (84.7%). The results suggest that MSM were aware of the risks and used condom in commercial sex, but were more likely to conduct unprotected sex with their partners. Over 93% of MSM had received intervention and detection services, and over 98% had knowledge of AIDS/HIV prevention, indicating a good reach of education and control measures to this population.

Testing of other sexually transmitted diseases in MSM was also performed. Syphilis and venereal disease had an increasing trend (P=0.025 and <0.01 respectively). The HCV positive rates were very low throughout the study period and no trend was detected (Table **2**). The HCV positive rates in HIV positive and negative MSM were 1.58% and 0.57% respectively, and the difference was not statistically significant (Supplementary Table **1**).

3.3. HIV-1 Infection Dynamics in MSM

214 MSM were diagnosed as HIV-1 positive from 2800 participants during the period of 2011-2017. The overall HIV-1 positive rate was 6.57%, and there was an increasing trend in HIV-1 diagnosis rate (P<0.05) (Table **3**). However, the HIV-1 positive rate in MSM decreased in 2017 after increasing in the prior few years. Whether this decrease was the start of a trend or the result of fluctuation requires further monitoring.

BED test was carried out for HIV-1 positive MSM to determine recent infection. The total BED test positive rate was 34.46%, with the range from 24% to 45.83%, indicating that

Table 4.   The factors associated significantly to HIV-1 infection in logistic regression analysis.

Variables	B [1]	S.E [2]	Wald [3]	df [4]	Sig. [5]	OR [6]	EXP(B) 95% C.I [7]	
							Lower	Upper
local residence	-0.554	0.178	9.689	1	0.002	0.575	0.405	0.814
Inconsistently use condom in anal sex in the past six months	0.252	0.109	5.363	1	0.021	1.287	1.040	1.594
Received intervention services	-1.033	0.224	21.321	1	0.015	0.356	0.230	0.552
Syphilis positive	0.774	0.246	9.873	1	0.002	2.168	1.338	3.512

[1]coefficient of partial regression.
[2]standard error.
[3]chi-square value.
[4]degree of freedom
[5]*P,* calculated by using a binary logistic regression model.
[6]odds ratio, calculated by using a binary logistic regression model.
[7]confidence interval.

most HIV-1 positive MSM was not recently infected (Table **3**). A decreasing trend of positive rates in BED test among HIV-1 positive MSM was noted during the study period. The overall rate of recent infections among MSM as determined by a compatible pattern of viral load, CD4 cell count, and clinical history in addition to the BED test was also recorded and did not significantly change over the study period (Table **3**). This phenomenon may indicate that the increased HIV-1 positive detection among MSM was a result of increased identification of late-diagnosis HIV-1 infection.

3.4. Analysis of Factors Associated with HIV-1 Infection using Multivariate Logistic Regression

To assess the effect of each factor surveyed on the risk of HIV-1 infection, multivariate logistic regression analysis was performed. With the infection of HIV-1 as the dependent variable, factors including age, marriage, resident statue, ethnicity, education, drug usage, received intervention and detection services, anal intercourse and condom usage, commercial sex and condom usage, heterosexuality and condom usage were used as independent variables. Four factors were shown to be significantly associated with HIV-1 infection (Table **4**). Local resident statue (OR=0.575, 95% CI 0.405~0.814) and received intervention and detection services (OR=0.356, 95% CI 0.23~0.552) correlated with reduced risk of HIV-1 infection. In contrast, inconsistent condom use during anal intercourse (OR=1.287, 95% CI 1.04~1.594) and positive syphilis diagnosis (OR=2.168, 95% 1.338~3.512) was associated with increased risk of HIV-1 infection.

3.5. Sequencing Analysis

Subtypes of HIV-1 in MSM were analyzed for pol region from 24 samples collected in 2017 using the software MEGA 6.0 with maximum-likelihood (ML) method and GTR+ I+Γmodel with 500 bootstrap replications. Four subtypes were identified. Subtype CRF01_AE was the most common (50.0%), followed by subtype CRF07_BC (29.0%), B (17.0%) and CRF55_01B (4.0%) (Fig. **2**). Subtype CRF55_01B has not yet been described elsewhere. A phylogenetic analysis of HIV-1 CRF55_01B was conducted using
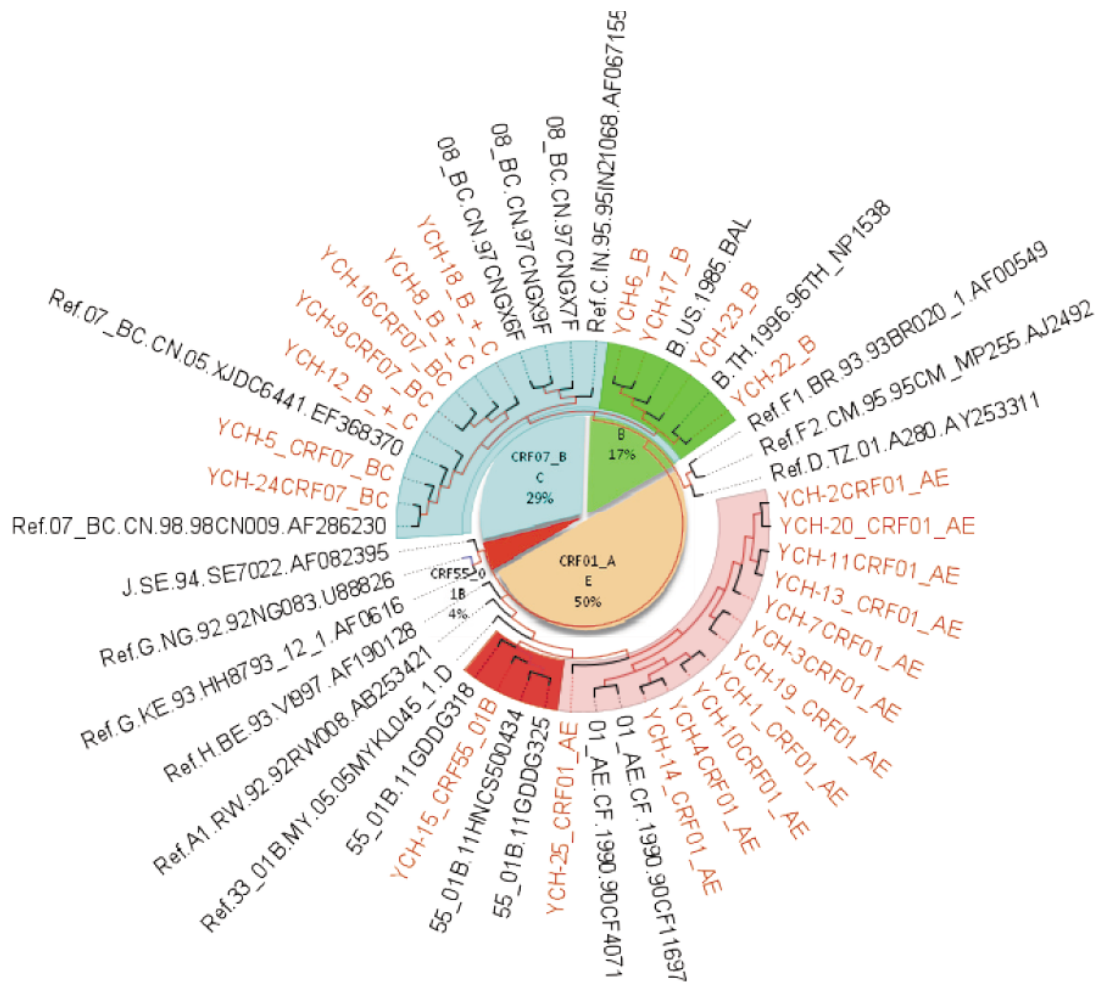
the Maximum Likelihood method based on the Jukes-Cantor model.

The result showed that the closest sequence of CRF55_01B in Yinchuan (YCH15) is the sequence identified in Changsha, Hunan province (Genebank number, JX574661) (Fig. **3**).

4. DISCUSSION

The increasing coverage of antiretroviral therapies and scaling-up of other HIV control measures have reduced the number of new infections each year globally. However, HIV infection in MSM have been increasing or remaining high in many areas, indicating that the current control measures have not successfully reached this population, or have not correctly adapted to the changes of this population [7, 23, 24]. Therefore, monitoring of this population became extremely important to identify the risk factors that cause the high rate of new HIV infections around the world [25]. This report analyzed data of consecutive cross-sectional studies among MSM in Yinchuan from 2011 to 2017 and provided important information that could be used to improve the control measures in this high-risk population for Ningxia, and other areas in a similar situation.

Firstly, the demographic characteristics of MSM in Yinchuan have been changing. During the study period from 2011-2017, the average age of MSM decreased. The trend of decreasing age of MSM was also reported from another city in northern China, Tianjin, but no trend was found in a city in southwest China, Chengdu, and an opposite trend that was found in Beijing, the capital city of China, indicating a diversity in characteristics of MSM in different areas of China [7, 26, 27]. The education levels of MSM increased over the study period with the proportions of people with a college or above education significantly increased from 43.3% in 2011 to 70.6% in 2017 in Yinchuan. The similar trend was also reported in another city in Southwest China, Chengdu [26]. However, the education level was not identified as an associated risk factor for HIV infection in our multivariate logistic regression analysis. This suggests that the increased proportion of MSM with a higher level of education may be a result

Fig. (2). HIV-1 subtype distribution from 24 HIV positive MSM in Yinchuan. The circular phylogenetic tree was generated using the software MEGA7.0 [59], with maximum-likelihood (ML) method and GTR+I+Γmodel with 500 bootstrap replications based on the pol sequences. HIV-1sequences were named by "city of serum collection" and "ID number", where YCH represents Yinchuan.

of increased numbers of students getting higher education in the general population, and/or higher education leads to an increase in tolerance/acceptance of diverse sexual orientations [28, 29]. An increased proportion of MSM staying in unmarried statues (single, divorced or widowed) was noted over the study period. These demographic changes of MSM should continue to be monitored in the future to determine if it is a continuing trend. Meanwhile, the control measures should be improved to meet these changes. Mixed ethnicities of Han and Hui is a unique demographic of Yinchuan. Compared to the proportion of ethnic Hui in the general population in Yinchuan, our results show that the proportion of ethnic Hui in MSM was lower than ethnic Han, and decreased during the study period. The reason for this lower proportion of MSM in ethnic Hui is not clear, but it may be linked with the cultural and religious beliefs of people of the Muslim faith [30-32].

Secondly, sexual behaviors of MSM were also changing. The study investigated three aspects of sexual behaviors for MSM, anal sex with male partners, commercial anal sex with another male, and heterosexual sex with women. Interestingly, condom usage between these sexual activities was quite different. Condom usage in commercial anal sex re-

mained relatively high (~85%) over the study period, while the condom usage in sex with a male (~38%) and female (~45%) partners was significantly lower and decreased over the study period. The low condom usage in sex with partners may be the result of a perception that their partner does not have any sexually transmitted diseases. This phenomenon was also reported from other studies [33, 34]. However, unprotected sex is a risk factor for HIV-1 transmission as identified in this study and other reports [35].

Thirdly, there was a different trend between HIV positive rates and recent infection rates in the MSM population. The increasing HIV-1 prevalence in MSM also was found in other parts of China [9, 12]. Interestingly, the recent infection rates in HIV positive MSM tended to decrease and resulted in relatively stable rates of recent infection among MSM. This phenomenon is likely explained by increased identification of late-diagnosed HIV infections as a result of scaling-up control and detection measures. The direct evidence for these interpretations is that receiving intervention and detection services remained at very high levels and was associated with reduced HIV infection by multivariate logistic regression analysis.
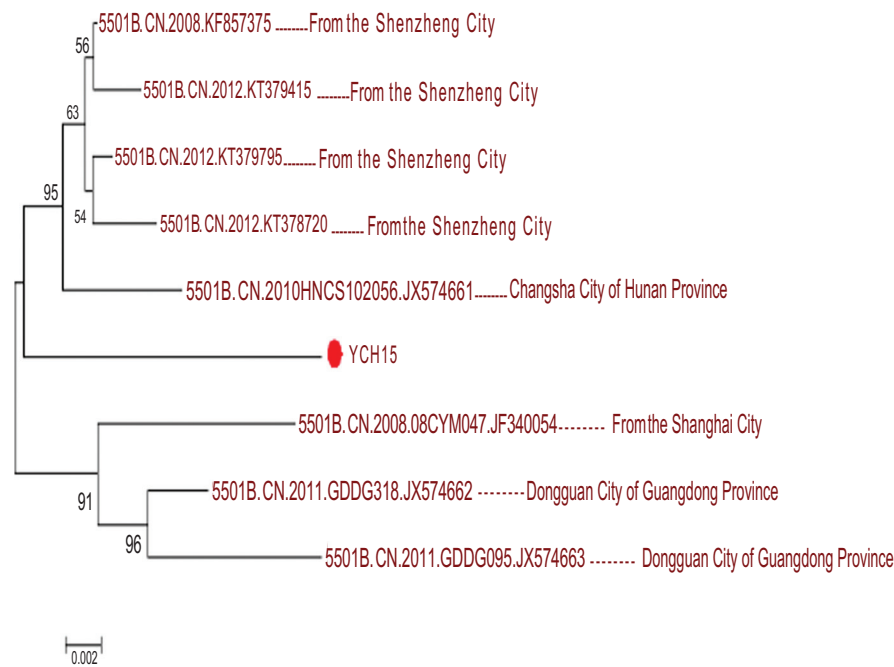
Fig. (3). Molecular phylogenetic analysis of the HIV-1 CRF55_01B of Yinchuan15 isolate. The evolutionary history was inferred by using the Maximum Likelihood method based on the Jukes-Cantor model. All the reference strains of subtype CRF55_01B were retrieved from the Los Alamos National Laboratory HIV Sequence Database (http://hiv-web.lanl.gov/). The Yinchuan15 (YCH15) isolates are labelled with a solid red circle. Evolutionary analyses were conducted in MEGA7 [59].

The multivariate logistic regression analysis identified two risk factors associated with HIV infection, which were inconsistent condom usage in anal sex and syphilis infection. These two factors are linked as syphilis infection is an indicator of unprotected sex [36, 37]. This finding is also supported by the fact that very low condom usage in anal sex among MSM in Yinchuan is consistent with studies conducted in other parts of China [4, 38, 39]. The analysis also identified that local resident status and detection and prevention services were correlated with reduced HIV infection. This suggests that temporary resident status is a risk factor. A previous study from another part of China also reported that temporary residents had the highest rate of multiple sexual partners and highest risk with HIV transmission [40]. This finding agrees with another report that immigrants have higher rates of HIV infection [32]. It is an encouraging sign that people who received prevention and detection services were overall less susceptible for HIV infection, and this suggests that the current control measures in Yinchuan is helping restrain HIV transmission in MSM.

CRF55_01B is CRF (Circulating Recombinant Form) which is composed of CRF01_AE and subtype B [41-46]. CRF55_01B was first identified from three epidemiologically unlinked MSM in China [47, 48]. It was then found in many places in China, including Shanxi, Fujian, Changsha, Nanjing and Kunming [28, 49-52]. A large-scale survey in MSM showed that CRF55_01B was found in 7 from 11 studied provinces in China [53, 54]. A recent paper reported that an outbreak prevalence of the CRF55_01B strains among MSM has formed in Shenzhen, a large city in Southern China [53, 55, 56]. The identification of CRF55_01B in MSM in Yinchuan indicates that MSM is the most at-risk population for the emergence of new HIV subtypes. The

spreading of CRF55_01B in MSM and other populations in Ningxia should be carefully monitored.

The study has several limitations and caution is required when interpreting the results. As MSM is a difficult-to-access population in China, snowball sampling is widely used in MSM studies and it is impossible to make unbiased estimates from snowball samples [57]. We used snowball sampling to recruit MSM in Yianchuan in our study. We recruited MSM through various MSM communities to increase representativeness and this analysis covered the data from 2011 to 2017 which may minimize sampling bias. Also, cross-sectional studies also have limitations in measuring the prevalence of outcomes [58]. Another limitation is that we only partially sequenced HIV from HIV positive MSM, with only the pol region being analyzed. Therefore, whole genome subtype information and changes cannot be assessed.

CONCLUSION

The HIV-1 prevalence among MSM in Yinchuan, China, had an increasing trend during 2011-2017, while the diagnosis of recent HIV infection in HIV-1 positive MSM tend to be decreasing. The rates of unprotected anal sex remained high and this sexual behavior was identified to be the main risk factor for HIV infection. Prevention and detection services were identified as a protective factor against HIV infection, which may be a positive sign that the current control measures are effective. This study reveals an increasing trend of unsafe sexual behavior that was also identified as a risk factor for HIV infection, highlighting the urgent need for implementing measures to reduce this behavior to stop HIV-1 transmission in this population. Compared to the propor-

tion of the general population, the proportions of ethnic Hui in MSM were lower and tended to decrease during the study period. It is worth to continually monitor this trend and to investigate the reasons to better understand the MSM population in Ningxia.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Research and Ethics Committee of Ningxia Centre of Diseases Control, China.

HUMAN AND ANIMAL RIGHTS

No animals were used in the study. All humans research procedures followed were in accordance with the standards set forth in the Declaration of Helsinki principles of 1975, as revised in 2008 (http://www.wma.net/en/20activities/ 10 ethics/10helsinki/).

CONSENT FOR PUBLICATION

Written informed consents were obtained from all recruited individuals.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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AVAILABILITY OF DATA AND MATERIALS

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

AUTHOR CONTRIBUTIONS

ZW, GG and JZ designed and organized the project. ZW performed experiments. ZW and DJR wrote the manuscript. ZW, GG, JZ, XM, XW, DY, PL and MC were involved in recruitment and interview of study subjects, blood sample collection.

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publishers web site along with the published article.

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