BioMed Research International

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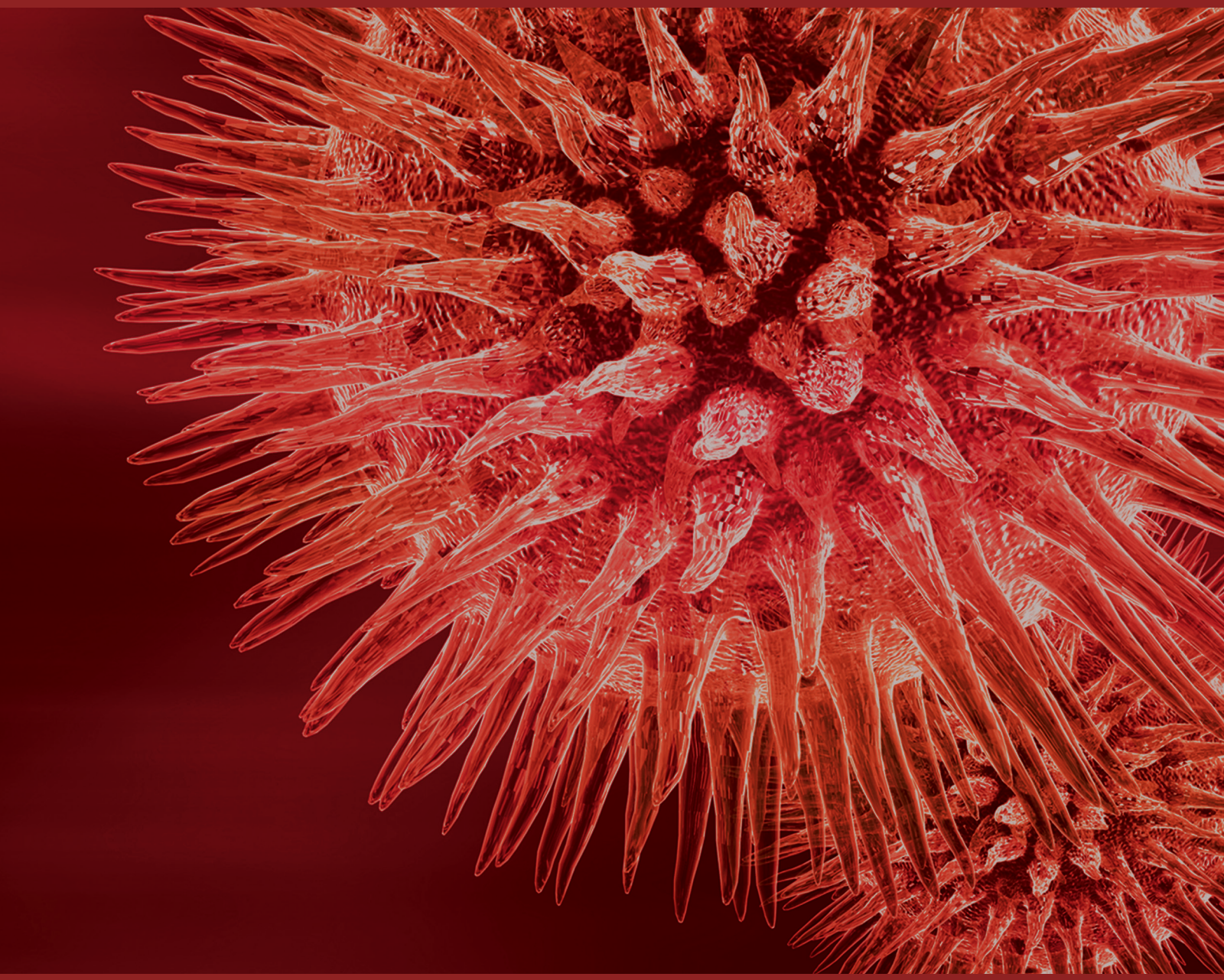
Guest Editors: Huachun Zou, Lei Zhang, Eric P. F. Chow, Weiming Tang, and Zixin Wang



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Editorial

Testing for HIV/STIs in China: Challenges, Opportunities, and Innovations

Huachun Zou,[1,2] Lei Zhang,[3,4,5,6] Eric P. F. Chow,[3,4] Weiming Tang,[7] and Zixin Wang[8,9]

[1] *Department of Medical Statistics and Epidemiology, School of Public Health, Sun Yat-sen University, Guangzhou 510080, China*
[2] *Kirby Institute, The University of New South Wales, Sydney, NSW 2052, Australia*
[3] *Melbourne Sexual Health Centre, Alfred Health, Melbourne, VIC 3053, Australia*
[4] *Central Clinical School, Monash University, Melbourne, VIC 3004, Australia*
[5] *School of Public Health and Preventive Medicine, Faculty of Medicine, Monash University, Melbourne, VIC 3004, Australia*
[6] *Research Centre for Public Health, Tsinghua University, Beijing 100084, China*
[7] *University of North Carolina Project-China, Guangzhou 510085, China*
[8] *JC School of Public Health and Primary Care, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, Hong Kong*
[9] *Shenzhen Research Institute, The Chinese University of Hong Kong, Shenzhen 518057, China*

Correspondence should be addressed to Huachun Zou; zouhuachun@mail.sysu.edu.cn

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In this special issue, five articles are included. The research topics include the trend of late presentation in HIV-infected populations in Guangzhou, China, positivity of human papillomavirus (HPV) among HIV-infected and HIV-uninfected men who have sex with men (MSM) in Guangzhou, China, association between age at first anal/vaginal sex and HIV infection among MSM in Shenzhen, China, impacts of online sex-seeking on HIV transmission among MSM in Shenyang, China, and the prevalence of antiretroviral drug resistance in people living with HIV in Jiangsu, China. Factors that are associated with HIV infection and challenges encountered by people living with HIV in different geographical areas of China are discussed, with MSM being the focus. These articles will hopefully provide evidence and insights to policy makers for HIV/STI control and prevention in China.

Using data extracted from the Chinese HIV/AIDS Comprehensive Response Information Management System (CRIMS), W. Cheng et al. examined the prevalence and trend of late presentation of HIV infection and explored the roles of different testing strategies [including voluntary counseling and testing (VCT) centers, provider-initiated testing and counseling (PITC) services at general hospitals and clinics, and Mandatory HIV Testing (MHT) at detention facilities] in HIV diagnosis in Guangzhou, China. The results showed that, in metropolitan city of Guangzhou, late presentation of HIV infection, defined as CD4 cell count below 350 cells/μl, decreased from 63% in 2008 to 43% in 2013. Likewise, presentation with an advanced HIV disease decreased from 40% in 2008 to 15% in 2013. Despite these reductions, late presentation of HIV infection at diagnosis remained high. The results revealed that, compared to PITC and MHT, VCT was a more efficient platform in early HIV diagnosis. PITC did not facilitate more timely diagnosis, which highlighted the gap of intervention to raise HIV awareness among key populations attending general health care facilities. The results also suggested that MHT allowed healthcare providers to provide early intervention to the key populations and to provide opportunities for testing, substance abuse treatment, and antiretroviral treatment (ART), which played an important role in the control of HIV epidemic. Recently, the updated national ART guideline recommends free ART for all PLWH upon diagnosis. Early identification of HIV-infected cases facilitates early treatment, which can in turn reduce the chance of onward HIV transmission.

X. Ren et al. reported on the positivity of anal HPV among HIV-positive and HIV-negative men who have sex with men (MSM) attending a sexual health clinic based at a gay community organization in Guangzhou. This study was timely to add to the knowledge of the epidemic of HPV among MSM in China. The sample was predominantly young

(median 26 years) and sexually experienced (40% had two or more partners in the past three months) MSM. Positivity of HPV of any type, any high-risk type, any low-risk type, any 4-valent vaccine type, and any 9-valent vaccine type in HIV-infected MSM, was almost twice as high as that in their HIV-uninfected counterparts. This study also found that prevalent anal bacterial infections, including chlamydia, gonorrhea, and *Mycoplasma genitalium*, contributed to much higher anal HPV positivity and multiplicity of anal HPV types, which implied the importance of timely detection and treatment of bacterial infections. The vast majority of HPV-infected MSM were infected with 9-valent vaccine types (59 out of 64 HIV-infected ones and 31 out of 41 HIV-uninfected ones). The high positivities of high-risk types including HPV 16, 18, and 52 and low-risk types including HPV 6 and 11 in the anal canal in MSM, especially in those infected with HIV, suggested high burdens of corresponding morbidities in the foreseeable future, including anal warts that may relapse, anal intraepithelial neoplasia, and even anal cancer. The study results warranted early HPV vaccination and regular anal exams in MSM attending sexual health clinics in Guangzhou. In 2016 the health authority in China approved the bivalent HPV vaccine manufactured by GlaxoSmithKline to be used in women. However HPV vaccination in males is rarely discussed. The acceptability of HPV vaccination in Chinese MSM is high, especially when it is provided free of charge. A study in Hong Kong, a city close to Guangzhou, found that over 80% of MSM were willing to take up free HPV vaccines [1]. However, the unavailability of policies with regard to HPV vaccination in men and targeted HPV vaccination in MSM in China remains to be an issue for the advocacy of policy changes.

R. Xu et al. reported the association between age at first anal sex with a man or vaginal sex with a woman and HIV infection among 533 MSM attending a sexual health clinic in Shenzhen. This sample was relatively young (median 32 years), commenced sex relatively late (21 years for vaginal sex with a woman and 22 years for anal sex with a man), and had a high rate of HIV infection (24%). A large proportion (66%) had ever had sex with women. MSM who also have sex with women is a bridge population transmitting HIV/STIs from high-risk population to low-risk population (e.g., female sex partners). Being younger, being unmarried/divorced/widowed, having lower education level, and being socioeconomically disconnected were associated with a relatively younger age at first sexual intercourse. Commencing anal sex before 14 was associated with higher rate of HIV infection. The proportion of consistent condom use in MSM in China is still suboptimal. This study called for the promotion of consistent condom use in anal sex and early education and interventions to be conducted among MSM. But given the availability of alternative biomedical and behavioral interventions for HIV, including preexposure prophylaxis and serosorting, it is quite difficult to increase condom use.

S. Pan et al. conducted a 6-year serial cross-sectional survey to study on the impacts of online sex-seeking (including using smartphone-based geosocial networking apps) and HIV infection among MSM in Shenyang, China. Around half of the participants (1000/1981) sought sex partners mainly through the Internet (Internet-based MSM, IBM) and this rate increased from 43% in 2009 to 62% in 2014. This study confirmed results from a meta-analysis on global data: IBM were younger, had a higher level of education, and had higher income, compared to non-IBM [2]. This study found a changing trend in HIV prevalence among IBM compared to non-IBM. Before 2010, the prevalence of HIV among IBM was slightly lower than non-IBM. But from 2010 to 2014, HIV prevalence in IBM increased from 12.5% to 20.7%, while that in non-IBM was stable (from 13.5% to 14.7%). Since May 2009, MSM have been increasingly using mobile apps to socialize in the gay community [2]. Sex-seeking using smartphone-based geosocial networking apps may have contributed to the dramatic increase of number of partners and unprotected anal sex. IBM preferred receptive anal sex and had a higher rate of STD symptoms. MSM engaging in receptive anal sex were 7 times more likely to be infected with HIV compared to MSM engaging in insertive anal sex [3]. All these factors contributed to a higher HIV prevalence among IBM. The study results can help public health policy makers to understand the HIV transmission routes among MSM and provide effective and comprehensive interventions. Given that most MSM are now using apps, interventions can potentially be done via apps.

Y. Zhou et al. analyzed the prevalence of ART drug resistance and its impact on HIV-1 virological failures in Jiangsu, China. A total of 2223 HIV-infected patients who had received ART for at least one year were included. Just over 10% had a viral load of >1,000 copies/mL (i.e., virologic failure), half of whom had drug resistance to either nucleoside reverse transcriptase inhibitors (NRTIs), nucleoside reverse transcriptase inhibitors (NNRTIs), or protease inhibitors (PIs). The overall prevalence of drug resistance was 5% (101/2223). Among these patients, the highest frequency drug resistance mutations were NNRTIs (47%), followed by NRTIs (37%), and PIs (6%). The most common mutations associated with drug resistance in NRTIs and NNRTIs were M184V (79%) and K103N (34%). Four PI-associated mutations were observed in 11 individuals. This study called for the scale-up of testing for transmitted drug resistance (TDR) in treatment-naive HIV-1 infected patients, to better monitor the epidemic. MSM tended to have higher drug resistance rates and worse treatment outcomes compared to other populations, and thus additional monitoring and further assessments of the drug resistance profile in this population are necessary.

Huachun Zou
Lei Zhang
Eric P. F. Chow
Weiming Tang
Zixin Wang

References

[1] J. T. F. Lau, Z. Wang, M. Lau, and C. H. Y. Lai, "Perceptions of HPV, genital warts, and penile/anal cancer and high-risk sexual behaviors among men who have sex with men in Hong Kong," *Archives of Sexual Behavior*, vol. 43, no. 4, pp. 789–800, 2014.

[2] H. Zou and S. Fan, "Characteristics of men who have sex with men who use smartphone geosocial networking applications and implications for HIV interventions: a systematic review and meta-analysis," *Archives of Sexual Behavior*, pp. 1–10, 2016.

[3] X. Meng, H. Zou, S. Fan et al., "Relative risk for HIV infection among men who have sex with men engaging in different roles in anal sex: a systematic review and meta-analysis on global data," *AIDS and Behavior*, vol. 19, no. 5, pp. 882–889, 2015.

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Research Article

Human Papillomavirus Positivity in the Anal Canal in HIV-Infected and HIV-Uninfected Men Who Have Anal Sex with Men in Guangzhou, China: Implication for Anal Exams and Early Vaccination

Xuqi Ren,[1] Wujian Ke,[1] Heping Zheng,[1] Ligang Yang,[1] Shujie Huang,[1] Xiaolin Qin,[1] Bin Yang,[1] and Huachun Zou[2,3]

[1]*Guangdong Provincial Center for Skin Disease & STI Control, Guangzhou, Guangdong 510091, China*
[2]*School of Public Health, Sun Yat-sen University, Guangzhou, Guangdong 510085, China*
[3]*Kirby Institute, The University of New South Wales, Sydney, NSW 2052, Australia*

Correspondence should be addressed to Bin Yang; yangbin101@hotmail.com and Huachun Zou; zouhuachun@mail.sysu.edu.cn

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Background. The epidemiology of HPV in men who have sex with men (MSM) in Guangzhou, China, had not been reported previously. *Methods.* HIV-infected and HIV-uninfected MSM were recruited from a Guangzhou-based MSM clinic in 2013. Sociodemographic characteristics and sexual behaviors were collected. An anal cytological sample was taken for HPV testing. *Results.* We recruited 79 HIV-infected and 85 HIV-uninfected MSM. The median age was 26 years in both groups. The positivities of anal HPV of any type (81.0% versus 48.2%), any high risk type (50.6% versus 27.1%), any low risk type (55.7% versus 31.8%), and any 9-valent vaccine type (74.7% versus 36.5%) were all significantly higher among HIV-infected compared to that among HIV-negative MSM (p for all < 0.05). The great majority of HPV-infected MSM were infected with 9-valent vaccine types (59 out of 64 HIV-infected and 31 out of 41 HIV-uninfected). Anal bacterial infections were associated with higher anal HPV positivity and greater number of anal HPV types. *Conclusion.* Sexually active MSM in Guangzhou, especially those infected with HIV, had high and multiple HPV detections. The majority of these cases were potentially preventable by HPV vaccine. Regular anal exams and early HPV vaccination are warranted in this population.

1. Introduction

Human papillomavirus (HPV) is one of the most common sexually transmitted infections (STIs) seen in the anogenital area, with high prevalence and incidence among men who have sex with men (MSM) [1–3]. A meta-analysis of global data on HPV in MSM found prevalence of anal HPV to be 93% in HIV-positive MSM and 64% in HIV-negative MSM and these rates did not decrease with increasing age [4]. HPV can cause symptoms such as anogenital warts and intraepithelial neoplasia and squamous-cell carcinoma in the anal canal [5]. These symptoms cause high psychological and financial burdens in the MSM population. Risk factors, including unprotected anal intercourse (UAI) and multiple sex partners, render MSM vulnerable to anal HPV infection, especially among those who are infected with HIV [6, 7]. A meta-analysis found significant difference in the incidence of anal cancer between HIV-infected and HIV-uninfected MSM: 46 versus 5 per 100,000 person-years [4].

Studies in China reported high prevalence of anal HPV among MSM irrespective of HIV status: 65.3–71.4% in Beijing [8–10], 73.0% in Taiyuan [11], 52.7% in Xi'an [12], 68.7% in Chengdu [11], and 36.4% in Shenzhen [13]. Among HIV-infected MSM the HPV prevalence was even higher, ranging from 71.4% in Shenzhen [13] to 99.0% in Xi'an [12]. Guangzhou is the largest city in southern China with a population of over 13 million in 2014 [14]. It was estimated that there were around 25,000 MSM living in Guangzhou [15].

However until now there had been no study that reported on HPV infection among MSM in this city. Our study aimed to clarify the severity of and factors associated with HPV infection among MSM in Guangzhou, comparing HIV-infected to HIV-uninfected ones.

2. Methods

2.1. Study Population. This study was conducted between January and October 2013 at the STD Clinic of the Lingnan Fellows Health Support Center, the largest MSM health support NGO group in Guangzhou. We recruited HIV-infected MSM from those who attended the clinic for periodical CD4+ T cell count test and HIV-uninfected MSM from those who attended the clinic for HIV testing and who tested negative. Eligible participants were males aged 18 years or older who had had receptive anal sex with another man in the past 12 months. The study was reviewed and monitored by the Ethics Committees of Guangdong Provincial Dermatology Hospital, Guangzhou, China. Written informed consent was obtained from all participants before data and sample collection.

2.2. Data Collection. A self-administered questionnaire was collected. The questionnaire included information on sociodemographic characteristics (e.g., age, ethnicity, education, marital status, and career), sexual behaviors (e.g., gender of first sex partner, heterosexual and homosexual behaviors, and condom use in the past three months), knowledge about sexual partner's STD status, experience of commercial sex, and alcohol use. A unique code was used to link questionnaire to specimens of a participant. Personal contact information, which was blinded to researchers, was kept by the STD Clinic of Lingnan Fellows Health Support Center for test results feedback and data validation.

2.3. Sample Processing. A blood sample was collected from HIV-negative/unknown subjects for HIV serological assessment. The HIV serologic status was determined by enzyme-linked immunoassay (ELISA, Wantai Biological Medicine Company, Beijing, China). Positive samples by ELISA were further confirmed by using the HIV-1/2 Western blot assay (HIV Blot 2.2 WB; Genelabs Diagnostics, Singapore). HIV serology was performed at the Guangzhou Center for Disease Control. An anal swab sample was taken to test for chlamydia, gonorrhea, and *Mycoplasma genitalium* using nucleic acid amplification test (DaAn Gene Co., Guangzhou, China) as previously described [16].

A clinician checked warts in the anal canal with the help of an anal speculum, after the collection of a cytological sample. A cytological sample was obtained around the dentate line in the anal canal using a small soft cytology brush (HybriBio, Chaozhou, China). The swab sample was coded and stored at −70°C before further processing. HPV DNA was extracted by Cell Lysate Kit (HybrioBio, Chaozhou, China) according to the manufacturer's instructions. HPV DNA was amplified by using MY09/11 primers [17] and the amplified fragment was about 450 bp. The DNA sequences within the L1 region of HPV were used to detect generic HPV DNA. Amplification was carried out in 50 μL of reaction mixture (1x GoTaq PCR buffer (Promega, USA), 0.2 mM deoxynucleotide triphosphates (dNTPs, Thermo, USA), 1.5 mM $MgCl_2$ (Sigma-Aldrich, USA), 1 μmole of each primer, 1.25 units (U) of GoTaq DNA Polymerase (Promega, USA) and 2 μL of DNA sample). The amplified condition was 95°C for 2 min of denaturation and then 40 cycles of amplification: 95°C for 1 min of denaturation, 55°C for 1 min of annealing, 72°C for 1 min of extension, and final extension at 72°C for 10 min by using the GeneAmp RCR System 9600 (Perkin Elmer, United States). PCR products were analyzed on 2.0% agarose gel with GeneGreen Nucleic Acid Dye (TianGen, China), visualized by the Tanon 5299 Multi (Ewell, China), and their molecular weights were determined by comparison with a DL 1000-bp DNA ladder (TaKaRa, Japan). All samples were tested by PCR methods three single times and confirmed by Sangon Biotech, China.

HPV genotype screening was conducted using (1) sequencing tests: HPV positive PCR products were purified by PureLink Quick Gel Extraction Kit (Invitrogen, USA) and the sequencing services were purchased from Sangon Biotech, China. The assembled sequences were submitted to BLAST alignment (https://www.ncbi.nlm.nih.gov/blast), against sequences available in GenBank; (2) HPV reverse dot blot genotyping: HPV genotyping was detected according to the manufacturer's instructions by using the HPV reverse dot blot genotyping kit and the automatic nucleic acid hybridization device (Guangzhou LBP Medical Technology Co., Ltd., Guangzhou, China), which can detect 28 HPV subtypes (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 66, 68, 73, 81, 82, and 84). We classified HPV types into high risk (HR) types (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82) and low risk (LR) types (6, 11, 40, 42, 43, 44, 54, 61, 81, and 84), based on their association with cervical cancer [18].

2.4. Statistical Analysis. A sample size of 79 HIV-infected and 86 HIV-uninfected MSM was required to provide acceptable 95% confidence intervals (CIs) around the expected proportion of men with anal HPV. Median and interquartile range (IQR) were used for continuous variables. Proportions were used for categorical variables. The chi-squared test was used to compare proportions between groups. Rank sum test was used to compare median of anal HPV type detected between groups. Univariate and multivariate logistic regression models were used to calculate odds ratios to estimate the association between potential risk factors and HPV infection. Variables with a $p < 0.1$ using univariate models were entered into the multivariate regression model. Variables with a $p > 0.1$ using univariate models yet deemed as potential confounders were also entered into the multivariate regression model, including age at enrollment, number of male sex partners in the past 3 months, and frequency of condom use in anal sex in the past 3 months. Data were entered using EpiData version 3.0 (The EpiData Association Odense, Denmark) and statistical analyses conducted using STATA version 13.0 (StataCorp, Texas).

Table 1: Demographic characteristics among HIV-infected and HIV-uninfected men who have sex with men in Guangzhou.

Demographic characteristic	HIV-infected		HIV-uninfected		All	
	Number/median	IQR/%	Number/median	IQR/%	Number/median	IQR/%
Age at recruitment (year)	26.0	20–38	26.0	20–39	26.0	19–40
18–24	29	36.7	35	41.2	64	39.0
25–34	42	53.2	44	51.8	86	52.4
≥35	8	10.1	6	7.1	14	8.5
Education level*						
Middle school or less	19	24.1	13	15.3	32	19.5
Technical diploma	26	32.9	21	24.7	47	28.7
University or above	34	43.0	51	60.0	85	51.8
Ethnicity						
Han	76	96.2	79	92.9	155	94.5
Other	3	3.8	6	7.1	9	5.5
Profession						
Student	12	15.2	18	21.2	30	18.3
Service industry	16	20.3	21	24.7	37	22.6
Private company	15	19.0	10	11.8	25	15.2
Other	36	45.6	36	42.4	72	43.9
Marital status						
Unmarried/divorced	71	89.9	79	92.9	14	8.5
Married	8	10.1	6	7.1	150	91.5
Frequency of drinking						
Every day or often	5	6.3	6	7.1	11	6.7
Sometimes	25	31.7	30	35.3	55	33.5
Seldom or never	49	62.0	49	57.7	98	59.8

Notes. *$0.05 < p < 0.10$.

3. Results

3.1. Participant Characteristics. We recruited 79 HIV-infected and 85 HIV-uninfected MSM. As shown in Table 1, HIV-infected MSM and HIV-uninfected MSM were comparable in age (median 26 years for both), ethnicity (96.2% versus 92.9% were Han ethnic), profession (15.2% versus 21.2% were students), marital status (89.9% versus 92.9% were unmarried), and frequency of drinking (p for all > 0.1). HIV-infected MSM had slightly less education compared to HIV-uninfected MSM (43.0% versus 60.0% had university education or above, $p < 0.1$). Anal warts were detected in 10.6% of HIV-infected MSM and 3.8% of HIV-uninfected MSM ($p = 0.095$). Sixty-three point three percent of HIV-infected MSM and 30.6% of HIV-uninfected MSM were detected with either anal chlamydia, gonorrhea, or mycoplasma ($p < 0.001$).

3.2. Sexual Behaviors. As shown in Table 2, the majority of both HIV-infected (81.0%) and HIV-uninfected MSM (83.5%) had sex with men only. Median age at first anal intercourse (AFAI) with a man (23.7% versus 21.7% with AFAI at 18 years or younger), frequency of condom use in anal sex in the past 3 months (46.8% versus 57.7% always used a condom), sexual experience with a woman in the past 3 months (6.3% versus 8.2%), and commercial sex experience (8.9% versus 4.7%) were similar in both groups of men (p for all > 0.1). HIV-infected MSM had slightly less male sex partners in the past 3 months compared to HIV-uninfected MSM, even though the difference was not statistically significant ($p < 0.1$).

3.3. Positivity of HPV DNA. All samples were beta-globin positive. The proportion of men with each of the 28 HPV types detected by PCR is shown in Table 3. The positivities of anal HPV of any type (81.0% versus 48.2%, $p < 0.001$), any high risk type (50.6% versus 27.1%, $p = 0.002$), any low risk type (55.7% versus 31.8%, $p = 0.002$), any 4-valent vaccine type (64.6% versus 32.9%, $p < 0.001$), and any 9-valent vaccine type (74.7% versus 36.5%, $p < 0.001$) were all significantly higher among HIV-infected MSM compared to those among HIV-negative MSM. This tendency also applied to HPV 18, HPV 6/11, and HPV 16/18 (p for all < 0.05). Other individual HPV types including HPV 6, 33, 39, 52, and 84 were slightly higher among HIV-infected MSM compared to those among HIV-negative MSM, even though the difference was not statistically significant ($p < 0.1$). In the meantime, multiple HPV infection was seen in 30.4% of HIV-infected MSM and 16.4% of HIV-uninfected MSM ($p =$

Table 2: Sexual behaviors among HIV-infected and HIV-uninfected men who have sex with men in Guangzhou.

Sexual behaviors	HIV-infected		HIV-uninfected		All	
	n	%	*n*	%	*N*	%
Gender of sex partner						
Mostly with women and sometimes with men	4	5.1	6	7.1	10	6.1
Mostly with men and sometimes with women	11	13.9	8	9.4	19	11.6
With men only	64	81.0	71	83.5	135	82.3
AFAI (year)	Median = 19		Median = 19		Median = 19	
≤18	18	23.7	18	21.7	36	22.6
>18	58	76.3	65	78.3	123	77.4
Type of first sex						
Mutual masturbation	33	41.8	41	48.2	74	45.1
Oral sex	15	19.0	16	18.8	31	18.9
Anal sex	28	35.4	25	29.4	53	32.3
Cervical sex	3	3.8	3	3.5	6	3.7
*Sex with a man in p3m**						
No	13	16.5	6	7.1	145	88.4
Yes	66	83.5	79	92.9	19	11.6
*Number of male sex partners in p3m**	Median = 2		Median = 2		Median = 2	
0	8	11.1	3	3.7	11	7.1
1	32	44.4	50	61.0	82	53.3
≥2	32	44.4	29	35.4	61	39.6
Frequency of condom use with men in p3m						
Always	37	46.8	49	57.7	86	52.4
Sometimes	33	41.8	25	29.4	58	35.4
Never	9	11.4	11	12.9	20	12.2
Sex with a woman in p3m						
Yes	5	6.3	7	8.2	12	7.3
No	74	93.7	78	91.8	152	92.7
Commercial sex experience						
Yes	7	8.9	4	4.7	11	6.7
No	72	91.1	81	95.3	153	93.3

Notes. *$0.05 < p < 0.10$.

0.035). HIV-infected MSM had significantly more types of any HPV infection, any 4-valent vaccine type HPV infection, and any 9-valent vaccine type HPV infection compared to HIV-uninfected MSM (median 1 versus 0, p for both < 0.05). The great majority of HPV-infected MSM were infected with 9-valent vaccine types (59 out of 64 HIV-infected ones and 31 out of 41 HIV-uninfected ones).

3.4. Factors Associated with Anal HPV Positivity. In the multivariate logistic regression model as shown in Table 4, after accounting for other potential confounders: MSM who had an STI detection (including anal chlamydia, anal gonorrhea, or anal mycoplasma) were 2.5 (95% CI: 1.1–6.0) times more likely to be infected with any anal HPV compared to those who had no STI detection; and MSM who had HIV were 4.1 (95% CI: 1.8–9.3) times more likely to be infected with any anal HPV compared to those HIV-uninfected. Rank sum test showed that MSM infected with anal chlamydia ($p = 0.003$) and anal gonorrhea ($p = 0.058$) had greater number of anal HPV types detected.

4. Discussion

This was the first study to report anal HPV positivity among MSM in Guangzhou, China. We found, among these predominantly young and sexually experienced MSM, anal

Table 3: Detection of anal HPV among HIV-infected and HIV-uninfected men who have sex with men in Guangzhou.

(a) DNA positivity

	n, %					
	HIV-infected (N = 79)		HIV-uninfected (N = 85)		All (N = 164)	
Any type tested**	64	81.0	41	48.2	105	64.0
High risk types						
Any high risk types**	40	50.6	23	27.1	63	38.4
HPV 16	17	21.5	12	14.1	29	17.7
HPV 18**	8	10.1	2	2.4	10	6.1
HPV 26	2	2.5	1	1.2	3	1.8
HPV 31	2	2.5	0	0	2	1.2
HPV 33*	7	8.9	2	2.4	9	5.5
HPV 35	1	1.3	1	1.2	2	1.2
HPV 39*	3	3.8	0	0	3	1.8
HPV 45	1	1.3	1	1.2	2	1.2
HPV 51	2	2.5	2	2.4	4	2.4
HPV 52*	8	10.1	3	3.5	11	6.7
HPV 53	2	2.5	3	3.5	5	3.1
HPV 56	2	2.5	0	0	2	1.2
HPV 58	6	7.6	2	2.4	8	4.9
HPV 59	5	5.1	2	2.4	6	3.7
HPV 66	2	2.5	2	2.4	4	2.4
HPV 68	5	6.3	3	3.5	8	4.9
HPV 73	0	0	1	1.2	1	0.6
HPV 82	1	1.3	2	2.4	3	1.8
Low risk types						
Any low risk type**	44	55.7	27	31.8	71	43.3
HPV 6*	23	29.1	11	12.9	34	20.7
HPV 11	15	19.0	9	10.6	24	14.6
HPV 40	3	3.8	1	1.2	4	2.4
HPV 42	4	5.1	1	1.2	5	3.1
HPV 43	3	3.8	2	2.4	5	3.1
HPV 44	2	2.5	1	1.2	3	1.8
HPV 54	2	2.5	1	1.2	3	1.8
HPV 61	2	2.5	3	3.5	5	3.1
HPV 81	0	0	2	2.4	2	1.2
HPV 84*	0	0	3	3.5	3	1.8
HPV 6/11**	36	45.6	18	21.2	54	32.9
HPV 16/18**	25	31.7	14	16.5	39	23.8
Any 4-valent vaccine type**	51	64.6	28	32.9	79	48.2
HPV 16/18/31/33/45/52/58**	35	44.3	19	22.4	54	32.9
Any 9-valent vaccine type**	59	74.7	31	36.5	90	54.9

(b) Detection of multiple HPV types

	n, %		
	HIV-infected (N = 79)	HIV-uninfected (N = 85)	All (N = 164)
Any type			
Median, IQR**	1, 0–5	0, 0–4	1, 0–6

(b) Continued.

	n, %					
	HIV-infected (N = 79)		HIV-uninfected (N = 85)		All (N = 164)	
0	15	19.0	44	51.8	59	36.0
1	40	50.6	27	31.8	67	40.9
2	8	10.1	7	8.2	15	9.2
3	6	7.6	3	3.5	9	5.5
4+	10	12.7	4	4.7	14	8.5
4-valent vaccine types						
Median, IQR**	1, 0–2		0, 0–2		0, 0–2	
0	28	35.4	57	60.1	85	51.8
1	41	51.9	23	27.1	64	39.0
2	8	10.1	4	4.7	12	7.3
3	2	2.5	1	1.2	3	1.8
9-valent vaccine types						
Median, IQR**	1, 0–3		0, 0–2		1, 0–3	
0	20	25.3	54	63.5	74	45.1
1	41	51.9	23	27.1	64	39.0
2	10	12.7	6	7.1	16	9.8
3	7	8.9	1	1.2	8	4.9
4+	1	1.3	1	1.2	2	1.2

Notes. *$0.05 < p < 0.10$. **$p < 0.05$.

HPV positivity in HIV-infected ones was almost twice as high as that in HIV-uninfected ones, and detection of anal chlamydia, gonorrhea, or mycoplasma was associated with both higher anal HPV positivity and greater number of anal HPV types detected.

The rates of anal HPV positivity among both HIV-infected and HIV-uninfected MSM in our study were similar to that reported among MSM in northern and middle China [8–10, 12]. However these rates were higher than that reported among MSM in Shenzhen (71.4% and 33.8%), another large city right next to Guangzhou [13]. The reason might be that men in our study had more sex partners compared to that study (median 2 versus 1.5 in the past 3 months). The Shenzhen study did not recruit HIV-infected and HIV-uninfected MSM separately and only recruited 28 HIV-infected MSM. As a result the point estimate of HPV positivity among HIV-infected MSM might not be reliable.

Many MSM, especially those infected with HIV, had multiple anal HPV types detected. HIV compromises the immunological responses of the body and results in even greater HPV positivity among HIV-infected MSM. Higher reinfection rates and lower clearance rates in HIV-positive MSM may also play a role [19]. The contribution of bacterial infections to HPV infection has not been well documented. Bacterial infections can cause inflammation in the mucosa of the anal canal and understandably facilitate the deposition of HPV in this area. Our study showed that anal bacterial infections, including chlamydia, gonorrhea, and mycoplasma, were associated with both higher anal HPV positivity and multiplicity of anal HPV types. Timely detection and treatment of bacterial infections may help reduce HPV transmission.

The positivities of high risk types including HPV 16, 18, and 52 and low risk types including HPV 6 and 11 were very high in the anal canal in participants, especially in those infected with HIV. The infections of these HPV types suggest high burdens of corresponding morbidities in the foreseeable future, including anal warts that may relapse, anal intraepithelial neoplasia, and even anal cancer. However anal pap smears are not widely available yet in many clinical settings. A review found that only 2 out of 30 national HIV treatment guidelines included anal cancer screening for HIV-infected people [20]. A mathematical modelling demonstrated that screening for anal cancer by incorporating regular anal examinations into routine care for HIV-infected MSM is likely to be cost-effective by conventional standards. Regular anal exams for MSM, especially those living with HIV, to detect anal cancer earlier should be implemented [21]. It is noticeable that the great majority of HPV types detected were covered by both 4-valent and 9-valent HPV vaccines. High immunogenicity and tolerability of both 4-valent and 9-valent HPV vaccines was found in MSM aged 19–26 years. This implied that if vaccinated before sex debut, most of HPV cases in MSM in Guangzhou would be prevented by the two HPV vaccines. However currently in China, the 2-valent HPV vaccine Cervarix manufactured by GlaxoSmithKline was recently approved by the China Food and Drug Administration (CFDA) and this will be the only HPV vaccine that

Table 4: Factors associated with detection of any HPV among men who have sex with men in Guangzhou.

Risk factors	% (number of those with HPV/number of men)	Crude odds ratio (95% CI)	Adjusted odds ratio (95% CI)
Age at enrollment			
18–24	57.8 (37/64)	Ref	Ref
25–34	66.3 (57/86)	1.43 (0.74–2.80)	1.45 (0.64–3.29)
≥35	78.6 (11/14)	2.68 (0.68–10.53)	3.07 (0.63–14.99)
Education level			
Middle school or less	78.1 (25/32)	Ref	
Technical diploma	61.7 (29/47)	0.45 (0.16–1.26)	
University or above	60.0 (51/85)	0.42 (0.16–1.08)	
Number of male sex partners in p3m*			
0	63.6 (7/11)	Ref	Ref
1	61.0 (50/82)	0.89 (0.24–3.30)	1.51 (0.33–7.04)
≥2	67.2 (41/61)	1.17 (0.31–4.47)	1.17 (0.24–5.72)
Frequency of condom use with male sex partners in p3m			
Always	64.0 (55/86)	Ref	Ref
Sometimes	72.4 (42/58)	1.48 (0.72–3.05)	1.15 (0.50–2.67)
Never	40.0 (8/20)	0.38 (0.14–1.02)	0.41 (0.12–1.40)
Commercial sex experience			
Yes	45.5 (5/11)	0.44 (0.13–1.52)	
No	65.4 (100/153)	Ref	
STI detection#			
Yes	74.4 (62/83)	**2.61 (1.35–5.05)****	**2.51 (1.06–5.95)****
No	53.1 (43/81)	Ref	Ref
HIV status			
Positive	81.0 (64/79)	**4.58 (2.26–9.27)****	**4.12 (1.82–9.32)****
Negative	48.2 (41/85)	Ref	Ref
Frequency of drinking			
Seldom or never	65.3 (64/98)	Ref	
Sometimes	60.0 (33/55)	0.80 (0.40–1.57)	
Every day or often	72.7 (8/11)	1.42 (0.35–5.69)	

Notes. ** $p < 0.05$. #STIs include anal chlamydia, anal gonorrhea, and anal mycoplasma. *$0.05 < p < 0.10$.

is available in the Chinese market. MSM in China are likely to take this vaccine and actively seek opportunities to get the 4-valent or 9-valent vaccine via other routes. Given that the age at first anal sex has been steadily decreasing [22], many MSM may miss the best age—an age before they commence their sexual life—to be vaccinated against HPV [23]. MSM media and MSM community organizations and clinicians should stand in the forefront of health education about HPV prevention in MSM.

The results in our study should be interpreted with cautions. We used convenience sampling method to recruit participants and our sample may not represent general MSM in Guangzhou. The sample size was comparatively small which limited the statistical power in detecting additional factors associated with HPV positivity. As cross-sectional detection of HPV may be transient deposition only, it was hard to predict how many of the cases would persist. Our study attracted many MSM who experienced receptive anal sex. The majority of men (72%) in our study had receptive anal sex while only 44% had insertive anal sex, in the past 3 months. This may contribute to an overestimated anal HPV infection. Tabaco use had been demonstrated to be an independent risk factor for HPV infection in MSM [24]. We did not ask questions about cigarette smoking and were unable to account for this confounder. Studies showed that smoking rates in both HIV-infected and HIV-uninfected MSM were similar [25]. The missing of this variable was unlikely to cause biased risk for HPV infection when comparing the two groups of men.

Our study found high HPV positivity among MSM in Guangzhou, especially among those infected with HIV. The majority of these cases were potentially preventable by the

9-valent HPV vaccine. Regular anal exams and early HPV vaccination are warranted in MSM attending sexual health clinics in Guangzhou.

Competing Interests

All authors declare no competing interests.

Authors' Contributions

This study was conceived and designed by Xuqi Ren, Wujian Ke, and Huachun Zou in consultation with the other authors. Xuqi Ren and Wujian Ke drafted the protocol. Ligang Yang and Bin Yang provided insights on clinic recruitment. Shujie Huang and Xiaolin Qin proofread the laboratory testing design. Xuqi Ren, Wujian Ke, and Huachun Zou drafted the manuscript with all authors critically reviewing the manuscript. This manuscript has not been previously published nor submitted elsewhere. Xuqi Ren and Wujian Ke contributed equally to this manuscript.

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References

[1] H. Zou, C. K. Fairley, J. S. Hocking, S. M. Garland, A. E. Grulich, and M. Y. Chen, "The prevalence of anal human papillomavirus among young HIV negative men who have sex with men," *BMC Infectious Diseases*, vol. 12, article 341, 2012.

[2] H. Zou, S. N. Tabrizi, A. E. Grulich et al., "Site-specific human papillomavirus infection in adolescent men who have sex with men (HYPER): an observational cohort study," *The Lancet Infectious Diseases*, vol. 15, no. 1, pp. 65–73, 2015.

[3] A. G. Nyitray, R. J. Carvalho da Silva, M. L. Baggio et al., "Age-specific prevalence of and risk factors for anal human papillomavirus (HPV) among men who have sex with women and men who have sex with men: the HPV in men (HIM) study," *The Journal of Infectious Diseases*, vol. 203, no. 1, pp. 49–57, 2011.

[4] D. A. Machalek, M. Poynten, F. Jin et al., "Anal human papillomavirus infection and associated neoplastic lesions in men who have sex with men: a systematic review and meta-analysis," *The Lancet Oncology*, vol. 13, no. 5, pp. 487–500, 2012.

[5] M. A. Stanley, D. M. Winder, J. C. Sterling, and P. K. C. Goon, "HPV infection, anal intra-epithelial neoplasia (AIN) and anal cancer: current issues," *BMC Cancer*, vol. 12, article 398, 4 pages, 2012.

[6] L. S. Arroyo, M. Basaras, E. Arrese, S. Hernáez, V. Esteban, and R. Cisterna, "Distribution of genital human papillomavirus genotypes in benign clinical manifestations among men from Northern Spain," *BMC Public Health*, vol. 16, article 81, 8 pages, 2016.

[7] M. F. Schim van der Loeff, S. H. Mooij, O. Richel, H. J. C. de Vries, and J. M. Prins, "HPV and anal cancer in HIV-infected individuals: a review," *Current HIV/AIDS Reports*, vol. 11, no. 3, pp. 250–262, 2014.

[8] Y. Hu, H.-Z. Qian, J. Sun et al., "Anal human papillomavirus infection among HIV-infected and uninfected men who have sex with men in Beijing, China," *Journal of Acquired Immune Deficiency Syndromes*, vol. 64, no. 1, pp. 103–114, 2013.

[9] Y. Yang, X. Li, Z. Zhang et al., "Association of human papillomavirus infection and abnormal anal cytology among HIV-infected MSM in Beijing, China," *PLoS ONE*, vol. 7, no. 4, Article ID e35983, 2012.

[10] X. Zhang, J. Yu, M. Li et al., "Prevalence and related risk behaviors of HIV, syphilis, and anal HPV infection among men who have sex with men from Beijing, China," *AIDS and Behavior*, vol. 17, no. 3, pp. 1129–1136, 2013.

[11] X. Li, M. Li, Y. Yang et al., "Anal HPV/HIV co-infection among men who have sex with men: a cross-sectional survey from three cities in China," *Scientific Reports*, vol. 6, Article ID 21368, 2016.

[12] Z. Li, H. Zhang, X. Li et al., "Anal human papillomavirus genotyping among HIV-positive men who have sex with men in Xi'an, China," *PLoS ONE*, vol. 10, no. 4, Article ID e0125120, 2015.

[13] D.-Y. Zhang, Y.-P. Yin, T.-J. Feng et al., "HPV infections among MSM in Shenzhen, China," *PLoS ONE*, vol. 9, no. 5, Article ID e96364, 2014.

[14] Guangzhou Economic and Social Development Statistics Bulletin 2015, (Chinese), http://www.gzstats.gov.cn/gzsq/201112/t20111229_431.html.

[15] C. Wang, L. Yang, A. Chen, Q. He, and L. Lin, "Qualitative investigation and multiplier methods on AIDS related information among the male homosexual in Guangzhou," *Journal of Diagnosis and Therapy on Dermato-venereology*, vol. 21, pp. 333–336, 2014.

[16] X. Qin, H. Zheng, J. Huang et al., "Study on the infection and pathogenicity of Mycoplasma genitalium in sexually transmitted diseases clinic patients of Guangzhou," *International Journal of Epidemiology and Infectious Disease*, vol. 42, no. 2, pp. 99–102, 2015.

[17] M. M. Manos, Y. Ting, D. K. Wright, J. Lewis, T. R. Broker, and S. M. Wolinsky, "The use of polymerase chain reaction amplification for the detection of genital human papillomaviruses," *Cancer Cells*, vol. 7, pp. 209–214, 1989.

[18] M. Schiffman, G. Clifford, and F. M. Buonaguro, "Classification of weakly carcinogenic human papillomavirus types: addressing the limits of epidemiology at the borderline," *Infectious Agents and Cancer*, vol. 4, article 8, 2009.

[19] A. A. R. Tobian, G. Kigozi, P. E. Gravitt et al., "Human papillomavirus incidence and clearance among HIV-positive and HIV-negative men in sub-Saharan Africa," *AIDS*, vol. 26, no. 12, pp. 1555–1565, 2012.

[20] J. J. Ong, M. Chen, A. E. Grulich, and C. K. Fairley, "Regional and national guideline recommendations for digital ano-rectal

examination as a means for anal cancer screening in HIV positive men who have sex with men: a systematic review," *BMC Cancer*, vol. 14, article 557, pp. 1–7, 2014.

[21] J. J. Ong, C. K. Fairley, S. Carroll et al., "Cost-effectiveness of screening for anal cancer using regular digital ano-rectal examinations in men who have sex with men living with HIV," *Journal of the International AIDS Society*, vol. 19, no. 1, Article ID 20514, 2016.

[22] H. Zou, J. Xu, Q. Hu et al., "Decreasing age at first anal intercourse among men who have sex with men in China: a multicentre cross-sectional survey," *Journal of the International AIDS Society*, vol. 19, no. 1, Article ID 20792, 2016.

[23] H. Zou, A. E. Grulich, A. M. Cornall et al., "How very young men who have sex with men view vaccination against human papillomavirus," *Vaccine*, vol. 32, no. 31, pp. 3936–3941, 2014.

[24] H. Zou, S. N. Tabrizi, A. E. Grulich et al., "Early acquisition of anogenital human papillomavirus among teenage men who have sex with men," *Journal of Infectious Diseases*, vol. 209, no. 5, pp. 642–651, 2014.

[25] W. Z. Akhtar-Khaleel, R. L. Cook, S. Shoptaw et al., "Trends and predictors of cigarette smoking among HIV seropositive and seronegative men: the multicenter aids cohort study," *AIDS and Behavior*, vol. 20, no. 3, pp. 622–632, 2016.

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Research Article

Internet-Based Sex-Seeking Behavior Promotes HIV Infection Risk: A 6-Year Serial Cross-Sectional Survey to MSM in Shenyang, China

Shi Pan,[1,2] Jun-Jie Xu,[1,2] Xiao-Xu Han,[1,2] Jing Zhang,[1,2] Qing-Hai Hu,[1,2] Zhen-Xing Chu,[1,2] Yan-Qiu Hai,[1,2] Xiang Mao,[1,2] Yan-Qiu Yu,[1,2] Wen-Qing Geng,[1,2] Yong-Jun Jiang,[1,2] and Hong Shang[1,2]

[1] *Key Laboratory of AIDS Immunology of National Health and Family Planning Commission, Department of Laboratory Medicine, The First Affiliated Hospital, China Medical University, Shenyang 110001, China*
[2] *Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, Hangzhou, China*

Correspondence should be addressed to Hong Shang; hongshang100@hotmail.com

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HIV prevalence is still rapidly increasing among Chinese men who have sex with men (MSM). The Internet also makes it easier for MSM to have casual partners. This study aims to evaluate the trend of Internet-based sex-seeking behavior of MSM and its impact on HIV prevalence, the distribution of HIV subtype strains, and transmitted drug resistance rates. A serial cross-sectional study was conducted from 2009 to 2014. Of the 1,981 MSM, 50.5% (1,000/1,981) mainly sought homosexual partners through the Internet (Internet-based MSM, IBM). The proportion of IBM among total MSM subjects increased from 43.3% to 61.5% ($p < 0.001$). HIV prevalence of IBM increased from 5.7% to 20.7%, while that of non-Internet-based MSM (NIBM) increased from 7.0% to 14.7%. A relative higher proportion of NIBM were infected with HIV CRF01_AE subtype than IBM (79.5% versus 72.2%, $p = 0.52$). Multivariable analysis found IBM had a significantly higher HIV prevalence than NIBM (13.2% versus 10.5%, aOR = 1.4, 95% CI [1.0–1.9]). Being a migrant non-Shenyang resident MSM (aOR = 1.9, 95% CI [1.3–2.9]) and occasionally/never using condoms with casual homosexual partners (aOR = 1.7, 95% CI [1.1–2.6]) were two distinct risk factors for HIV infection in IBM. More efforts should be targeted towards developing interventions aimed at IBM, particularly migrant MSM and who engage in UAI with casual homosexual partners.

1. Introduction

Globally, men who have sex with men (MSM) suffer disproportionately with HIV infection. The prevalence of HIV in China continues to increase, with rates in Chinese MSM increasing from 0.9% to 7.3% from 2003 to 2013 [1]. Out of the newly reported HIV/AIDS cases in China in 2015, 28.3% were from the MSM population [2], while in 2011, MSM only accounted for 13.7% of newly reported cases [3].

With the development of Internet and mobile technology, the ways of socializing and seeking homosexual partners have changed dramatically with MSM. More and more MSM use Internet- or mobile-based geosocial networking (GSN) applications to socialize and seek partners. The Internet makes it easier for MSM to seek casual homosexual partners. But Internet-based MSM [IBM] also have distinct characteristics which promotes their high-risk sexual behaviors [4–7]. As a result, there are great concerns for the potential effect of Internet-based socializing on HIV infection and transmission [4, 6].

The majority of published studies on IBM were completed via questionnaires done online or over the smart phone application [4–7]. These studies examined HIV testing and sexual behaviors and mainly found that IBM are usually younger, have higher educational level, and are more likely to test for HIV compared with non-Internet-based MSM [NIBM] [6–11]. A few studies compared the prevalence of HIV and sexually transmitted infections (STIs) between IBM

and NIBM, but with inconclusive evidence on whether online partner-seeking increased the prevalence of HIV and or STIs [5, 9]. One meta-analysis showed that the pooled prevalence of both gonorrhea and chlamydia was significantly higher among IBM than NIBM [6], but the prevalence of HIV was not found significantly different between above two groups by recent surveys [8, 10, 12, 13]. Hence, there is an urgent need for longitudinal studies on biological measures of HIV and other STDs between IBM and NIBM, to confirm the real impact of Internet socializing of MSM on their HIV infection risk [4–6].

The subtypes of HIV are quite diverse in the MSM population, with the subtypes correlating with geographic location, transmission routes, and even sexual characteristics [14–16]. HIV bioinformatics analysis has been used to elucidate the transmission origin, transmission routes, and evolutionary dynamics of HIV in MSM and other populations [17]. However, scarce information is available about the molecular characteristics of HIV in IBM.

The main objectives of this study are to investigate the dynamics of MSM seeking homosexual partners through the Internet, the prevalence of HIV and its trends over time among IBM and NIBM, factors correlating with HIV infection among IBM and NIBM, and the difference in HIV subtypes and HIV transmitted drug resistance (TDR) rates between IBM and NIBM in Shenyang, China.

2. Materials and Methods

2.1. Study Design. This study was conducted in the city of Shenyang in China. Shenyang is the provincial capital of Liaoning Province and one of the largest cities in China, with a population of approximately 8.1 million. Shenyang serves as the commercial and transportation hub of China's northeast region, with frequent trade between Russia, Japan, and Korea.

A six-year serial cross-sectional study was conducted in Shenyang, China, from February 2009 to December 2014. The Institutional Review Boards of the First Affiliated Hospital of CMU approved the study protocol and informed consent forms.

2.2. Study Participant Enrollment. The Voluntary Counseling and Testing (VCT) clinic of the First Affiliated Hospital of China Medical University (CMU) in Shenyang was chosen as the study site. The VCT clinic provides the largest free HIV testing and counseling service in Shenyang. Demographics, types, and numbers of sexual partners and high-risk HIV behaviors were collected from MSM who came to the VCT clinic for the first time. Fingerprinting was used as identification for participants. Eligible entry criteria were (1) being biologically male, (2) at least 18 years old, (3) self-reported as having anal or oral sex with another male in the past 12 months, (4) self-reported as never testing positive for HIV, and (5) being able to provide written informed consent. The exclusion criteria of this study were (1) not meeting one of five entry criteria or (2) MSM who already tested positive for HIV at VCT.

2.3. Interview Process. Participants were interviewed by using a face-to-face, structured questionnaire. The following data was collected: marriage status, occupation, monthly salary, age of male sexual behavior debut, number of male sexual partners, condom use with male sexual partners, preferred sexual roles, sexual orientation, and STI symptoms in the past 12 months (pain during urination, sensation of burning in the genitals, or abnormal urethral discharge) [18]. Ways of seeking homosexual partners were collected from each subject individually; those subjects who self-reported mainly sought male sexual partners through Internet or GSN applications in past 12 months were defined as IBM, otherwise were defined as NIBM.

2.4. Laboratory Testing. Five ml of venous blood was drawn to test antibodies of HIV-1 and syphilis. Blood specimens positive for HIV were further tested for HIV subtypes and transmitted HIV drug resistance.

2.4.1. Antibodies to HIV-1 and Syphilis. HIV-1 antibody was first screened by enzyme-linked immune sorbent assay (ELISA) (Vironostika HIV-1 Microelisa System; BioMerieux, Durham, NC). Positive specimens were further confirmed by HIV-1/2 Western blot assay (HIV Blot 2.2 WB; Genelabs Diagnostics, Singapore).

Syphilis was tested by rapid plasma regain (RPR) (Diagnosis; Shanghai, Kehua, China), and any RPR positive samples were then tested by treponema pallidum particle assay (TPPA) (Serodia, Japan). Samples that tested positive for both RPR and TPPA were determined to be currently infected with syphilis.

2.4.2. HIV Molecular Analysis. Ribonucleic acid (RNA) was extracted to synthesize cDNA from plasma. Individual genome amplification and sequencing amplified the $3'$ or $5'$ halves of viral cDNA. Single sequence fragments of each amplification was combined and edited by the sequencher program (version 4.9). We used Bioedit Software Alignment followed by the Los Alamos HIV Sequence Database. Subtyping analysis was performed by the neighbor-joining method in MEGA version 4.0. The Stanford HIV Drug Resistance Database (https://hivdb.stanford.edu/) was used to analyze mutation profiles and predict genotypic resistance.

All the above laboratory tests were conducted at the Key Laboratory of AIDS and Immunology of National Health and Family Planning Commission at CMU.

2.5. Data Analysis. Questionnaires were entered using EpiData 3.1 software. Data were analyzed using SPSS (SPSS version 20.0, IL, USA). Chi-square test was used to compare differences in categorical variables. Multivariable logistic regression analysis was used to determine independent factors correlated with HIV infection. Potential confounding factors, including age, monthly income, and education level, were all adjusted in the multivariable logistic models. Trend analysis of IBM and HIV prevalence over time was performed by chi-square trend test (linear-by-linear association). p value < 0.05 was considered statistically significant in the analysis.

2.6. Ethical Issues. A six-digit number was used to identify the participant and to link the questionnaire and laboratory test results with the participant. Written informed consent was obtained from each participant. The participants could refuse to answer any of the questions. Those who tested positive for HIV or syphilis received an immediate referral to professional HIV clinics and STI clinics. All documents with identifying information were placed in a locked file cabinet, which cannot be accessed by anyone else except for the principal investigator and study staff.

3. Results

3.1. Characteristics of IBM Participants. Among 1998 MSM who were approached to take part in the study, 10 refused to attend the study and 7 were excluded for had been tested HIV positive before attending the study. Among 1,981 eligible MSM, 50.5% (1000/1981) reported using the Internet as their main method for seeking homosexual partners. Compared with NIBM subjects, IBM subjects were younger, had more proportion of local residents, had a high school level education or above, and earned a higher income (each $p < 0.05$). IBM participants had more proportion of homosexual orientation, earlier age of male sexual behavior debut, used condoms more frequently, and more preferred receptive anal sexual roles (each $p < 0.05$). But the ethnicity and the number of homosexual partners in the past year had no statistical difference between IBM and NIBM populations (Table 1).

3.2. Factors Correlated with Internet-Based Sex-Seeking Behavior. After adjusting for age, monthly incomes, and level of education, we found that IBM was associated with having Shenyang city as a place for residence (aOR = 1.2, 95% CI [1.0–1.5], $p = 0.040$), being single (aOR = 2.0, 95% CI [1.6–2.5], $p < 0.001$), having anal intercourse at age of 25 years or younger (less than 18 years [aOR = 2.5, 95% CI [1.7–3.6], $p < 0.001$]; between 18 and 25 years [aOR = 1.9, 95% CI [1.4–2.6], $p < 0.001$]), preferring receptive anal intercourse (aOR = 1.5, 95% CI [1.1–2.0], $p = 0.004$), being of homosexual orientation (aOR = 1.3, 95% CI [1.1–1.6], $p = 0.006$), and having STD symptoms in the past year (aOR = 1.6, 95% CI [1.0–2.6], $p = 0.036$) (Table 1).

3.3. HIV/Syphilis Prevalence and Molecular Characteristics. Between 2009 and 2014, the proportion of IBM among total recruited MSM showed a rapid increased trend (from 43.3% to 61.5%, trend $p < 0.001$). Between 2009 and 2010, the increment of HIV prevalence among IBM (from 5.7% to 12.5%) and NIBM (from 7% to 13.5%) was similar. While, from 2010 to 2014, the increment of HIV prevalence among IBM was more rapid among IBM (from 12.5% to 20.7%, trend $p < 0.001$) than NIBM (from 13.5% to 14.7%, trend $p = 0.014$) (Figure 1).

HIV-1 nucleotide sequences were amplified and sequenced from drawn HIV positive specimens during 2012 to 2014, in which 93.9% (123/131) were successfully amplified. The proportion of HIV subtypes had no statistical difference between the IBM group and the NIBM group, although the former had slightly lower proportion of CRF01_AE (72.2% versus 79.5%, $p = 0.520$). The total proportion of TDR (5.1% versus 6.8%, $p = 0.487$), protease inhibitors (PIs) resistance rate (3.8% versus 2.3%, $p = 0.549$) and nonnucleoside reverse-transcriptase inhibitors (NNRTIs) resistance rate (1.3% versus 4.5%, $p = 0.291$) also had no statistical difference between the two groups (Tables 2 and 3).

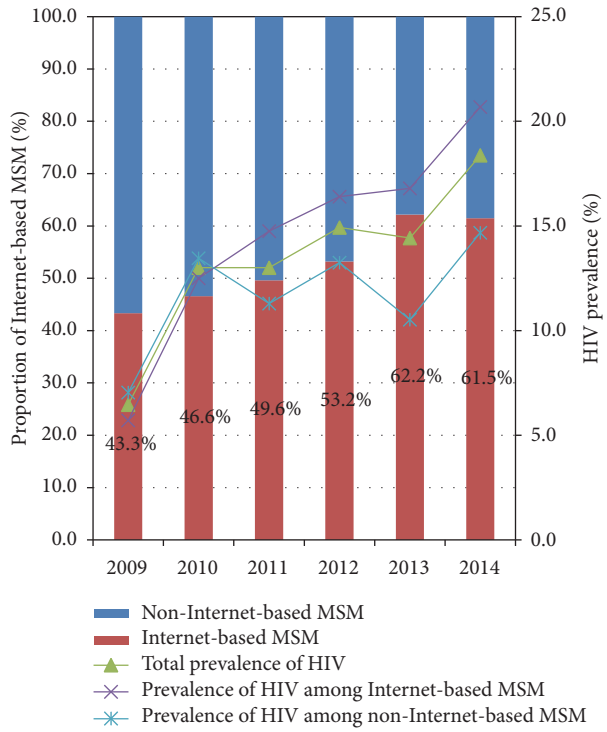

Figure 1: The annual prevalence of HIV among Internet-based MSM was 5.7% (18/315), 12.5% (17/136), 14.8% (9/61), 16.4% (31/189), 16.8% (21/125), and 20.7% (36/174). And the annual prevalence of HIV among non-Internet-based MSM was 7.0% (29/412), 13.5% (21/156), 11.3% (7/62), 13.3% (22/166), 10.5% (8/76), and 14.7% (16/981).

3.4. Associated Factors between HIV Infection and Internet Use to Seek Partners. Multivariable logistic analysis showed that, after adjusting for age, monthly income, and education level, MSM who sought partners through the Internet had significantly higher odds of HIV infection compared to MSM who did not use the Internet to seek partners (aOR = 1.4, 95% CI [1.0–1.9], $p = 0.032$) (Table 4).

Among the IBM population, the independent and statistically significant ($p < 0.05$) risk factors correlated with HIV infection included not being a resident of Shenyang (aOR,1.9), occasionally or never using condoms with steady male partners and casual male partners (aOR,1.9 and aOR,1.7, resp.), preferring both insertive and receptive anal sexual roles (aOR,2.0), preferring only receptive anal sexual role (aOR,2.5), and testing positive for syphilis (aOR,4.3) (Table 4).

Table 1: Univariate and multivariate logistic regression analyses of factors associated with using the Internet to seek male partners among Shenyang MSM [N = 1981][a].

Variables	Using Internet MSM (n = 1000)		Non-Internet MSM (n = 981)		cOR (95% CI)	aOR (95% CI)
	n	%	n	%		
Age						
≤25 years	600	60.0%	422	43.0%	Ref	
>25 years	400	40.0%	559	57.0%	2.0 (1.7–2.4)***	NA
Monthly income						
None	321	32.1%	219	22.3%	Ref	
1–300 $	321	32.1%	480	48.9%	0.5 (0.4–0.6)***	NA
>300 $	358	35.8%	282	28.7%	0.9 (0.7–1.1)	NA
Education level						
Junior high school and below	229	22.9%	539	54.9%	Ref	
Senior high school and above	771	77.1%	442	45.1%	4.1 (3.4–5.0)***	NA
Residence place						
Other cities	633	63.3%	708	72.2%	Ref	Ref
Shenyang	367	36.7%	273	27.8%	1.5 (1.2–1.8)***	1.2 (1.0–1.5)*
Ethnicity						
Non-Han	148	14.8%	161	16.4%	Ref	Ref
Han	852	85.2%	820	83.6%	1.1 (0.9–1.4)	1.0 (0.8–1.3)
Marriage status						
Others	181	18.1%	336	34.3%	Ref	Ref
Single	819	81.9%	645	65.7%	2.4 (1.9–2.9)***	2.0 (1.6–2.5)***
Sexual orientation						
Bisexual orientation	281	28.1%	366	37.3%	Ref	Ref
Homosexual orientation	704	70.4%	584	59.5%	1.6 (1.3–1.9)***	1.3 (1.1–1.6)**
Heterosexual or not sure	15	1.5%	31	3.2%	0.6 (0.3–1.2)	0.6 (0.3–1.1)
Age of male sexual behavior debut						
>25 years	90	9.0%	218	22.5%	Ref	Ref
18–25 years	678	68.0%	598	61.6%	2.8 (2.1–3.6)***	1.9 (1.4–2.6)***
<18 years	229	23.0%	155	16.0%	3.6 (2.6–4.9)***	2.5 (1.7–3.6)***

Table 1: Continued.

Variables	Using Internet MSM (n = 1000)		Non-Internet MSM (n = 981)		cOR (95% CI)	aOR (95% CI)
	n	%	n	%		
Condom use with steady male sex partners in the past year						
Occasionally or never use	371	58.1%	385	65.3%	Ref	Ref
Always use	268	41.9%	205	34.7%	1.4 (1.1–1.7)**	1.3 (1.0–1.7)*
Condom use with casual male sex partners in the past year						
Occasionally or never use	384	47.5%	402	57.8%	Ref	Ref
Always use	425	52.5%	294	42.2%	1.5 (1.2–1.9)***	1.4 (1.1–1.7)**
Preferred intercourse sexual roles with male partners						
Insertive anal sexual role	295	29.7%	339	35.2%	Ref	Ref
Receptive anal sexual role	204	20.5%	148	15.4%	1.6 (1.2–2.1)***	1.5 (1.1–2.0)**
Both insertive and receptive	495	49.8%	475	49.4%	1.2 (1.0–1.5)	1.2 (1.0–1.5)
Number of male sexual partners in the past year						
>10	272	27.2%	311	31.7%	Ref	Ref
4–10	343	34.3%	296	30.2%	1.3 (1.1–1.7)*	1.2 (0.9–1.5)
0–3	385	38.5%	374	38.1%	1.2 (1.0–1.5)	0.9 (0.7–1.1)
STD symptoms in the past year						
No	946	94.6%	946	96.4%	Ref	Ref
Yes	54	5.4%	35	3.6%	1.5 (1.0–2.4)	1.6 (1.0–2.6)*

a: adjusted for age, monthly income, and education level. cOR: crude odds ratio. aOR: adjusted odds ratio. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. NA, not applicable.

Table 2: HIV/syphilis prevalence and molecular characteristics of HIV of Shenyang MSM from 2009 to 2014 [$N = 1981$].

Variables	Using Internet MSM		Non-Internet MSM		Total	%
	n	%	n	%		
Syphilis infection						
Positive	114	11.4%	118	12.0%	232	11.7%
Negative	886	88.6%	863	88.0%	1749	88.3%
HIV infection						
Positive	132	13.2%	103	10.5%	235	11.9%
Negative	868	86.8%	878	89.5%	1746	88.1%

Table 3: Molecular characteristics of HIV of Shenyang MSM from 2012 to 2014 [$N = 123$].

Variables	Using Internet MSM		Non-Internet MSM		Total	%
	n	%	n	%		
Subtype						
CRF01_AE	57	72.2%	35	79.5%	92	74.8%
CRF07_BC	9	11.4%	5	11.4%	14	11.4%
Other subtypes	13	16.5%	4	9.1%	17	13.8%
Drug resistance						
Yes	4	5.1%	3	6.8%	7	5.7%
No	75	94.9%	41	93.2%	116	94.3%
PIs resistance rate						
Yes	3	3.8%	1	2.3%	4	3.3%
No	76	96.2%	43	97.7%	119	96.7%
NNRTIs resistance rate						
Yes	1	1.3%	2	4.5%	3	2.4%
No	78	98.7%	42	95.5%	120	97.6%

Among the NIBM population, the independent and statistically significant ($p < 0.05$) risk factors correlated with HIV infection included being nonsingle (aOR,1.7), occasionally and never using condoms with steady male partners (aOR,3.0), preferring both insertive and receptive anal sexual role (aOR,2.3), and testing positive for syphilis (aOR,4.7) (Table 4).

4. Discussion

To our knowledge, this is the first longitudinal study examining the relationship between Internet-based partner-seeking behavior and HIV infection risk. Our study expanded on the recently published literature on Internet-based partner-seeking behavior of MSM and confirmed that the IBM population had higher HIV prevalence than the NIBM population. Furthermore, the HIV prevalence in the IBM population increased more rapidly than NIBM over a 5-year time period. Our findings indicate that using the Internet to seek partners and sex is associated with an increased HIV infection and transmission risk and is correlated with distinct virtual sexual network and HIV strains.

Previous studies have failed to find a difference in age, education, and income between IBM and NIBM populations [5]. However, four peer surveys have found that, compared to NIBM, IBM population was younger, received a higher level of education, and had higher income [4, 9–11]. Our study results confirmed the findings from the four peer surveys. Furthermore, our results show that IBM tend to be single and local residents, in study site (Shenyang city).

There was also a lack of consensus in the literature about whether the Internet can increase the number of homosexual partners and the rate of unprotected anal intercourse (UAI). Grosskopf et al. found no difference in the rate of UAI and the number of homosexual partners between IBM and NIBM [7, 10, 11]. Andovitz's survey reported that the rate of UAI was significantly lower in IBM than in NIBM [8]. However, Tang's survey showed that IBM engaged in more UAI, in addition to group sex and commercial sex. Our study found that IBM had less UAI with both steady male partners and casual male partners. In regard to number of homosexual partners, Phillips [13], Lehmiller [5], and Tang [4]'s surveys indicated that IBM subpopulation had a greater number of homosexual partners. Our findings showed that the number of homosexual partners did not differ between IBM and NIBM. One possible explanation over our findings' discrepancies with previous studies' findings is that this study used venue-based recruitment method, while most of the above surveys used Internet or mobile application-based methods.

It is worth mentioning that this serial cross-sectional survey found that IBM had a statistically significant higher HIV prevalence than NIBM after adjusting for potential study confounders. We found IBM preferred receptive anal sexual roles and that more STD symptoms occurred in IBM.

Table 4: Univariate and multivariate logistic regression analysis of factors associated with HIV infection[a].

Variables	Using Internet MSM (N = 1000)			Non-Internet MSM (N = 981)		
	HIV prevalence (%)	cOR (95% CI)	aOR (95% CI)	HIV prevalence (%)	cOR (95% CI)	aOR (95% CI)
Age						
≤25 years	11.0 (66/600)	Ref		9.5 (40/422)	Ref	
>25 years	16.5 (66/400)	1.6 (1.1–2.3)*	NA	11.3 (63/559)	1.2 (0.8–1.8)	NA
Monthly income						
None	10.3 (33/321)	Ref		8.7 (19/219)	Ref	
1–300 $	13.1 (42/321)	1.3 (0.8–2.1)	NA	10.6 (51/480)	1.3 (0.7–2.2)	NA
>300 $	15.9 (57/358)	1.7 (1.1–2.6)*	NA	11.7 (33/282)	1.4 (0.8–2.5)	NA
Education level						
Junior high school and below	14.8 (34/229)	Ref		10.2 (55/539)	Ref	
Senior high school and above	12.7 (98/771)	0.8 (0.5–1.3)	NA	10.9 (48/442)	1.1 (0.7–1.6)	NA
Residence place						
Shenyang	9.0 (33/367)	Ref	Ref	10.6 (29/273)	Ref	Ref
Other cities	15.6 (99/633)	1.9 (1.2–2.9)**	1.9 (1.3–2.9)**	10.5 (74/708)	1.0 (0.6–1.6)	1.0 (0.6–1.6)
Ethnicity						
Non-Han	11.5 (17/148)	Ref	Ref	11.8 (19/161)	Ref	Ref
Han	13.5 (115/852)	1.2 (0.7–2.1)	1.2 (0.7–2.1)	10.2 (84/820)	0.9 (0.5–1.4)	0.8 (0.5–1.4)
Marriage status						
Single	12.5 (102/819)	Ref	Ref	8.7 (56/645)	Ref	Ref
Others	16.6 (30/181)	1.4 (0.9–2.2)	1.2 (0.7–1.9)	14.0 (47/336)	1.7 (1.1–2.6)*	1.7 (1.1–2.5)*
Sexual orientation						
Bisexual orientation	12.5 (35/281)	Ref	Ref	8.5 (31/366)	Ref	Ref
Homosexual orientation	13.6 (96/704)	1.1 (0.7–1.7)	1.2 (0.8–1.9)	12.0 (70/584)	1.5 (1.0–2.3)	1.5 (1.0–2.4)
Heterosexual or not sure	6.7 (1/15)	0.5 (0.1–3.9)	0.5 (0.1–4.1)	6.5 (2/31)	0.7 (0.2–3.3)	0.7 (0.2–3.2)
Age of male sexual behavior debut						
<18 years	12.7 (29/229)	Ref	Ref	13.5 (21/155)	Ref	Ref
18–25 years	12.1 (82/678)	1.0 (0.6–1.5)	0.9 (0.6–1.4)	8.9 (53/598)	0.6 (0.4–1.1)	0.6 (0.4–1.0)
>25 years	22.2 (20/90)	2.0 (1.1–3.7)*	1.5 (0.8–3.1)	13.3 (29/218)	1.0 (0.5–1.8)	0.9 (0.5–1.8)

Table 4: Continued.

Variables	Using Internet MSM (N = 1000)			Non-Internet MSM (N = 981)		
	HIV prevalence (%)	cOR (95% CI)	aOR (95% CI)	HIV prevalence (%)	cOR (95% CI)	aOR (95% CI)
Condom use with steady male sex partners in the past year						
Always use	9.3 (25/268)	Ref	Ref	5.4 (11/205)	Ref	Ref
Occasionally or never use	16.2 (60/371)	1.9 (1.1–3.1)*	1.9 (1.2–3.1)*	14.0 (54/385)	2.9 (1.5–5.6)**	3.0 (1.5–5.9)***
Condom use with casual male sex partners in the past year						
Always use	11.1 (47/425)	Ref	Ref	9.2 (27/294)	Ref	Ref
Occasionally or never use	16.7 (64/384)	1.6 (1.1–2.4)*	1.7 (1.1–2.6)*	12.2 (49/402)	1.4 (0.8–2.3)	1.4 (0.9–2.4)
Preferred intercourse sexual roles with male partners						
Insertive anal sexual role	8.5 (25/295)	Ref	Ref	6.8 (23/339)	Ref	Ref
Both insertive and receptive roles	14.5 (72/495)	1.8 (1.1–3.0)*	2.0 (1.3–3.3)**	13.7 (65/475)	2.2 (1.3–3.6)**	2.3 (1.4–3.8)***
Receptive anal sexual role	17.2 (35/204)	2.2 (1.3–3.9)**	2.5 (1.5–4.5)***	9.5 (14/148)	1.4 (0.7–2.9)	1.5 (0.7–3.1)
Number of male sexual partners in the past year						
>10	11.8 (32/272)	Ref	Ref	8.7 (27/311)	Ref	Ref
4–10	15.5 (53/343)	1.4 (0.9–2.2)	1.4 (0.9–2.3)	11.1 (33/296)	1.3 (0.8–2.3)	1.3 (0.8–2.2)
0–3	12.2 (47/385)	1.0 (0.7–1.7)	1.1 (0.7–1.7)	11.5 (43/374)	1.4 (0.8–2.3)	1.3 (0.8–2.3)
STD symptoms in the past year						
No	12.9 (122/946)	Ref	Ref	10.3 (97/946)	Ref	Ref
Yes	18.5 (10/54)	1.5 (0.8–3.1)	1.5 (0.7–3.0)	17.1 (6/35)	1.8 (0.7–4.5)	1.8 (0.7–4.4)
Syphilis infection						
Negative	10.5 (93/886)	Ref	Ref	8.0 (69/863)	Ref	Ref
Positive	34.2 (39/114)	4.4 (2.9–6.9)***	4.3 (2.8–6.7)***	28.8 (34/118)	4.7 (2.9–7.4)***	4.7 (2.9–7.5)***

a: adjusted for age, monthly income, and education level. cOR: crude odds ratio. aOR: adjusted odds ratio. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. NA, not applicable.

Both of these factors can significantly increase IBM's risk for acquiring HIV [19–22]. This study also was the first to show that there was a great increase in the Internet seeking behavior in Shenyang during the six-year survey, in which the proportion of IBM increased from 43.3% to 61.5%. Moreover, our study also found a changing trend in the prevalence of HIV in IBM compared to NIBM. The year of 2010 was a boundary time point. Before 2010, the prevalence of HIV among IBM was slightly lower than NIBM. But from 2010 to 2014, the HIV prevalence rate in IBM increased from 12.5% to 20.7%, while the HIV prevalence in NIBM did not have a significant change (from 13.5% to 14.7%). Compared to previous studies on the relationship of Internet-based partner-seeking behavior and HIV infection risk in MSM, this study was able provide laboratory testing results as well. However, this study was conducted in MSM who sought HIV counseling and testing in one big health center of Shenyang city. More longitudinal studies are needed to further examine the relationship of Internet-based partner-seeking behavior of MSM and HIV infection risk.

In the setting of continuous implementation of large scale of free antiviral therapy, the HIV TDR rate of MSM increased from 3.8% in 2004 to 4.7% in 2014 [23, 24]. This study revealed that the HIV TDR rate of Shenyang MSM is all higher than the national rate of MSM (total TDR rate, 5.7% versus 3.6%; PIs resistance rat, 3.3% versus 1.7% NNRTIs resistance rat, 2.4% versus 0.5%) [25]. The majority of HIV TDR and subtypes are CRF01_AE and PIs in Shenyang MSM, which was similar to previous study results in the same city [26]. Our study failed to found statistical differences between IBM and NIBM subpopulations in HIV TDR rates and HIV subtypes, although slightly fewer HIV CRF01_AE subtypes existed in IBM in contrast with NIBM (72.2% versus 79.5%). It showed that currently the Internet-based partner-seeking behavior have not substantial impact for their distribution of HIV subtypes and TDR. And the statistical power to determine a difference in HIV TDR and subtypes may be relatively lower due to the relatively small sample size of HIV positive subjects. Hence, HIV TDR and subtypes of IBM and NIBM should be continually monitored to provide molecular biomarker proof about transmission routes and clustering characteristics of IBM subpopulation.

We found similar independent risk factors for HIV infection in both IBM and NIBM populations, which were syphilis infection, preferring both receptive and insertive anal intercourse with steady male partners, and occasionally or never using condoms with steady sexual partners. These HIV infection risk factors were also reported by previous studies in MSM [17, 20, 27, 28]. Our findings indicate that public health officials should pay attention to relevant contextual factors that may promote HIV infection, including UAI with homosexual partners and concurrent STD infection. In addition, HIV/AIDS health education and condoms should be provided to MSM groups who prefer receptive anal role in order to raise their condom use and negotiation skills with their homosexual partners [29, 30].

We also found some distinct independent HIV infection risk factors in IBM. Among the IBM population, migrant MSM had a higher HIV prevalence than local MSM. Migrant MSM are more likely to engage in higher risk sexual risk behaviors and had lower level of HIV/AIDS knowledge [31]. They are also more likely to be the victims of sexual violence [32]. Moreover, they might lack knowledge about the availability of free HIV testing sites [31, 33]. As a result, HIV/AIDS intervention programs should be targeted towards migrant MSM, in particular those who seek partners through the Internet.

Our findings also show that IBM who occasionally or never use condoms with casual male sexual partners had significantly higher HIV prevalence. Many of these IBM will use Internet applications designed to help MSM to find casual sexual partners [5]. Thus, the combination of UAI with casual sexual partners has a great impact on HIV infection risk for IBM. To increase IBM's condom use with casual partners, more HIV/AIDS knowledge should be provided, particularly about the long asymptomatic period of HIV infection and the fact that HIV positive individuals can appear healthy.

Several strengths exist in this study. First, it is one of the few venue-based surveys about the relationship between Internet-based partner-seeking behavior of MSM and HIV infection. Thus, the HIV prevalence data was more reliable and accurate than self-reported HIV/STI testing results [5, 8, 10, 13, 34]. Previously only one study reported lab-confirmed HIV/STD infections [9]. Second, this is the first serial cross-sectional study, which can provide stronger evidence about trends of MSM seeking homosexual partners through the Internet. Third, this study also revealed differences in HIV subtype distribution and TDR rate between IBM and NIBM, which can help public health policy makers to understand the HIV transmission routes of Chinese IBM and provide effective and comprehensive interventions.

Some limitations to this study exist. This study used convenience sampling to recruit MSM subjects, so it cannot fully represent all of the characteristics of MSM in Shenyang. We used an interview questionnaire to collect information on some sexual behaviors, including the number of sexual partners and whether they engaged in UAI, so recall bias and social desirability bias may exist. In this survey, we did not collect the detailed frequency of using the Internet to seek sexual partners among studied subjects, and there may be some connection between the frequency and HIV infection; further studies should be conducted for future researches. We only tested for syphilis, and other STDs were not tested; more kinds of STDs should be tested to be explored in the future research to well understand the relationship between STDs and HIV infection of MSM subjects. Blood samples were not available to conduct HIV molecular testing from 2009 to 2011, so HIV subtypes and drug resistance rates derived from this study may have been different with MSM participants during that time. Despite the limitations, this study identifies the trends of Chinese MSM seeking sexual partners through the Internet and reports that Internet partner-seeking behavior is associated with significantly increased HIV prevalence. Our results have implications for behavioral interventions targeted at IBM.

Internet has become an increasingly popular means for Chinese MSM to socialize and seek partners. Our study showed that the IBM subpopulation had quite different

demographics compared with NIBM subpopulation. The former is younger and single, with a higher educational level and a higher income. A greater proportion of IBM preferred receptive anal sexual role and STD symptoms than NIBM, which may increase their HIV infection risk. Prevalence of HIV increased more rapidly in IBM than NIBM over the course of the study. IBM also had a different distribution of HIV subtypes and TDR. More longitudinal surveys, in particular prospective cohort surveys, are needed to further examine the relationship between Internet partner-seeking behavior, sexual behaviors, and HIV infection in MSM. Gay application developers should try to integrate HIV/AIDS messaging into their platforms to promote HIV/STIs testing, condom usage, risk behavior reduction counseling, and other necessary services for the MSM community.

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

Authors' Contributions

Shi Pan and Jun-Jie Xu are equal contributors.

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References

[1] National Health and Family Planning Commission of China, UNAIDS, and World Health Organization, "China AIDS Response Progress Report," 2014, http://www.unaids.org/sites/default/files/documents/CHN_narrative_report_2014.pdf.

[2] NCAIDS, NCSTD, and China CDC, "Update on the AIDS/STD epidemic in China and main response in control and prevention in December, 2015," *Chinese Journal of AIDS & STD*, vol. 22, no. 2, p. 69, 2016.

[3] NCAIDS and China CDC, "Update on the AIDS/STD epidemic in China and main response in control and prevention in 2011," *Chinese Journal of AIDS & STD*, vol. 18, no. 1, p. 64, 2012.

[4] W. M. Tang, B. John, Y. Zhang et al., "Gay mobile apps and the evolving virtual risk environment: a cross-sectional online survey among men who have sex with men in China," *Sexually Transmitted Infections*, vol. 92, no. 7, pp. 508–514, 2016.

[5] J. J. Lehmiller and M. Ioerger, "Social networking smartphone applications and sexual health outcomes among men who have sex with men," *PLoS ONE*, vol. 9, no. 1, Article ID e86603, 2014.

[6] H. C. Zou and S. Fan, "Characteristics of men who have sex with men who use smartphone geosocial networking applications and implications for HIV interventions: a systematic review and meta-analysis," *Archives of Sexual Behavior*, 2016.

[7] N. A. Grosskopf, M. T. LeVasseur, and D. B. Glaser, "Use of the Internet and mobile-based 'apps' for sex-seeking among men who have sex with men in New York City," *American Journal of Men's Health*, vol. 8, no. 6, pp. 510–520, 2014.

[8] R. J. Landovitz, C.-H. Tseng, M. Weissman et al., "Epidemiology, sexual risk behavior, and HIV prevention practices of men who have sex with men using GRINDR in Los Angeles, California," *Journal of Urban Health*, vol. 90, no. 4, pp. 729–739, 2013.

[9] M. R. Beymer, R. E. Weiss, R. K. Bolan et al., "Sex on demand: geosocial networking phone apps and risk of sexually transmitted infections among a cross-sectional sample of men who have sex with men in Los Angeles county," *Sexually Transmitted Infections*, vol. 90, no. 7, pp. 567–572, 2014.

[10] C. H. Bien, J. M. Best, K. E. Muessig, C. Wei, L. Han, and J. D. Tucker, "Gay apps for seeking sex partners in China: implications for MSM sexual health," *AIDS & Behavior*, vol. 19, no. 6, pp. 941–946, 2015.

[11] E. R. Burrell, H. A. Pines, E. Robbie et al., "Use of the location-based social networking application GRINDR as a recruitment tool in rectal microbicide development research," *AIDS & Behavior*, vol. 16, no. 7, pp. 1816–1820, 2012.

[12] H. J. Rendina, R. H. Jimenez, C. Grov, A. Ventuneac, and J. T. Parsons, "Patterns of lifetime and recent HIV testing among men who have sex with men in New York city who use grindr," *AIDS & Behavior*, vol. 18, no. 1, pp. 41–49, 2014.

[13] G. Phillips, C. Grov, and B. Mustanski, "Engagement in group sex among geosocial networking mobile application-using men who have sex with men," *Sexual Health*, vol. 12, no. 6, pp. 495–500, 2015.

[14] J. H.-K. Chen, K.-H. Wong, K. C.-W. Chan, S. W.-C. To, Z. Chen, and W.-C. Yam, "Phylodynamics of HIV-1 subtype B among the men-having-sex-with-men (MSM) population in Hong Kong," *PLoS ONE*, vol. 6, no. 9, Article ID e25286, 2011.

[15] B. Zhao, X. Han, J. Xu et al., "Increase of RT-related transmitted drug resistance in non-CRF01_AE among HIV type 1–infected men who have sex with men in the 7 cities of China," *Journal of Acquired Immune Deficiency Syndromes*, vol. 68, no. 3, pp. 250–255, 2015.

[16] Z. Li, L. Liao, Y. Feng et al., "Trends of HIV subtypes and phylogenetic dynamics among young men who have sex with men in China, 2009–2014," *Scientific Reports*, vol. 5, article 16708, 2015.

[17] J. Xu, M. An, X. Han et al., "Prospective cohort study of HIV incidence and molecular characteristics of HIV among men who have sex with men(MSM) in Yunnan Province, China," *BMC Infectious Diseases*, vol. 13, article 3, 2013.

[18] J.-J. Xu, K. H. Reilly, C.-M. Lu et al., "A cross-sectional study of HIV and syphilis infections among male students who have sex with men (MSM) in northeast China: implications for implementing HIV screening and intervention programs," *BMC Public Health*, vol. 11, article no. 287, 2011.

[19] Q.-Q. Wang, X.-S. Chen, Y.-P. Yin et al., "HIV prevalence, incidence and risk behaviours among men who have sex with men in Yangzhou and Guangzhou, China: a cohort study," *Journal of the International AIDS Society*, vol. 17, no. 4, Article ID 18849, 2014.

[20] J.-J. Xu, M. Zhang, K. Brown et al., "Syphilis and HIV seroconversion among a 12-month prospective cohort of men who

have sex with men in Shenyang, China," *Sexually Transmitted Diseases*, vol. 37, no. 7, pp. 432–439, 2010.

[21] X. Meng, H. Zou, S. Fan et al., "Relative risk for HIV infection among men who have sex with men engaging in different roles in anal sex: a systematic review and meta-analysis on global data," *AIDS & Behavior*, vol. 19, no. 5, pp. 882–889, 2015.

[22] P. Pathela, K. Jamison, S. L. Braunstein, J. A. Schillinger, J. K. Varma, and S. Blank, "Incidence and predictors of HIV infection among men who have sex with men attending public sexually transmitted disease clinics, New York City, 2007–2012," *AIDS & Behavior*, 2016.

[23] L. Liao, H. Xing, H. Shang et al., "The prevalence of transmitted antiretroviral drug resistance in treatment-naive HIV-infected individuals in China," *JAIDS Journal of Acquired Immune Deficiency Syndromes*, vol. 53, pp. S10–S14, 2010.

[24] D.-J. Liu, M.-X. Feng, and M. Liu, "Primary drug resistance of human immunodeficiency virus(HIV) among the treatment-naïve individuals with HIV in China: a meta-analysis," *Journal of Peking University (Health Sciences)*, vol. 47, no. 3, pp. 474–482, 2015.

[25] J. Wu and N. Wang, "The prevalence of HIV primary drug resistance among men who have sex with men in China: a meta-analysis," *Chinese Journal of AIDS & STD*, vol. 20, no. 7, pp. 470–473, 2014.

[26] B. Zhao, X. Han, D. Dai et al., "New trends of primary drug resistance among HIV type 1-infected men who have sex with men in Liaoning Province, China," *AIDS Research & Human Retroviruses*, vol. 27, no. 10, pp. 1047–1053, 2011.

[27] W. Zhang, J. Xu, H. Zou, J. Zhang, N. Wang, and H. Shang, "HIV incidence and associated risk factors in men who have sex with men in Mainland China: an updated systematic review and meta-analysis," *Sexual Health*, vol. 13, no. 4, pp. 373–382, 2016.

[28] J.-J. Xu, C. Zhang, Q.-H. Hu et al., "Recreational drug use and risks of HIV and sexually transmitted infections among Chinese men who have sex with men: mediation through multiple sexual partnerships," *BMC Infectious Diseases*, vol. 14, no. 1, article 642, 7 pages, 2014.

[29] A. L. Herrick, S. H. Lim, C. Wei et al., "Resilience as an untapped resource in behavioral intervention design for gay men," *AIDS & Behavior*, vol. 15, supplement 1, pp. S25–S29, 2011.

[30] B. T. Mausbach, S. J. Semple, S. A. Strathdee, J. Zians, and T. L. Patterson, "Efficacy of a behavioral intervention for increasing safer sex behaviors in HIV-negative, heterosexual methamphetamine users: results from the fast-lane study," *Annals of Behavioral Medicine*, vol. 34, no. 3, pp. 263–274, 2007.

[31] J. Wu, H. Wu, P. Li, and C. Lu, "HIV/STIs risks between migrant MSM and local MSM: a cross-sectional comparison study in China," *PeerJ*, vol. 4, Article ID e2169, 2016.

[32] N. He, F. Y. Wong, Z. J. Huang et al., "HIV risks among two types of male migrants in Shanghai, China: money boys vs. general male migrants," *AIDS*, vol. 21, supplement 8, pp. S73–S79, 2007.

[33] Y. Song, X. Li, L. Zhang et al., "HIV-testing behavior among young migrant men who have sex with men (MSM) in Beijing, China," *AIDS Care*, vol. 23, no. 2, pp. 179–186, 2011.

[34] H. Winetrobe, E. Rice, J. Bauermeister, R. Petering, and I. W. Holloway, "Associations of unprotected anal intercourse with Grindr-met partners among Grindr-using young men who have sex with men in Los Angeles," *AIDS Care*, vol. 26, no. 10, pp. 1303–1308, 2014.

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Research Article

Early Sexual Debut and HIV Infection among Men Who Have Sex with Men in Shenzhen, China

Ruiwei Xu,[1,2] Wenjie Dai,[1,2] Guanglu Zhao,[2] Dan Tu,[3] Lin Yang,[1,2] Feng Wang,[2] Yumao Cai,[2] Lina Lan,[2] Hongzhuan Tan,[1] Aizhong Liu,[1] Tiejian Feng,[2] and Atipatsa C. Kaminga[1,4]

[1] *Department of Epidemiology and Health Statistics, Xiangya School of Public Health, Central South University, Hunan 410008, China*
[2] *Shenzhen Center for Chronic Disease Control, Guangdong 518021, China*
[3] *Graduate School, Shenzhen University, Guangdong 518060, China*
[4] *Department of Mathematics, Mzuzu University, Private Bag 201, Mzuzu 2, Malawi*

Correspondence should be addressed to Tiejian Feng; fengtiej@126.com

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Studies investigating the association between early sexual debut and human immunodeficiency virus (HIV) infection have mainly focused on Africans or females but rarely on men who have sex with men (MSM) in China. This study, therefore, mainly aimed at exploring the association between early sexual debut and HIV infection among MSM in Shenzhen, China. A total of 533 MSM were enrolled in this study using a convenience sampling method. Information about sociodemographic characteristics and risky sexual behaviors was collected. It was found that the prevalence of HIV infection was 24.2% among this study population and 66.4% of the MSM reported having had vaginal sexual intercourse with females. The mean ages at first vaginal sexual intercourse, first anal sexual intercourse, and first sexual intercourse were 21.38, 22.43, and 19.87 years, respectively. Multivariable logistic regression analyses showed that the MSM who experienced early anal sexual debut were more likely to be infected with HIV than those who did not. The results indicate that HIV infection is quite prevalent among MSM in Shenzhen. Early and efficient intervention strategies should be taken, and the MSM experiencing early anal sexual debut should be given special attention.

1. Introduction

In China, the number of individuals reported with human immunodeficiency virus (HIV) has alarmingly increased in recent years, especially among men who have sex with men (MSM) [1]. According to a recent meta-analysis involving twenty-five eligible articles, the pooled HIV incidence among MSM in Mainland China was 5.50/100 person-years in 2012–2014, and MSM only accounted for 21.4% of new HIV infections in China in 2013, but this proportion increased to 25.8% in 2014 [2]. The rapid increase of HIV infection among MSM has brought great challenges to the prevention of HIV infections [3].

Age at first anal sexual intercourse among MSM decreased greatly in the past few decades in China. For instance, among the MSM born in 1990–1996, the median age at first anal sexual intercourse was 18 years, which was less than 33 years, the median age at first anal sexual intercourse among the MSM born in 1940–1959 [4]. Corresponding to the decrease in the age at first anal sexual intercourse was an increase in the number of MSM who experienced early sexual debut, defined as having first sexual intercourse (anal or vaginal) at or before the age of 14 [5]. Early sexual debut may be related to many subsequent risky sexual behaviors, such as having more sexual partners, improper or less condom use during sexual intercourse, and drug use [6, 7]. Thus, individuals with early sexual debut may be more susceptible to HIV infection than those without early sexual debut [5].

Studies investigating the association between early sexual debut and HIV infection have mostly focused on Africans or females but rarely on men who have sex with men (MSM) in China [8, 9]. For example, in rural Malawi and in Nairobi, early vaginal sexual debut (VSD) was significantly associated with the increased risk of HIV infection among ever-married

women and HIV-seropositive adolescent girls, respectively [10, 11]. Moreover, in Kenya, men who had sexual debut at the age between 16 and 21 were 1.83 times more likely to be infected with HIV, compared with those who never had sex [12]. In China, early anal sexual debut (ASD) was found to be a risk factor for HIV infection among the MSM without commercial sexual behavior [13].

There were cases in some previous study which indicated that certain proportion of MSM may have bisexual behaviors [14]. Thus, among these MSM, it could be reasonable to divide early sexual debut into early VSD and early ASD when investigating the effect of early sexual debut on HIV infection. Currently, it is not clear in Shenzhen in China if early anal or vaginal sexual debut would be associated with HIV infection among MSM. Therefore, this study mainly aimed at exploring the association between early sexual debut, including early ASD and early VSD, and HIV infection among MSM in Shenzhen, China. Also examined was the association between HIV infection status and their sociodemographic variables and risky sexual behaviors. Age at first sexual intercourse and some sociodemographic factors associated with younger age at first sexual intercourse were also examined.

2. Materials and Methods

2.1. Ethics Statement. This study was approved by the Ethics Committee of Shenzhen Center for Chronic Disease Control. Written informed consent was obtained from all participants.

2.2. Participants. This study was conducted between March 2013 and August 2015 in Shenzhen city of China. Site based convenience sampling method was used to recruit participants. The MSM who came to the sexually transmitted disease (STD) clinic of Shenzhen Center for Chronic Disease Control for treatment or consultation every Monday between March 2013 and August 2015 were considered for enrollment in this study. The inclusion criteria for participants were as follows: (1) being biologically male; (2) self-reporting of having had anal sexual intercourse with at least one biological male in the previous year; (3) age $\geq$ 18 years; and (4) being willing to provide informed consent. Exclusion criteria included MSM with any kind of mental disease, a history of drug use or prostitution, or severe medical illness. In addition, since this study was a virtually two-year survey, in order to avoid recruiting the same individuals, participants were asked whether or not they had participated in this study before. If the answer was yes, they were excluded.

2.3. Data Collection. Data were collected by well-qualified investigators who either had worked for Shenzhen Center for Chronic Disease Control or had studied in a medical school. Before data collection, all investigators went through a unified training which was guided by a written investigation manual. After that, they interviewed the participants face-to-face with an anonymous questionnaire to collect data.

2.4. Measurements

2.4.1. Sociodemographic Variables. Sociodemographic variables included in this study were present age, ethnicity, marital status, educational level, socioeconomic disconnection (neither being currently employed nor being a student [15]), monthly income, and length of stay in Shenzhen.

2.4.2. Risky Sexual Variables. Risky sexual variables included the experience of vaginal sexual intercourse, age at first sexual intercourse, age at first anal sexual intercourse, age at first vaginal sexual intercourse, fixed homosexual partner, number of anal sexual partners in the past six months, role in anal sexual intercourse (insertive, receptive, or both insertive and receptive), and condom use in sexual intercourse in the past six months.

Early sexual debut in this study was defined as having first sexual intercourse at the age of 14 or less. Similarly, early ASD and early VSD were, respectively, defined as having first anal sexual intercourse and having first vaginal sexual intercourse at the age of 14 or less. Besides, having a fixed homosexual partner was defined as having an anal sexual relationship with only one and the same homosexual partner for at least six months. Moreover, having no anal sexual intercourse in the past 6 months or using condom every time having anal sexual intercourse with homosexual partners was categorized as always using condom in the past 6 months.

2.4.3. Knowledge of HIV. Eight HIV-related questions were administered to evaluate the participants' knowledge of HIV [16]. The questions included the following: could HIV be transmitted via mosquito bites? Do individuals infected with HIV look healthy? Could HIV be transmitted via blood transfusion or using blood products? Could HIV be transmitted via sharing a meal with a person infected with HIV? Could HIV be transmitted to an unborn child via delivery? Could HIV be transmitted via sharing a needle with someone who was infected with HIV? Could the risk of being infected with HIV be reduced by having sex with a steady sexual partner? And, could HIV be transmitted if you correctly use a condom every time you have sex? One point was given to each correct answer and zero point was given to each wrong answer. Thus the total score, which ranged from 0 to 8 points, was equal to the total number of correct answers. Participants who scored a total of at least 6 points were regarded as having a comprehensive knowledge of HIV [17].

2.4.4. HIV and Syphilis Tests. Blood samples were collected from all respondents for HIV and syphilis tests. HIV was tested using enzyme-linked immunosorbent assay (ELISA) (Wantai Biotech Inc, Beijing, China) for screening and the screening results were confirmed using Western blot (MP Diagnostics, Singapore). Syphilis was tested with ELISA (Lizhu Biotech Inc, Zhuhai, China) for screening and then the screening results were confirmed by toluidine red unheated serum test (TRUST) (Rongsheng Biotech Inc, Shanghai, China). Participants with ELISA positive and Western blot positive were diagnosed as HIV positive (HIV infection); otherwise they were diagnosed as HIV negative. Similarly, participants with ELISA positive and TRUST positive were diagnosed as having syphilis infection.

2.5. Data Analyses. Statistical analyses were performed using Statistical Package for the Social Science (SPSS) version 19.0 (IBM Corp, Armonk, NY). All statistical tests were two-tailed and were considered significant if $P < 0.05$. For categorical variables, frequency (%) was presented, while mean and standard deviation (SD) were presented to describe a continuous variable. The chi-square (χ^2) test was used to compare the distribution of sociodemographic and risky sexual variables between HIV positive and HIV negative MSM. Also, it was used to compare the distribution of early sexual debut between HIV positive and HIV negative MSM. The independent sample student t-test was used to compare age at first sexual intercourse by sociodemographic characteristics of MSM. Univariable logistic regression analyses were used to assess the effect of early sexual debut on HIV infection status. Multivariable logistic regression analyses were performed to explore the contribution of early sexual debut to HIV infection status adjusting for the covariates including sociodemographic variables, risky sexual variables, syphilis infection status, and knowledge of HIV. Each logistic regression model included only one indicator of early sexual debut so as to avoid multicollinearity.

3. Results

3.1. Participants' Characteristics. A total of 537 participants were interviewed. After excluding 4 invalid questionnaires, 533 participants were finally enrolled in this study.

Table 1 presents sociodemographic and risky sexual characteristics of the participants. Among all participants, 499 (93.6%) were of Han nationality, 160 (30.0%) had attended junior middle school at most, and 52 (9.8%) were categorized as having socioeconomic disconnection. The mean (SD) present age of the participants was 32.09 (8.41) years.

Furthermore, 354 (66.4%) reported having ever had vaginal sexual intercourse and 368 (69.0%) reported having a fixed homosexual partner. There were 214 (42.5%) participants who reported always using condoms in anal sexual intercourse in the past six months and 378 (70.9%) participants who reported having a comprehensive knowledge of HIV. Those who were confirmed as having syphilis infection were 159 (29.8%) of the participants.

3.2. Prevalence of HIV Infection. Prevalence rate of HIV infection among MSM in this study was 24.2% (129/533). The association between HIV infection and sociodemographic variables and the association between HIV infection and risky sexual variables are presented in Table 1. Role in the anal sexual intercourse was significantly associated with HIV infection ($P < 0.05$), and MSM with syphilis infection were more likely to be infected with HIV than those without syphilis infection (44.0% versus 15.8%, $P < 0.05$). Besides, those who always used condom in anal sexual intercourse in the past 6 months were less prone to be infected with HIV, compared with those who did not always use condom in anal sexual intercourse in the past 6 months (18.2% versus 27.7%, $P < 0.05$).

3.3. Age at First Sexual Intercourse. Table 2 presents age (mean ± SD) of MSM at first sexual intercourse with respect to sociodemographic variables. The mean (SD) ages at first vaginal sexual intercourse, first anal sexual intercourse, and first sexual intercourse were 21.38 (3.93), 22.43 (6.40), and 19.87 (3.80) years, respectively. Age at first sexual intercourse was significantly associated with some sociodemographic variables, namely, present age, marital status, educational level, and socioeconomic disconnection ($P < 0.05$). Compared with participants with present age of at least 25, participants with present age younger than 25 had earlier age at first sexual intercourse (20.30 versus 17.81, $P < 0.05$), first vaginal sexual intercourse (23.28 versus 18.41, $P < 0.05$), and first anal sexual intercourse (21.85 versus 17.73, $P < 0.05$). In addition, compared with the married individuals, those who were never married/divorced/widowed had earlier age at first sexual intercourse (20.88 versus 19.54, $P < 0.05$), first vaginal sexual intercourse (22.65 versus 20.67, $P < 0.05$), and first anal sexual intercourse (27.29 versus 20.88, $P < 0.05$).

3.4. Association between Early Sexual Debut and HIV Infection. Table 3 presents the distribution of early sexual debut by HIV infection status. The proportions of participants reported having experienced sexual debut and anal sexual debut at the age of 14 or less were 5.6% and 4.5%, respectively. In addition, among 354 MSM who had ever had sex with females, 2.0% reported having vaginal sexual debut at the age of 14 or less. Early VSD and early sexual debut were not significantly associated with HIV infection ($P > 0.05$). However, the prevalence of HIV infection was significantly higher among individuals who reported having experienced early ASD than among those who did not experience early ASD (41.7% versus 23.4%, $P < 0.05$).

Logistic regression analyses were performed to examine some factors which might be associated with HIV infection in this group of MSM. The results are shown in Table 4. Univariable logistic regression analyses showed that early ASD (odds ratio (OR) = 2.34, 95% confidence interval (95% CI) = 1.02–5.41) was a risk factor for HIV infection and remained a significant risk factor (OR = 2.88, 95% CI = 1.01–8.18) for HIV infection adjusting for the covariates listed in Table 1. However, both univariable and multivariable logistic regression analyses indicated that early sexual debut and early VSD were not significantly associated with HIV infection status ($P > 0.5$).

4. Discussion

Although some studies indicated that early sexual debut was associated with HIV infection [18], only a handful of studies investigated early sexual debut and HIV infection among MSM [7]. Motivated by the fact that some MSM also have vaginal sexual intercourse with women, to the best of our knowledge, this may be the first study in China to classify early sexual debut into early anal sexual debut and early vaginal sexual debut when exploring the association between early sexual debut and HIV infection among the Chinese MSM in China.

Table 1: Sociodemographic and risky sexual characteristics of MSM by HIV infection status.

Variables	Category	Total sample (%)	HIV positive (%)	HIV negative (%)	χ^2 value	P value
Present age					2.141	0.143
	<25	93 (17.4)	28 (30.1)	65 (69.9)		
	≥25	440 (82.6)	101 (23.0)	339 (77.0)		
Ethnicity					0.537	0.464
	Han	499 (93.6)	119 (23.8)	380 (76.2)		
	Minority	34 (6.4)	10 (29.4)	24 (70.6)		
Marital status					2.158	0.142
	Married	129 (24.2)	25 (19.4)	104 (80.6)		
	Never married/divorced/widowed	404 (75.8)	104 (25.7)	300 (74.3)		
Educational level					2.577	0.108
	≤junior middle school	160 (30.0)	46 (28.8)	114 (71.2)		
	>junior middle school	373 (70.0)	83 (22.3)	290 (77.7)		
Socioeconomic disconnection					0.232	0.630
	Yes	52 (9.8)	14 (26.9)	38 (73.1)		
	No	481 (90.2)	115 (23.9)	366 (76.1)		
Monthly income (RMB)					2.460	0.117
	<2500	73 (13.7)	23 (31.5)	50 (68.5)		
	≥2500	460 (86.3)	106 (23.0)	354 (77.0)		
Length of stay in Shenzhen					0.100	0.752
	<1 month	38 (7.1)	10 (26.3)	28 (73.7)		
	≥1 month	495 (92.9)	119 (24.0)	376 (76.0)		
Syphilis infection					48.539	<0.001
	Yes	159 (29.8)	70 (44.0)	89 (56.0)		
	No	374 (70.2)	59 (15.8)	315 (84.2)		
Ever had vaginal sexual intercourse					0.620	0.431
	Yes	354 (66.4)	82 (23.2)	272 (76.8)		
	No	179 (33.6)	47 (26.3)	132 (73.7)		
Fixed homosexual partner					0.054	0.816
	Yes	368 (69.0)	88 (23.9)	280 (76.1)		
	No	165 (31.0)	41 (24.8)	124 (75.2)		
Number of anal sexual partners in the past 6 months					5.285	0.071
	≤2	217 (40.7)	44 (20.3)	173 (79.7)		
	3–5	150 (28.1)	46 (30.7)	104 (69.3)		
	>5	166 (31.2)	39 (23.5)	127 (76.5)		
Role in anal sexual intercourse*					44.861	<0.001
	Insertive	212 (41.1)	21 (9.9)	191 (90.1)		
	Receptive	123 (23.8)	50 (40.7)	73 (59.3)		
	Both	181 (35.1)	54 (29.8)	127 (70.2)		
Always use condom in anal sexual intercourse in the past 6 months*					6.089	0.014
	Yes	214 (42.5)	39 (18.2)	175 (81.8)		
	No	289 (57.5)	80 (27.7)	209 (72.3)		
With a comprehensive knowledge of HIV					0.114	0.736
	Yes	378 (70.9)	93 (24.6)	285 (75.4)		
	No	155 (29.1)	36 (23.2)	119 (76.8)		

*Data may not add up to 533 because of missing value.

Table 2: Age (mean ± SD) at first sexual intercourse by sociodemographic characteristics.

Variables	Category	Age at first vaginal sexual intercourse	Age at first anal sexual intercourse	Age at first sexual intercourse
Present age				
	<25	18.41 ± 2.76	17.73 ± 2.32	17.81 ± 2.72
	≥25	23.28 ± 6.63*	21.85 ± 3.84*	20.30 ± 3.85*
Ethnicity				
	Han	21.46 ± 3.90	22.48 ± 6.37	19.92 ± 3.77
	Minority	20.21 ± 4.19	21.76 ± 6.98	19.10 ± 4.21
Marital status				
	Married	22.65 ± 4.11	27.29 ± 8.46	20.88± 3.95
	Never married/divorced/widowed	20.67 ± 3.64*	20.88 ± 4.63*	19.54 ± 3.70*
Educational level				
	≤junior middle school	20.79 ± 3.70	22.65 ± 7.57	19.16 ± 3.34
	>junior middle school	21.66 ± 4.01	22.34 ± 5.84	20.17 ± 3.95*
Socioeconomic disconnection				
	Yes	19.97 ± 3.10	21.96 ± 8.17	18.62 ± 2.82
	No	21.51 ± 3.97*	22.48 ± 6.19	20.00 ± 3.87*
Monthly income (RMB)				
	<2500	22.15 ± 3.86	24.05 ± 9.26	19.33 ± 4.07
	≥2500	21.26 ± 3.93	22.17 ± 5.79*	19.95 ± 3.75
Length of stay in Shenzhen				
	<1 month	21.19 ± 3.75	21.58 ± 5.84	19.79 ± 3.43
	≥1 month	21.39 ± 3.94	22.50 ± 6.45	19.88 ± 3.83

Analysis of t-test, $^{*}P < 0.05$.

Table 3: Distribution of early sexual debut by HIV infection status.

Variables	Category	Total sample (%)	HIV positive (%)	HIV negative (%)	χ^2 value	P value
Early VSD					0.117	0.732
	Yes	7 (2.0)	2 (28.6)	5 (71.4)		
	No	347 (98.0)	80 (23.1)	267 (76.9)		
Early ASD					4.178	0.041
	Yes	24 (4.5)	10 (41.7)	14 (58.3)		
	No	509 (95.5)	119 (23.4)	390 (76.6)		
Early sexual debut					2.692	0.101
	Yes	30 (5.6)	11 (36.7)	19 (63.3)		
	No	503 (94.4)	118 (23.5)	385 (76.5)		

The prevalence of HIV infection among MSM in this study population was 24.2%, which is higher than the prevalence, 3.3–5.3%, of HIV infection found among the general MSM in the same area [19–21]. This is mainly due to the fact that the prevalence of STDs among MSM recruited from an STD clinic was much higher than that among the general MSM [19]. Besides, the HIV infection prevalence found in this study was almost 5 times higher than that (4.2%) reported in a previous study which enrolled 743 MSM from the STD clinic of Shenzhen Institute of Dermatology in 2008-2009 [19]. Thus, the prevalence of HIV infection in this study supports the results of a previous study which indicated an alarming increase of HIV infections among MSM in China in recent years [1] and, hence, the suggestions made by this previous study to urgently implement more efficient preventions and more comprehensive interventions of HIV infections among MSM are supported.

Some risky sexual behaviors, including infrequent condom use in anal sexual intercourse in the past 6 months and role in anal sexual intercourse, were correlated with HIV infection. This finding was consistent with previous studies which investigated risk factors for HIV infection

Table 4: Logistic regression analyses for assessing the effect of early sexual debut on HIV infection status.

Variables	Category	Univariable analysis		Multivariable analysis	
		OR (95% CI)	*P* value	OR (95% CI)	*P* value
Early VSD			0.733		0.885
	No	1		1	
	Yes	1.34 (0.25–7.01)		1.15 (0.18–7.57)	
Early ASD			0.046		0.047
	No	1		1	
	Yes	2.34 (1.02–5.41)		2.88 (1.01–8.18)	
Early sexual debut			0.106		0.150
	No	1		1	
	Yes	1.89 (0.87–4.08)		2.00 (0.78–5.11)	

[2, 22]. Indeed, promoting condom use in anal sexual behavior is a pivotal approach in order to reduce the risk of HIV infection among MSM [23]. The significant association of syphilis infection with HIV infection in this study was also consistent with previous research findings which established a higher likelihood of HIV infection in someone with syphilis infection than in someone without syphilis infection [24, 25]. However, unlike in some previous researches [17, 22], comprehensive knowledge of HIV in this study was not significantly associated with HIV infection. The reason for the insignificant association could be due to even promotion of HIV knowledge by the local government or communities. Nevertheless, it is widely understood that increasing knowledge about HIV could reduce the prevalence of HIV infection [26].

Similar to a previous study [27], the proportion (66.4%) of the MSM who also have vaginal sexual intercourse with women in this study population was high, suggesting that potential risk factors for HIV infection among MSM should be considered from an HIV transmission web involving sexual intercourse activities among MSM and between MSM and women. It was also found that the mean ages at first vaginal sexual intercourse, first anal sexual intercourse, and first sexual intercourse were 21.38, 22.43, and 19.87 years, respectively. Consistent with a previous study [28], age at first sexual intercourse was significantly related to some sociodemographic characteristics. For example, having a younger present age (<25), being never married/divorced/widowed, having education level at most junior middle school, and having a socioeconomic disconnection were significantly associated with a relatively younger age at first sexual intercourse. Given that a relatively younger age at first sexual intercourse increased the likelihood of being infected with STDs in later life [7], it is necessary to give special concern to the MSM with those preceding associated factors for having first sexual intercourse at a relatively younger age.

Also found in this study is that early ASD was associated with HIV infection, while early VSD was not associated with HIV infection. This can be explained by the fact that compared with male sexual partners of MSM female sexual partners of MSM are usually fixed, and thus male sexual partners of MSM are more likely to transmit HIV or syphilis [29]. For example, it was found that having a male as the first sexual partner rather than a female was a risk factor for syphilis infection [30], and it has been widely understood that early ASD was related to many subsequent risky sexual behaviors, such as more anal sexual partners, more unprotected anal sexual intercourse, and more sex exchange [5, 8]. Thus, MSM with ASD, rather than VSD, are at higher risk for HIV infection.

There may be several ways in which early sexual debut affects one's susceptibility of being infected with HIV. Firstly, early sexual debut may increase one's risk of HIV infection by extending one's duration of sexual activity, since compared with those who do not have an early sexual debut individuals who commence sex early may have a relatively longer duration of sexual activity and therefore they would be potentially exposed to HIV infection for a longer period of time [31, 32]. Secondly, individuals who start sex early may also be more prone to be related to many subsequent risky sexual behaviors, such as having more sexual partners and less condom use [6, 33]. Finally, it is possible that individuals who commence sex early are more likely to have partners with higher risk of being infected with HIV than those who do not have an early sexual debut [5].

This study has some limitations. Firstly, the participants were recruited using convenience sampling method. Though widely used, this method is improper for random sampling, which may reduce the generalizability of our findings to other MSM populations. Secondly, participants in this study were recruited from the STD clinic, as a consequence of which selection bias may be induced. Thirdly, though managed by well-trained investigators, this survey was essentially retrospective, suggesting that some data from questions, such as "how many anal sexual partners did you have in the past 6 months?" and "how old were you at your first sexual intercourse?," could be approximations which might be affected by recall bias. Finally, the results of this study

may not be applicable to the MSM population with a history of drug use because MSM with a history of drug use were excluded from this study.

5. Conclusions

This cross-sectional study mainly explored the association between early sexual debut and HIV infection among MSM in Shenzhen, China. The prevalence of HIV infection among this group was 24.2% and 66.4% of the MSM reported having had vaginal sexual intercourse with females. The mean ages at first vaginal sexual intercourse, first anal sexual intercourse, and first sexual intercourse were 21.38, 22.43, and 19.87 years, respectively. Having a younger present age (<25), being never married/divorced/widowed, having attended junior middle school at most, and being socioeconomically disconnected were associated with a relatively younger age at first sexual intercourse. Compared with the MSM who had anal sexual debut at the age of greater than 14 years, those who had anal sexual debut at the age of 14 or less were more prone to be infected with HIV. Therefore, early and effective measures to reduce the transmission of HIV among MSM in Shenzhen should be taken, and MSM with early sexual debut should be given special consideration.

Competing Interests

The authors declare that they have no competing interests.

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References

[1] X. Ma, Q. Zhang, X. He et al., "Trends in prevalence of HIV, syphilis, hepatitis C, hepatitis B, and sexual risk behavior among men who have sex with men: results of 3 consecutive respondent-driven sampling surveys in Beijing, 2004 through 2006," *Journal of Acquired Immune Deficiency Syndromes*, vol. 45, no. 5, pp. 581–587, 2007.

[2] W. Zhang, J. J. Xu, H. Zou, J. Zhang, N. Wang, and H. Shang, "HIV incidence and associated risk factors in men who have sex with men in Mainland China: an updated systematic review and meta-analysis," *Sexual Health*, vol. 13, no. 4, pp. 373–382, 2016.

[3] S. D. Liu, J. Su, S. M. Zhang, H. P. Dong, H. Wang, and W. Luo, "Identification of HLA-A*11:01-restricted *Mycobacterium tuberculosis* $CD8^+$ T cell epitopes," *Journal of Cellular and Molecular Medicine*, vol. 20, no. 9, pp. 1718–1728, 2016.

[4] H. Zou, J. Xu, Q. Hu et al., "Decreasing age at first anal intercourse among men who have sex with men in China: a multicentre cross-sectional survey," *Journal of the International AIDS Society*, vol. 19, no. 1, Article ID 20792, 2016.

[5] H. Wand and G. Ramjee, "The relationship between age of coital debut and HIV seroprevalence among women in Durban, South Africa: a cohort study," *BMJ Open*, vol. 2, Article ID e000285, 2012.

[6] W. D. Manning, M. A. Longmore, and P. C. Giordano, "The relationship context of contraceptive use at first intercourse," *Family Planning Perspectives*, vol. 32, no. 3, pp. 104–110, 2000.

[7] A. Y. Outlaw, G. Phillips II, L. B. Hightow-Weidman et al., "Age of MSM sexual debut and risk factors: results from a multisite study of racial/ethnic minority YMSM living with HIV," *AIDS Patient Care and STDs*, vol. 25, supplement 1, pp. S23–S29, 2011.

[8] A. Pettifor, K. O'Brien, C. MacPhail, W. C. Miller, and H. Rees, "Early coital debut and associated HIV risk factors among young women and men in South Africa," *International Family Planning Perspectives*, vol. 35, no. 2, pp. 82–90, 2009.

[9] T. B. Hallett, J. J. C. Lewis, B. A. Lopman et al., "Age at first sex and HIV infection in rural Zimbabwe," *Studies in Family Planning*, vol. 38, no. 1, pp. 1–10, 2007.

[10] C. Boileau, S. Clark, S. Bignami-Van Assche et al., "Sexual and marital trajectories and HIV infection among ever-married women in rural Malawi," *Sexually Transmitted Infections*, vol. 85, no. 1, pp. i27–i33, 2009.

[11] A. F. Rositch, P. Cherutich, P. Brentlinger, J. N. Kiarie, R. Nduati, and C. Farquhar, "HIV infection and sexual partnerships and behaviour among adolescent girls in Nairobi, Kenya," *International Journal of STD & AIDS*, vol. 23, no. 7, pp. 468–474, 2012.

[12] E. O. Onsomu, J. K. Kimani, B. A. Abuya et al., "Delaying sexual debut as a strategy for reducing HIV epidemic in Kenya," *African Journal of Reproductive Health*, vol. 17, no. 2, pp. 46–57, 2013.

[13] J. Zhao, W.-D. Cai, Y.-X. Gan et al., "A comparison of HIV infection and related risk factors between money boys and noncommercial men who have sex with men in Shenzhen, China," *Sexually Transmitted Diseases*, vol. 39, no. 12, pp. 942–948, 2012.

[14] J.-P. Chen, M.-M. Han, Z.-J. Liao et al., "HIV-related behaviors, social support and health-related quality of life among men who have sex with men and women (MSMW): a cross-sectional study in Chongqing, China," *PLoS ONE*, vol. 10, no. 2, Article ID e0118651, 2015.

[15] T. A. Gayles, L. M. Kuhns, S. Kwon, B. Mustanski, and R. Garofalo, "Socioeconomic disconnection as a risk factor for increased HIV infection in young men who have sex with men," *LGBT Health*, vol. 3, no. 3, pp. 219–224, 2016.

[16] M. Jin, Z. Yang, Z. Dong, and J. Han, "Correlates of consistent condom use among men who have sex with men recruited through the Internet in Huzhou city: A Cross-Sectional Survey," *BMC Public Health*, vol. 13, no. 1, article 1101, 2013.

[17] Z. Yang, Z. Huang, Z. Dong et al., "Risk factors for HIV diagnosis among men who have sex with men: results of a case-control study in one sample of eastern China," *AIDS Research and Human Retroviruses*, 2016.

[18] K. Zuma, G. Setswe, T. Ketye, T. Mzolo, T. Rehle, and N. Mbelle, "Age at sexual debut: a determinant of multiple partnership among South African youth," *African Journal of Reproductive Health*, vol. 14, no. 2, pp. 47–54, 2010.

[19] F.-C. Hong, H. Zhou, Y.-M. Cai et al., "Prevalence of syphilis and HIV infections among men who have sex with men from different settings in Shenzhen, China: implications for HIV/STD surveillance," *Sexually Transmitted Infections*, vol. 85, no. 1, pp. 42–44, 2009.

[20] W.-D. Cai, J. Zhao, J.-K. Zhao et al., "HIV prevalence and related risk factors among male sex workers in Shenzhen, China: results from a time-location sampling survey," *Sexually Transmitted Infections*, vol. 86, no. 1, pp. 15–20, 2010.

[21] S. Liu, J. Zhao, K. Rou et al., "A survey of condom use behaviors and HIV/STI prevalence among venue-based money boys in Shenzhen, China," *AIDS and Behavior*, vol. 16, no. 4, pp. 835–846, 2012.

[22] X. Huan, C. Hao, H. Yan et al., "High prevalence of HIV and syphilis among men who have sex with men recruited by respondent-driven sampling in a city in Eastern China," *Asia-Pacific Journal of Public Health*, vol. 27, no. 2, pp. NP854–NP865, 2015.

[23] S. Ramanathan, V. Chakrapani, L. Ramakrishnan et al., "Consistent condom use with regular, paying, and casual male partners and associated factors among men who have sex with men in Tamil Nadu, India: findings from an assessment of a large-scale HIV prevention program," *BMC Public Health*, vol. 13, article 827, 2013.

[24] A. Das, J. Li, F. Zhong et al., "Factors associated with HIV and syphilis co-infection among men who have sex with men in seven Chinese cities," *International Journal of STD & AIDS*, vol. 26, no. 3, pp. 145–155, 2015.

[25] T. V. Nguyen, N. Van Khuu, P. D. Nguyen et al., "Sociodemographic factors, sexual behaviors, and alcohol and recreational drug use associated with HIV among men who have sex with men in Southern Vietnam," *AIDS and Behavior*, vol. 20, no. 10, pp. 2357–2371, 2016.

[26] C. Wejnert, B. Le, C. E. Rose, A. M. Oster, A. J. Smith, and J. Zhu, "HIV infection and awareness among men who have sex with men-20 cities, United States, 2008 and 2011," *PLoS ONE*, vol. 8, no. 10, Article ID e76878, 2013.

[27] J. N. Park, E. Papworth, S. Kassegne et al., "HIV prevalence and factors associated with HIV infection among men who have sex with men in Cameroon," *Journal of the International AIDS Society*, vol. 16, supplement 3, Article ID 18752, 2013.

[28] K. Zuma, T. Mzolo, and E. Makonko, "Determinants of age at sexual debut and associated risks among South African youths," *African Journal of AIDS Research*, vol. 10, no. 3, pp. 189–194, 2011.

[29] Z. Mor and U. Davidovich, "Same-sex sexual attraction, behavior, and practices of Jewish men in Israel and the association with HIV prevalence," *AIDS Care*, vol. 28, supplement 1, pp. 64–67, 2016.

[30] B. Wang, X. Li, B. Stanton, Y. Liu, and S. Jiang, "Sociodemographic and behavioral correlates for HIV and syphilis infections among migrant men who have sex with men in Beijing, China," *AIDS Care*, vol. 25, no. 2, pp. 249–257, 2013.

[31] S. J. S. Pascoe, L. F. Langhaug, J. Durawo et al., "Increased risk of HIV-infection among school-attending orphans in rural Zimbabwe," *AIDS Care*, vol. 22, no. 2, pp. 206–220, 2010.

[32] A. E. Pettifor, A. Van Der Straten, M. S. Dunbar, S. C. Shiboski, and N. S. Padian, "Early age of first sex: a risk factor for HIV infection among women in Zimbabwe," *AIDS*, vol. 18, no. 10, pp. 1435–1442, 2004.

[33] M. Ghebremichael, U. Larsen, and E. Paintsil, "Association of age at first sex with HIV-1, HSV-2, and other sexual transmitted infections among women in northern Tanzania," *Sexually Transmitted Diseases*, vol. 36, no. 9, pp. 570–576, 2009.

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Research Article

Prevalence of HIV Antiretroviral Drug Resistance and Its Impacts on HIV-1 Virological Failures in Jiangsu, China: A Cross-Sectional Study

Ying Zhou,[1] Jing Lu,[1] Jinge Wang,[2] Hongjing Yan,[1] Jianjun Li,[1] Xiaoqin Xu,[1] Zhi Zhang,[1] Tao Qiu,[1] Ping Ding,[1] Gengfeng Fu,[1] Xiping Huan,[1] and Haiyang Hu[1]

[1] *Jiangsu Provincial Center for Disease Prevention and Control, Nanjing, China*
[2] *College of Agriculture, Nanjing Agriculture University, Nanjing, China*

Correspondence should be addressed to Haiyang Hu; huhaiyang@jscdc.cn

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Antiretroviral therapy (ART) has been shown to improve survival of patients with Human Immunodeficiency Virus (HIV) infection and to reduce HIV-1 transmission. Therefore, the Chinese central government initiated a national program to provide ART free of charge to HIV-1 patients. We conducted a cross-sectional survey in Jiangsu province to determine the level of drug resistance (DR) in HIV-1 infected patients and the correlates of DR in virological failures in 2012. Approximately 10.4% of the HIV-1 patients in the study experienced virological failure after one year of ART and were divided into drug sensitive and drug resistant groups based on genotype determination. The viral loads (VLs) in the drug resistant group were significantly lower than the drug sensitive group. There were two independent predictors of virological failure: male gender and increasing duration of treatment. The primary mutations observed in the study were against nucleoside reverse transcriptase inhibitors (NRTIs) which were M184V (79.45%) and K103N (33.70%) in nonnucleoside reverse transcriptase inhibitors (NNRTIs). The overall rate of DR in Jiangsu province is still relatively low among treated patients. However, close monitoring of drug resistance in male patients in the early stages of treatment is vital to maintaining and increasing the benefits of HIV ART achieved to date.

1. Introduction

During 1996–2012, it was estimated that antiretroviral therapy (ART) prevented approximately 6.6 million deaths due to Acquired Immunodeficiency Syndrome (AIDS) worldwide, including 5.5 million deaths in low- and middle-income countries [1]. In addition, ART has been shown to reduce the risk of Human Immunodeficiency Virus (HIV) transmission [2–4]. Given the potential benefits of effective ART treatment, the Chinese central government launched the National Free ART Program in 2002. The program has expanded rapidly since 2004 [5], and by September 2013, the National Free ART Program has cumulatively treated approximately 260,000 patients [6]. It is estimated that during 2002 to 2009 the program reduced AIDS associated mortality from 39.3 to 14.2 cases per 100 infected persons [7]. The promising early results from the National Free ART Program have resulted in strong governmental supports for continuing to improve and expand the program. The treatment guidelines for the Chinese National Free ART Program have been carried out with zidovudine (AZT)/stavudine (D4T) + didanosine (DDI) + nevirapine (NVP) as the regimens in 2003 [8] and switched to AZT/D4T + 3TC + EFV/NVP as first-line therapy regimens in 2005. Because of the side effect of D4T, the first-line regimens were switched again to TDF + 3TC + NVP/EFV in 2012 lasting until now to remain current with the World Health Organization (WHO) ART guidelines, including the latest 2013 WHO guidelines [9, 10].

While increasing the availability of ART has improved the survival rates and quality of life for HIV/AIDS patients, resistance to antiretroviral drugs can undermine the success of ART programs [11]. A high number of ART resistant HIV-1

strains have emerged after ART in resource-limited countries and substantially offset the benefits from treatment programs [12, 13]. Similarly, a trend has been observed in China. There was a concomitant increase in the number of HIV patients with ART resistance during the ramp up of the National Free ART Program [14, 15]. To decrease the diminishing efficacy of the free antiretroviral treatment, China initiated the switch to second-line antiretroviral therapy for patients with failure of first-line treatment in 2009 [8]. However, second-line antiretroviral therapy is the last choice in China in HIV-1 treatment because of the drug deficiency.

Jiangsu is a representative province in eastern China for societal characteristic and the prevalence of HIV-1 disease [16]. Free ART was implemented in Jiangsu in 2005 as part of the Chinese National Free ART Program. The number of new reporting cases of HIV-1 rapidly increased from 78 in 2005 to 3841 in 2012. However, there was no comprehensive effort made to collect data about these patients, including basic information such as viral loads (VLs), and the prevalence of drug resistant HIV-1, until after they had been receiving ART for at least one year. In this study, we have undertaken the first comprehensive examination of the HIV-1 patients in Jiangsu province in 2012 to document the rates of DR in virological failures and correlates of DR in HIV-1 virological failures receiving ART for at least 12 months. The results of this study will help provide a baseline evaluation of the Chinese HIV-1 treatment program and may help inform evidence-based decision making for clinical care of HIV-1 patients in China.

2. Methods

2.1. Ethical Statement. This was approved by the Ethics Committee of Jiangsu Provincial Center for Disease Prevention and Control. Signed informed consent was obtained from each of the participants prior to the interviews and blood collection. Each participant was free to decline to participate or withdraw from the study at any point in time. All the methods (not just laboratory experiments) were carried out in accordance with the relevant guidelines, including any relevant details.

2.2. Study Design. We conducted a cross-sectional survey of all patients who were currently on ART for at least 12 months from January 1 to December 31, 2012, in Jiangsu province. Laboratory data and demographic information were collected to evaluate HIV drug resistance.

2.3. Sample Selection and Information Collection. All the laboratory experiments were carried out in accordance with approved guidelines. According to the guidelines of the National Free ART Handbook (version 3, 2012) [11], HIV-1 cases whose $CD4^+$ T-cell count ≤350 cells/mm^3 are eligible for ART. Free $CD4^+$ T-cell counts tests were conducted twice each year for all treated patients and free VLs testing was conducted once per year for patients who received ART for more than 12 months. Patients with VLs >1,000 copies/mL after 12 months of treatment were defined as virological failures and selected for further drug resistance genotyping test according to this ART Handbook too. The presence of any drug resistant mutation whatever it is in NRTIs, NNRTIs, or PIs is defined as drug resistance in this study. The inclusion criteria for this study were as follows: (1) patients had to be receiving ART for more than 12 months; (2) they had to have VLs testing results; and (3) there was access to serum samples at the Center of Jiangsu Provincial HIV/AIDS confirmatory laboratory, Jiangsu Provincial Center for Disease Prevention and Control (JSCDC). We allowed 16 cases who had been treated for between 11 and 12 months to participate in the study. All of the laboratory results and patient demographic information related to ART were entered into the web-based national ART information system. Demographic information including age, gender, and treatment duration and other relevant information required for the HIV drug resistance analysis were collected from the China Information System for HIV Control and Prevention.

2.4. Laboratory Measures. VLs and drug resistance testing were conducted at the provincial HIV/AIDS confirmatory laboratory. The VLs were quantified using the COBAS Ampliprep TaqMan 96 (Roche) and COBAS TaqMan HIV-1 Test v2.0 kit. HIV-1 RNA was extracted from the plasma using the QIAamp Viral RNA Mini Kit. Both VLs testing and HIV-1 RNA extraction were performed using the residual blood drawn for the $CD4^+$T-cell testing. The residual blood was stored at −80°C until it was analysed.

Nested-PCR was used to amplify the reverse transcriptase gene (codons 1–230) and protease gene (codons 1–99) from the virus isolated from the plasma [17]. The PCR products were sent to Company TaKaRa for sequencing and the resulting sequences were spliced together using the software Chromaspro. Interpretation and analysis of the sequences generated were undertaken using the Stanford University HIV Drug Resistance Database (http://hivdb.stanford.edu/).

2.5. Statistical Analysis. VLs were analysed with F test to compare variances using the Graphpad Prism4 software (Graphpad Software, Inc.) and $p < 0.05$ was considered as significant. The factors that could have been related to drug resistance were collected from the China Information System for HIV Control and Prevention. Univariate associations were evaluated using the chi-square test for categorized variables and the Student t-test for continuous variables and the two-sided test to estimate the level of significance. The selected variables of $p < 0.1$ in univariate analysis were entered in the logistic regression model to determine level of significance with SPSS version 20.0 (IBM).

3. Results

3.1. Patient Demographics. At the end of 2012, the VLs had been determined for 2,223 HIV-1 patients who had received ART for at least one year. Of these, 232 patients had VLs >1,000 copies/mL and were classified as virologic failures who needed to be further tested for drug resistance. The overall rate of virologic failure was 10.44% (232/2223). Of 232 cases of virological failure, 196 plasma samples were successfully taken to test for drug resistance by genotyping. Of these, 101 were considered drug resistant and 95 were drug sensitive.

Therefore, the overall rate of DR in Jiangsu HIV-1 patients sample was 4.54% (101/2223).

Next, we compared the VLs between drug sensitive and resistant groups. There was a statistically significant difference in the mean VLs between the drug sensitive (303,500 ± 201700 copies/mL) and the drug resistant (75,860 ± 15080 copies/mL; $p < 0.05$ F test to compare variances).

3.2. Factors Associated with Drug Resistance. The demographic characteristics of HIV-1 treated patients with virological failure for whom plasma samples could be obtained for resistance testing are shown in Table 1. Patients were most commonly male, 30–50 years old, and married, had contracted HIV via the heterosexual route, were classified as clinical stage I HIV by the WHO, had been treated for 1-2 years, and were frequently treated with AZT/D4T + 3TC + NVP/EFV (NRTIs + NNRTIs combination). A logistic regression analysis was conducted to identify independent predictors of drug resistance in this population. Patients who were female (RR = 0.362, 95% CI: 0.155–0.844) were at lower risk of ART resistance. In addition, by comparing the relative risk of drug resistance between patients treated for 1-2 years (RR = 13.616, 95% CI: 1.715–108.109), 2-3 years (RR = 19.556, 95% CI: 2.278–167.857), and more than 3 years (RR = 50.579, 95% CI: 4.855–526.891) we concluded that there was a significantly greater possibility of HIV DR emerging with longer treatment duration (Table 2).

To better understand the types of DR observed in Jiangsu, we further subdivided the drug resistant cases by resistance to nucleoside reverse transcriptase inhibitors (NRTIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs), or protease inhibitors (PIs) based on mutations in the viral genome. The highest frequency drug resistance mutations were NNRTIs (46.94%), followed by NRTIs (37.25%), and PIs (5.61%). These results suggested that NNRTIs drug use caused the most severe resistance in Jiangsu. We then compared the frequency of multidrug resistance (NRTIs + NNRTIs, NRTIs + PIs, and NNRTIs + PIs). The combination with the highest frequency of multidrug resistance was the NRTIs and NNRTIs (35.17%) combination (Figure 1).

3.3. DR Mutations Profiles. The most common mutations associated with drug resistance in NRTIs were M184V (79.45%), M41L (23.29%), M184I (10.96%), and K70R (10.96%) (Figure 2(a)). K103N, Y181C, G190A, and V108I were the most prevalent mutations associated with NNRTIs resistance and the frequencies were 33.70%, 29.35%, 27.17%, and 27.17%, respectively (Figure 2(b)). The most often combination of NRTIs mutations is M184V + M41L, and its frequency is 6.63%; however, the pattern of NNRTIs mutation combination is an even distribution. 69.30% of patients (70/101) harbored resistance to both NRTIs and NNRTIs. Four PI-associated mutations were observed in 11 individuals in this study whithout phenotypic resistance to PI drugs.

4. Discussion

Jiangsu is the largest province in eastern China; therefore, incomplete data about the efficacy of the National Free ART Program in Jiangsu affects the integrity of the data for China as a whole. Here, we have analysed the prevalence of DR in Jiangsu and characterised the factors associated with drug resistance in virological failure in 2012. This particular year was selected because there were a large number of samples available and there was a more complete collection of data for each case available. This study provides a baseline for further study on the efficacy of the ART program. We found that failure occurred overall in about 10.44% of cases and that the overall rate of DR was about 4.54%. The prevalence of HIV DR is low compared to the WHO estimate suggesting the emergence of drug resistance in <30% of HIV-1 infected patients with virological failure [18]. Furthermore, this figure indicates that the ART treatment regimens in Jiangsu are efficacious. Plasma VLs and blood CD4 cell counts are most widely used to monitor the success of antiretroviral therapy. Surprisingly, in those cases where virological failure occurred, we observed higher VLs results in patients with the drug sensitive virus. It is reported that the replication capacity of HIV drug resistant strains would be decreased during the course of viral RNA synthesis in contrast to the wild type HIV virus [19]. In addition, it has been shown in other studies that the risk of selection of resistance peaks around 10,000 copies/mL [20]. All these findings above are in accordance with our results of VLs in drug-resistant group which is lower than that in drug-susceptible group. But there is no evidence demonstrating that VLs show correlation with drug resistance incidence [21].

Mutations associated with DR were found in approximately half (51.5%) of the plasma samples from patients with virological failure. This estimation is similar to nationwide

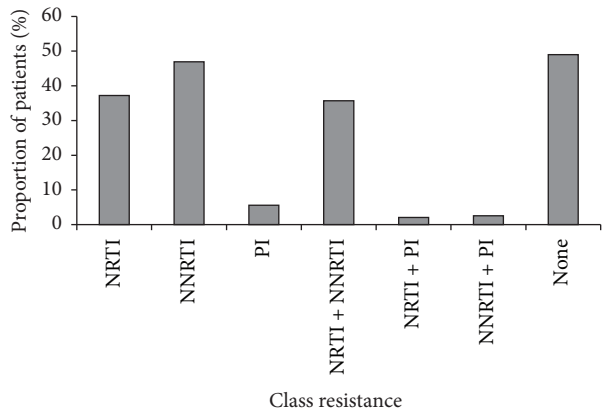

Figure 1: The percentage of patients with specific antiviral mutations. Based on the genotype results, the drug resistant strains were divided into nucleoside reverse transcriptase inhibitor (NRTI); nonnucleoside reverse transcriptase inhibitor (NNRTI); and protease inhibitor (PI) resistant strains using the Stanford website. The percentage of patients with NRTI, NNRTI, or PI resistant strains is shown. The denominator in all cases is 196 patients, all those who experienced virological failure and had serum samples available. The numerators for each column were NRTI = 73 (including multiple drug resistant strains); NNRTI = 92 (including multiple drug resistant strains); PI = 11 (including multiple drug resistant strains); NRTI + NNRTI = 70; PI + NRTI = 4; and PI + NNRTI = 5. In addition, 95 strains had none of the drug resistance mutations.

Table 1: Demographic characteristics of HIV-1 treated patients with virological failure on ART and univariate analyses for correlates of drug resistance.

Variables	Virologic failure (n = 196)	%	Drug resistance % (n)	χ^2 value	p value
Age (years)				0.050	0.975
<30	38	19.4	50 (19)		
30–50	121	61.7	52.1 (63)		
>50	37	18.9	51.4 (19)		
Gender				3.424	0.064
Male	159	81.1	54.7 (87)		
Female	37	18.9	37.8 (14)		
Marital status				0.161	0.923
Single	52	26.5	53.8 (28)		
Married	110	56.1	50.9 (56)		
Other	34	17.3	50.0 (17)		
Routes of infection				4.876	0.300
Blood	11	5.6	63.6 (7)		
IDU	6	3.1	66.7 (4)		
MSM	68	34.7	57.4 (39)		
Hetro	96	49.0	43.8 (42)		
Other	15	7.7	60.0 (9)		
WHO clinical stages				10.442	0.015
I	101	51.5	40.6 (41)		
II	50	25.5	60.0 (30)		
III	28	14.3	67.9 (19)		
IV	17	8.7	64.7 (11)		
Treatment duration				18.376	0.000
<1	16	8.2	6.2 (1)		
1-2	120	61.2	52.5 (63)		
2-3	39	19.9	53.8 (21)		
>3	21	10.7	76.2 (16)		
Side effect				3.594	0.058
Yes	13	6.6	76.9 (10)		
No	183	93.4	49.7 (91)		
SMZ taken				4.280	0.039
Yes	11	5.6	81.8 (9)		
No	185	94.4	49.7 (92)		
Treatment regimen				1.909	0.592
AZT/D4T + 3TC + NVP/EFV	176	89.8	51.1 (90)		
TDF + 3TC + NVP/EFV	14	7.1	50.0 (7)		
AZT + 3TC + LPV/r	4	2.0	50.0 (2)		
AZT + TDF + LPV/r	2	1.0	100 (2)		

IDU: intravenous drug use; MSM: men who have sex with men; hetro: heterosexual; SMZ: compound sulfamethoxazole; AZT: zidovudine; TDF: tenofovir; 3TC: lamivudine; NVP: nevirapine; LPV/r: lopinavir + ritonavir.

rates of HIV drug resistance among patients with treatment failure (57%) [22]. Also consistent with previous studies, NNRTIs, NRTIs, and the NRTIs + NNRTIs combination regimens showed high drug resistance [22]. The majority of patients enrolled in our study received treatment with a NRTIs + NNRTIs combination regimen for 1-2 years. In fact, studies showed that drug resistance most often emerged in the first 6–12 months after ART initiation [23]. However, it is hard to practice this surveillance guideline in resource-limited countries. Therefore, the WHO has developed comprehensive drug resistance surveillance and monitoring strategy based on public health principles [24],

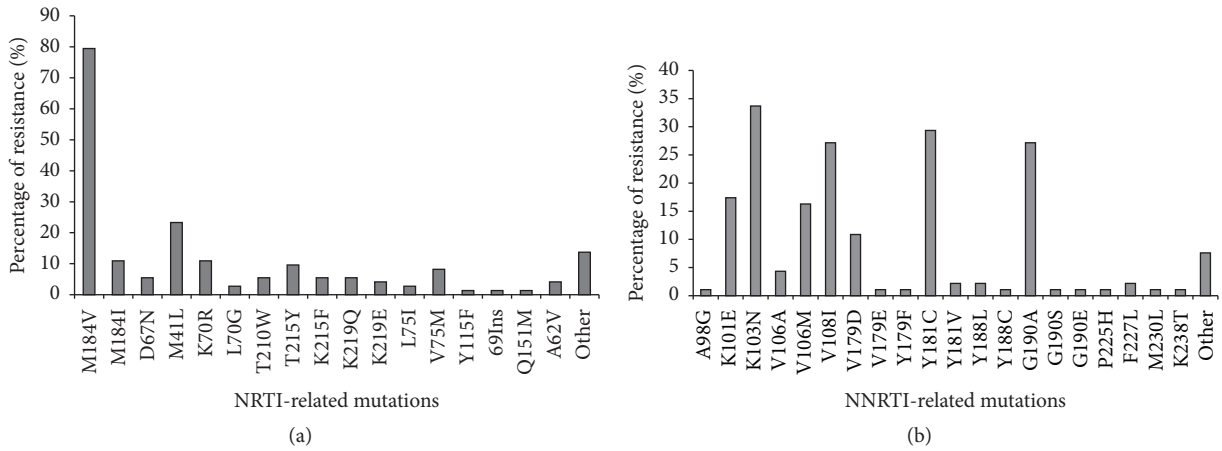

Figure 2: The frequencies of resistant mutations associated with NRTI. The results of these mutations presented here are all from the patients' sequence alignment on Stanford University HIV Drug Resistance Database. The percentages of mutation were showed in the figures. (a) The denominator in these cases is the number of patients who get any NRTI mutations ($n = 73$). M184V ($n = 58$), followed by M41L ($n = 17$), M184I ($n = 8$), and K70R ($n = 8$) in sequence. (b) The denominator in these cases is the number of patients who get any NNRTI mutations ($n = 92$). The most numerous mutation in NNRTI is K103N ($n = 31$), followed by Y181C ($n = 27$), G190A ($n = 25$), and V108I ($n = 25$).

Table 2: Logistic regression analysis of factors associated with HIV-1 drug resistance.

Factors	Variables	p value	RR	95% CI for RR	
				Lower	Upper
Gender	Male		1		
	Female	0.019	0.362	0.155	0.844
WHO clinical stages	I	0.297			
	II	0.085	1.926	0.913	4.063
	III	0.224	1.815	0.694	4.742
	IV	0.859	1.125	0.308	4.102
Treated duration	<1	0.009			
	1-2	0.014	13.616	1.715	108.109
	2-3	0.007	19.556	2.278	167.857
	>3	0.001	50.579	4.855	526.891
Side effect	Yes		1		
	No	0.186	20576	0.634	10.468
SMZ taken	Yes		1		
	No	0.123	3.707	0.700	19.633

SMZ: compound sulfamethoxazole.

to address concerns about emergence of drug resistance, and developed early warning indicators (EWI) for use in resource-limited settings to limit the development and spread of preventable drug resistance [25]. Therefore, it would likely benefit the Chinese program to introduce the WHO EWI to limit the possibility of further drug resistance emerging in Jiangsu. The high rate of resistance to the first-line regimen also suggests that we should be aware of the possibility of transmitted drug resistance (TDR) and test for drug resistance in treatment-naïve HIV-1 infected patients.

The resistance mutations identified in this study are not surprising for a developing country treatment program based on NRTIs and NNRTIs, with second-line PI regimens not yet scaled up. M184V could cause high-level resistance to 3TC and low-level resistance to ddI. M41L belongs to TAMs (thymidine analogue-associated mutations) that were selected by AZT and D4T. K103N, Y181C were the most prevalent mutations associated with NNRTI resistance in our study. K103N causes high-level resistance to NVP and EFV and Y181C can cause high-level resistance to NVP and intermediate resistance to EFV. All the mutations identified are in accordance with our first-line therapy regimen. Of concern, in 101ART treated patients with drug resistance mutations, 69.30% harbored resistance to both NRTI and NNRTI, much higher than the incidence around 50% in other studies in China [26, 27]. The number of incidences in Africa is around 60% and it is much lower in North America or in Western Europe, which are both approximately 20% [28]. This number of double resistance to NRTIs and NNRTIs in our study was particularly high partly due to the sustaining, permanent ART regimen without new drugs alternative.

Logistic regression analysis showed that gender and treatment time were associated with DR. The findings show that male gender and long-term ART predicted drug resistance are similar to previous studies in the Chinese literature [29, 30]. In Jiangsu province, the main route of HIV transmission was through heterosexual transmission; however, homosexual transmission has surpassed heterosexual transmission in recent years [31]. In our study, 34.7% of male patients with virological failure were men who have sex with men (MSM). Compared with other demographic groups infected with HIV, MSM tend to have higher drug resistance rates and worse treatment outcomes [32, 33]. Thus, additional monitoring and further assessments of the drug resistance profile in this population are required to consolidate the efforts for ART treatment.

Our study is limited by several caveats. First, of the 232 patients that were virological failures, only 196 were successfully sequenced, meaning 15.5% (36/232) of the samples were missing for sequences analysis, and the estimated prevalence

of DR therefore may not be accurate and credible. Second, our conclusions only reflect the status of DR in 2012, additional studies are necessary to fully evaluate the consequences of the National Free ART Program, and the predicted severity of drug resistance is not sufficient. Despite these limitations, this study provides valid advice for clinical treatment, not only through survey analysis, but also based on laboratory data. The preliminary data support that the use of first-line ART regimens is effective for patients in Jiangsu. However, we should be conscious that drug resistance has been emerging. Thus, in the resource-limited settings in China with large numbers of patients, management of first-line ART and follow-up prescription guidelines is vital to prevent drug resistance from becoming uncontrollable.

Competing Interests

The authors declared no potential conflict of interests with respect to the research, authorship, and/or publication of this article.

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References

[1] UNAIDS, *Global Report: UNAIDS Report on the Global AIDS Epidemic 2013*, 2013, http://reliefweb.int/report/world/global-report-unaids-report-global-aids-epidemic-2013.

[2] N. T. T. Dang, H. Sivakumaran, D. Harrich, P. N. Shaw, N. Davis-Poynter, and A. G. A. Coombes, "Synergistic activity of tenofovir and nevirapine combinations released from polycaprolactone matrices for potential enhanced prevention of HIV infection through the vaginal route," *European Journal of Pharmaceutics and Biopharmaceutics*, vol. 88, no. 2, pp. 406–414, 2014.

[3] M. S. Cohen, Y. Q. Chen, M. McCauley et al., "Prevention of HIV-1 infection with early antiretroviral therapy," *The New England Journal of Medicine*, vol. 365, no. 6, pp. 493–505, 2011.

[4] M. S. Cohen, M. K. Smith, K. E. Muessig, T. B. Hallett, K. A. Powers, and A. D. Kashuba, "Antiretroviral treatment of HIV-1 prevents transmission of HIV-1: where do we go from here?" *The Lancet*, vol. 382, no. 9903, pp. 1515–1524, 2013.

[5] F. Zhang, J. E. Haberer, Y. Wang et al., "The Chinese free antiretroviral treatment program: challenges and responses," *AIDS*, vol. 21, supplement 8, pp. S143–S148, 2007.

[6] National Health and Family Planning Commission of the People's Republic of China, *Background of HIV/AIDS Prevention and Control in China*, 2013, http://www.nhfpc.gov.cn/jkj/s3586/201312/2b871ccd2ef446eb9542875d3d68bbca.shtml.

[7] F. Zhang, Z. Dou, Y. Ma et al., "Effect of earlier initiation of antiretroviral treatment and increased treatment coverage on HIV-related mortality in China: A National Observational Cohort Study," *The Lancet Infectious Diseases*, vol. 11, no. 7, pp. 516–524, 2011.

[8] M. Zhang, M. Shang, W. Yang, J. Chen, Z. Wang, and H. Shang, "Treatment effect and drug-resistant mutations in Chinese AIDS patients switching to second-line antiretroviral therapy," *PLoS ONE*, vol. 9, no. 10, Article ID e110259, 2014.

[9] F. Zhang, J. Wang, F. Wang et al., *National Free Antiretroviral Treatment Handbook (Version 3)*, People's Medical Publishing House, 2012.

[10] World Health Organization, "Consolidated guidelines on the use of antitrtroviral drugs for treating and preventing HIV infection Recommendations for a public health approcach," 2013, http://www.who.int/hiv/pub/guidelines/arv2013/en/.

[11] K. M. Stadeli and D. D. Richman, "Rates of emergence of HIV drug resistance in resource-limited settings: a systematic review," *Antiviral Therapy*, vol. 18, no. 1, pp. 115–123, 2013.

[12] D. B. Fofana, C. Soulié, A. Baldeé et al., "High level of HIV-1 resistance in patients failing long-term first-line antiretroviral therapy in Mali," *Journal of Antimicrobial Chemotherapy*, vol. 69, no. 9, pp. 2531–2535, 2014.

[13] C. A. Velasco-de-Castro, B. Grinsztejn, V. G. Veloso et al., "HIV-1 diversity and drug resistance mutations among people seeking HIV diagnosis in voluntary counseling and testing sites in Rio de Janeiro, Brazil," *PLoS ONE*, vol. 9, no. 1, Article ID e87622, 2014.

[14] J. Wang, Z. Wang, J. Liu et al., "Efficacy and HIV drug resistance profile of second-line ART among patients having received long-term first-line regimens in rural China," *Scientific Reports*, vol. 5, Article ID 14823, 2015.

[15] L.-J. Hou, H.-W. Wang, S.-P. Duan et al., "The prevalence and determinants of drug-resistance-associated mutations in the HIV-1-infected MSM population of Henan Province in China," *Archives of Virology*, vol. 160, no. 8, pp. 2051–2061, 2015.

[16] T. Qiu, P. Ding, X. Liu et al., "Epidemiological characteristics of HIV/AIDS patients newly received highly active antiretroviral therapy during 2005–2013 in Jiangsu province," *Zhonghua Liu Xing Bing Xue Za Zhi*, vol. 35, no. 12, pp. 1320–1323, 2014.

[17] P. Scott, E. Arnold, B. Evans et al., "Surveillance of HIV antiretroviral drug resistance in treated individuals in England: 1998–2000," *Journal of Antimicrobial Chemotherapy*, vol. 53, no. 3, pp. 469–473, 2004.

[18] M. R. Jordan, D. E. Bennett, S. Bertagnolio, C. F. Gilks, and D. Sutherland, "World Health Organization surveys to monitor HIV drug resistance prevention and associated factors in sentinel antiretroviral treatment sites," *Antiviral Therapy*, vol. 13, supplement 2, pp. 15–23, 2008.

[19] A. M. N. Tsibris and M. S. Hirsch, "Antiretroviral therapy in the clinic," *Journal of Virology*, vol. 84, no. 11, pp. 5458–5464, 2010.

[20] M. C. F. Prosperi, N. Mackie, S. Di Giambenedetto et al., "Detection of drug resistance mutations at low plasma HIV-1 RNA load in a European multicentre cohort study," *Journal of Antimicrobial Chemotherapy*, vol. 66, no. 8, pp. 1886–1896, 2011.

[21] C. Rokx, L. Gras, D. van de Vijver, A. Verbon, and B. Rijnders, "Virological responses to lamivudine or emtricitabine when combined with tenofovir and a protease inhibitor in treatment-naïve HIV-1-infected patients in the Dutch AIDS Therapy Evaluation in the Netherlands (ATHENA) cohort," *HIV Medicine*, vol. 17, no. 8, pp. 571–580, 2016.

[22] J. Wu, J. Norris, H. X. Liu et al., "The prevalence of HIV drug resistance among treatment-failure individuals and treatment-naïve individuals in China: a meta-Analysis," *Biomedical and Environmental Sciences*, vol. 27, no. 11, pp. 858–871, 2014.

[23] M. W. Tang and R. W. Shafer, "HIV-1 antiretroviral resistance: scientific principles and clinical applications," *Drugs*, vol. 72, no. 9, pp. e1–e25, 2012.

[24] D. E. Bennett, S. Bertagnolio, D. Sutherland, and C. F. Gilks, "The World Health Organization's global strategy for prevention and assessment of HIV drug resistance," *Antiviral Therapy*, vol. 13, supplement 2, pp. 1–13, 2008.

[25] J. Fokam, S. C. Billong, A. C. Bissek et al., "Declining trends in early warning indicators for HIV drug resistance in Cameroon from 2008–2010: lessons and challenges for low-resource settings," *BMC Public Health*, vol. 13, article 308, 2013.

[26] C. Yang, S. Yang, J. Li et al., "Genetic diversity and drug resistance among antiretroviral treatment-failed individuals from 2010 to 2012 in Honghe, China," *AIDS Research and Human Retroviruses*, vol. 31, no. 8, pp. 822–829, 2015.

[27] M. Zhong, B. Yang, and S. Yang, "Analysis of genotype resistance mutations sites among 123 antiretroviral-therapy failure in HIV/AIDS patients in Yunnan province, China," *Chinese Journal of Laboratory Medicine*, vol. 36, pp. 53–58, 2013.

[28] R. K. Gupta, "Global epidemiology of drug resistance after failure of WHO recommended first-line regimens for adult HIV-1 infection: a multicentre retrospective cohort study," *The Lancet Infectious Diseases*, vol. 16, no. 5, pp. 565–575, 2016.

[29] K. E. Muessig, M. M. McLaughlin, J. M. Nie et al., "Suboptimal antiretroviral therapy adherence among HIV-infected adults in Guangzhou, China," *AIDS Care*, vol. 26, no. 8, pp. 988–995, 2014.

[30] H. Liu, Y. Ma, Y. Su et al., "Emerging trends of HIV drug resistance in Chinese HIV-infected patients receiving first-line highly active antiretroviral therapy: a systematic review and meta-analysis," *Clinical Infectious Diseases*, vol. 59, no. 10, pp. 1495–1502, 2014.

[31] H. Haiyang, L. Xiaoyan, and H. Xiping, "Status of recent infection on new reported HIV/AIDS in Jiangsu province, 2011-2012," *Acta Universitatis Medicinalis Anhui*, vol. 49, pp. 40–43, 2014.

[32] L. Dai, N. Li, F. Wei et al., "Transmitted antiretroviral drug resistance in the men who have sex with men HIV patient cohort, Beijing, China, 2008–2011," *Viral Immunology*, vol. 27, no. 8, pp. 392–397, 2014.

[33] H. Yan, H. Yang, J. Li et al., "Emerging disparity in HIV/AIDS disease progression and mortality for men who have sex with men, Jiangsu Province, China," *AIDS and Behavior*, vol. 18, supplement 1, pp. S5–S10, 2014.

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Research Article

Late Presentation of HIV Infection: Prevalence, Trends, and the Role of HIV Testing Strategies in Guangzhou, China, 2008–2013

Weibin Cheng,[1] Weiming Tang,[2] Zhigang Han,[1] Thitikarn May Tangthanasup,[2] Fei Zhong,[1] Faju Qin,[1] and Huifang Xu[1]

[1] *Department of AIDS/STD Control and Prevention, Guangzhou Center for Disease Control and Prevention, No. 1, Qide Road, Jiahe, Baiyun District, Guangzhou, Guangdong Province 510440, China*
[2] *University of North Carolina Project-China, No. 2, Lujing Road, Yuexiu District, Guangzhou, Guangdong Province 510095, China*

Correspondence should be addressed to Huifang Xu; xuhuifang1027@21cn.com

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Background. The prevalence, trends, and the role of different HIV testing strategies in late presentation of HIV infection in China were unknown. *Methods*. Data of newly reported HIV cases in Guangzhou between 2008 and 2013 was analyzed to examine the prevalence, trends, and characteristics of late presentation of HIV infection by three types of HIV testing strategies. *Results*. Overall, 53.2% (1412/2653) and 27.3% (724/2653) met the criteria of late presentation and presentation with advanced HIV disease. The overall trend of late presentation of HIV infection within the study period was declining. Late presentation was 62.9% in 2008 and dropped to 43.3% in 2013 ($P < 0.001$); presentation with advanced HIV disease was 40.3% in 2008 and dropped to 15.2% in 2013 ($P < 0.001$). Of the three testing strategies, PITC presented higher odds of both late presentation [AOR (95% CI): PITC versus VCT: 1.37 (1.09, 1.73); PITC versus MHT: 3.09 (2.16, 4.42)] and presentation with advanced HIV disease [AOR (95% CI): PITC versus VCT: 1.65 (1.29, 2.11); PITC versus MHT: 13.14 (8.47, 20.39)]. *Conclusions*. Although the late presentation of HIV infection was declining, it was still high in Guangzhou. The worse situation among PITC cases urges the policy adjustment in medical settings to increase early HIV diagnosis.

1. Background

In October 2009, the European Late Presenter Consensus working group reached a consensus definition of late presentation of HIV infection [1]. According to the consensus, late presentation was defined as persons presenting for care with a CD4 cell count below 350 cells/μL or presenting with an AIDS-defining event, regardless of the CD4 cell count. Presentation with advanced HIV disease was defined as persons presenting for care with a CD4 cell count below 200 cells/μL or presenting with an AIDS-defining event, regardless of the CD4 cell count. Late presentation for HIV care is detrimental for infected persons and others [2–4]. Studies showed that late presentation not only has a serious impact on HIV transmission [5–8], but also has become the major concern for AIDS and AIDS-related deaths in western countries [9, 10]. Despite tremendous efforts, a substantial number of late HIV infections still persist worldwide [11–15]. Even though we already have essential data from the United States and European countries, there is a lack of data on late presentation of HIV infection in China and other Asian countries.

A number of studies documented that late presentation of HIV infection is generally more common among marginalized individuals (i.e., ethnic minorities, drug users, and immigrants), people with low perceived risk of HIV infection (e.g., the elderly and heterosexuals), and those of low socioeconomic status (e.g., lower educational attainment) [14, 16–24]. Late presentation of HIV infection reflected the lack of knowledge about HIV and/or cultural and social stigma and socioeconomic barriers which limited the access to healthcare. While HIV testing strategy is considered to be important for late diagnosis as it offers opportunities for HIV positive identification, there is scarce data about testing strategies.

In the past decade, with the help of international funding agencies, the availability of testing services in China has increased remarkably. In 2004, a free HIV voluntary counseling and testing (VCT) program was introduced to China, which followed the "Four Free, One Care" HIV policy [25, 26]. VCT services are mainly available at the Centers for Disease Control and Prevention (CDC) and primary healthcare centers that are equipped with trained staff. VCT is free of charge, targeting people who have experienced risk of HIV infection. However, people need to take the initiative to drop by the center to use the service. To increase the VCT service uptake, CDC has implemented a series of intervention programs among the at-risk population, such as men who have sex with men (MSM) and female sex workers. In 2005, mandatory HIV testing (MHT) was launched at detention centers, methadone maintenance treatment clinics, and reeducation/labour camps [27]. MHT was administered to all individuals when they entered into the detention settings. The purpose of MHT was to test at-risk groups including drug users, prisoners, and sex workers along with their clients. In 2006, provider-initiated testing and counseling (PITC) was introduced and implemented at general hospitals and STI clinics in China [26]. HIV test was prescribed by the clinical doctors in the hospital when patients presented with HIV clinical indicator diseases. Meanwhile, PITC included applying HIV testing to individuals who presented to STD clinics. PITC was a paid HIV testing service by using an "opt-out" approach. Patients were informed that HIV testing will take place along with other standard tests and that they must state whether they do not wish to be tested. These three HIV testing strategies are the main types of HIV testing strategies in China. However, according to our knowledge, the effectiveness of testing strategy in late HIV diagnosis has not been evaluated.

Given the clinical and public health benefits of early HIV diagnosis on timely antiretroviral therapy (ART) initiation, it is imperative to understand the situation of late presentation of HIV infection in China and to provide evidence on whichever strategies could facilitate early diagnosis of HIV infection. Thus, by analyzing the HIV case report data in Guangzhou in 2008 to 2013 that was extracted from the Chinese HIV/AIDS Comprehensive Response Information Management System (CRIMS) [28], we conducted this study to examine the prevalence and trend of late presentation of HIV infection and to explore the role of different testing strategies in late presentation of HIV infection.

2. Methods

HIV testing service in Guangzhou city is available within authorized CDC, medical institutes, primary healthcare centers, and collective settings. By the end of 2008, there were three HIV confirmatory test laboratories and ninety-one HIV screening laboratories (this increased to 147 in 2013).

Guangzhou started HIV/AIDS case reporting through the Internet in 2005. By the end of 2007, CRIMS was developed and put into use. It is a web-based real-time database system managed by the National Centre for AIDS/STD Control and Prevention (NCAIDS), China CDC [28]. CRIMS includes case report module and epidemiology follow-up module. An electronic record is created in CRIMS for each patient who tested positive for HIV. Case report and epidemiology follow-up services are conducted by trained staffs from hospitals, district level CDC, and primary healthcare centers.

2.1. Data Sources. The data included in our study was retrieved from CRIMS case report module and epidemiology follow-up module. The data included information on demographic characteristics, sexual and drug use behaviors, transmission routes, medical histories, and laboratory test results. In this study, we only included cases that were over 15 years old when identified and were identified between January 1, 2008, and December 31, 2013, in our data analysis.

2.2. Statistical Analysis. In this study, first-time CD4 cell count results (tested within two weeks after confirmatory HIV positive test) were used to define late presentation of HIV infection. Late presentation was defined as CD4 cell count below 350 cells/μL or presenting with an AIDS-defining event at the first follow-up, regardless of the CD4 cell count. Presentation with advanced HIV disease was defined as CD4 cell count below 200 cells/μL or presenting with an AIDS-defining event at the first follow-up, regardless of the CD4 cell count. Descriptive analyses were used to describe and compare (Chi squared test) demographic characteristics of late presentation of HIV infection cases identified through the three types of testing strategies. Trends of late presentation of HIV infection by three types of testing strategies during the study period were examined. Multivariate logistic regression models were used to compare the proportion of late presentation of HIV infection with different testing strategies. Demographic variables with $P < 0.20$ under Chi squared test analysis were adjusted for multivariate logistic regression models. Adjusted odds ratio (AOR) and 95% confidence intervals (95% CI) were also estimated. Results with a two-sided $P < 0.05$ were considered statistically significant. All statistical analyses were performed with SPSS 18.0 for Windows.

2.3. Ethics Statement. This study was based on data from the Chinese government HIV/AIDS CRIMS. All individuals signed a general consent form when they enrolled in CRIMS, and no additional study informed consent was needed for this current study. The study protocol was approved by Guangzhou CDC Ethics Committee. All personal identification information was removed prior to analysis.

3. Results

Of the 6,737 cases identified between 1 January 2008 and 31 December 2013, 2,653 (39.4%) cases that had CD4 cell count results at the first epidemiology follow-up were included in this study. Demographic characteristics of the cases identified through PITC were tendency to be older (42.1% aged over

Table 1: Characteristics and proportion of late presentation of HIV infection among newly diagnosed positives by three types of HIV testing strategies between 2008 and 2013 in Guangzhou, China.

Variable	Total (N = 2653)	VCT (N = 1275)	PITC (N = 832)	MHT (N = 546)	P
	n (%)				
Year					
2008	313 (11.8)	156 (12.2)	101 (12.1)	56 (10.3)	<0.001
2009	244 (9.2)	79 (6.2)	75 (9.0)	90 (16.5)	
2010	381 (14.4)	95 (7.5)	194 (23.3)	92 (16.8)	
2011	474 (17.8)	206 (16.1)	160 (19.2)	108 (19.8)	
2012	689 (26.0)	382 (30.0)	179 (21.5)	128 (23.4)	
2013	552 (20.8)	357 (28.0)	123 (14.9)	72 (13.2)	
Age (years)					
<20	75 (2.8)	41 (3.2)	28 (3.4)	6 (1.1)	<0.001
20~	860 (32.4)	535 (42.0)	175 (21.0)	150 (27.5)	
30~	981 (37.0)	438 (34.4)	279 (33.5)	264 (48.4)	
40~	490 (18.5)	180 (14.1)	207 (24.9)	103 (18.9)	
≥50	247 (9.3)	81 (6.4)	143 (17.2)	23 (4.2)	
Sex					
Male	2264 (85.3)	1131 (88.7)	612 (73.6)	521 (95.4)	<0.001
Female	389 (14.7)	144 (11.3)	220 (26.4)	25 (4.6)	
Marital status					
Single	1355 (51.1)	750 (58.8)	228 (27.4)	377 (69.0)	<0.001
Married	1015 (38.4)	396 (31.1)	501 (60.2)	118 (21.6)	
Divorced or widowed	244 (9.2)	117 (9.2)	91 (10.9)	36 (6.6)	
Unknown	39 (1.5)	12 (0.9)	12 (1.4)	15 (2.7)	
Educational attainment					
Primary school/lower	526 (19.8)	113 (8.9)	165 (19.8)	248 (45.4)	<0.001
Secondary school	1534 (57.8)	677 (53.1)	562 (67.5)	295 (54.0)	
College/above	593 (22.4)	485 (38.0)	105 (12.6)	3 (0.5)	
Route of HIV infection					
Male-to-male sexual contact	917 (34.6)	837 (65.6)	77 (9.3)	3 (0.5)	<0.001
Injecting drug use	654 (24.7)	85 (6.7)	99 (11.9)	470 (86.1)	
Heterosexual contact	967 (36.4)	326 (25.6)	581 (69.8)	60 (11.0)	
Others	115 (4.3)	27 (2.1)	75 (9.0)	13 (2.4)	
Place of living					
Urban	1384 (52.2)	827 (64.9)	316 (38.0)	241 (44.1)	<0.001
Rural	480 (18.1)	205 (17.1)	133 (4.4)	133 (24.2)	
Outside of Guangzhou	789 (29.7)	374 (45.0)	172 (31.5)	172 (31.5)	
Late presentation					
Yes	1412 (53.2)	659 (51.7)	590 (70.9)	206 (37.7)	<0.001
No	1241 (46.8)	616 (48.3)	242 (29.1)	340 (62.3)	
Presentation with advanced HIV disease					
Yes	724 (27.3)	254 (19.9)	416 (50.0)	54 (9.9)	<0.001
No	1929 (72.7)	1021 (80.1)	416 (50.0)	492 (90.1)	

VCT: HIV voluntary counseling and testing; PITC: provider-initiated testing and counseling; MHT: mandatory HIV testing.

40), being female (26.4%), being married (60.2%), and self-reporting being infected through heterosexual transmission (69.8%). VCT cases had higher educational attainment (38.0% had a college degree or above) and were infected through homosexual contact (65.6%). The majority of MHT cases were infected through injecting drug use (86.1%). Overall, 53.2% (1412/2653) and 27.3% (724/2653) were classified into late presentation and presentation with advanced HIV disease groups. PITC cases had a higher proportion of late presentation (70.9% and 51.7% for VCT and 37.7% for MHT) and presentation with advanced HIV disease (50.0% and 19.9% for VCT and 9.9% for MHT). All characteristics were significantly different among the three groups ($P < 0.001$) (Table 1).

Table 2: Changes in late presentation of HIV infection over time by three types of testing strategies in Guangzhou, 2008–2013.

	2008 (N = 313)	2009 (N = 244)	2010 (N = 381)	2011 (N = 474)	2012 (N = 689)	2013 (N = 552)	P
			Late presentation, n (%)				
Total	197 (62.9)	121 (49.6)	259 (68.0)	260 (54.9)	336 (48.8)	239 (43.3)	<0.001
Testing strategy							
VCT	102 (65.4)	37 (46.8)	55 (57.9)	114 (55.3)	172 (45.0)	136 (38.1)	<0.001
PITC	78 (77.2)	43 (57.3)	161 (83.0)	106 (66.3)	120 (67.0)	82 (66.7)	0.052
MHT	17 (30.4)	41 (45.6)	43 (46.7)	40 (37.0)	44 (34.4)	21 (29.2)	0.131
			Presentation with advanced HIV disease, n (%)				
Total	126 (40.3)	45 (18.4)	166 (43.6)	137 (28.9)	166 (24.1)	84 (15.2)	<0.001
Testing strategy							
VCT	63 (40.4)	11 (13.9)	26 (27.4)	49 (23.8)	63 (16.5)	42 (11.8)	<0.001
PITC	59 (58.4)	24 (32.0)	126 (64.9)	77 (48.1)	91 (50.8)	39 (31.7)	0.002
MHT	4 (7.1)	10 (11.1)	14 (15.2)	11 (10.2)	12 (9.4)	3 (4.2)	0.294

VCT: HIV voluntary counseling and testing; PITC: provider-initiated testing and counseling; MHT: mandatory HIV testing.

The annual proportions of late presentation from 2008 to 2013 were 62.9% (197/313), 49.6% (121/244), 68.0% (259/680), 54.9% (260/474), 48.8% (336/689), and 43.3% (239/552). Although the overall proportion of late presentation declined during the study period ($P < 0.001$), only the annual rate of late presentation among cases identified through VCT was significantly reduced (from 65.4% to 38.1%). Annual proportions of presentation with advanced HIV disease from 2008 to 2013 were 40.3% (126/313), 18.4% (45/244), 28.9% (1137/680), 24.1% (166/689), and 15.2% (84/552). The overall proportion of presentation with advanced HIV disease was declining ($P < 0.001$), while similar results were found for both VCT and PITC strategies (Table 2).

Two multivariate logistic regression models were developed to compare late presentation of HIV infection within three HIV testing strategies. Models were adjusted for year, age, sex, marital status, educational attainment, route of HIV infection, and place of living. Results showed that PITC has the highest odds of late presentation of HIV infection among the three strategies [late presentation, PITC versus VCT, AOR (95% CI): 1.37 (1.09, 1.73); PITC versus MHT: 3.09 (2.16, 4.42); presentation with advanced HIV disease, PITC versus VCT: 1.65 (1.29, 2.11); PITC versus MHT: 13.14 (8.47, 20.39)]. Among the three strategies, MHT has the lowest odds of late presentation of HIV infection ($P < 0.01$) (Table 3).

4. Discussion

In this study, we found that half of the HIV newly diagnosed cases showed late presentation of HIV infection. Among the late presenters, half showed presentation with advanced HIV disease. The epidemic of late presentation of HIV infection in China was high [3], compared to Europe, even though the situation has significantly dropped in the past few years. In addition, this study has compared three types of HIV testing strategies role in the late HIV diagnosis. Results showing a high proportion of late presentation of HIV infection among PITC cases urged the need for improving the capacity of early case detection in medical settings.

Delayed diagnosis of HIV infection may result in late initiation of ART, which subsequently could undermine the effectiveness of clinical outcomes. A recent study demonstrated that deferred initiation of ART at CD4 levels 251 to 350 cells/μL was associated with higher rates of AIDS and mortality, compared to starting ART at the range of 351 to 450 cells/μL (HR: 1.28, 95% CI: 1.04–1.57), and that the adverse effect of deferring treatment increased with the decreasing CD4 cell count threshold [29]. Meanwhile, late presentation of HIV infection reflects the inefficiency of the health providers in response to patients' need for access to HIV services, such as testing and treatment [30]. However, early identification presents an opportunity to encourage safer behavior, which in turn reduces the chance of HIV transmission. The seriousness of late HIV diagnosis in Guangzhou presented a great barrier to achieve the UNAIDS goal of ending AIDS epidemic by 2030.

In this study, robust associations were observed between HIV testing strategies and late presentation of HIV infection. Before that, few reports had explored the association between HIV testing strategies and late HIV diagnosis [18, 31, 32]. Consistent with previous studies, results suggested that PITC did not facilitate more timely diagnosis than targeted HIV counseling. As Duffus WA pointed out, clinical risk-based testing strategy (including PITC), even if implemented successfully in their facility, would still have missed an earlier diagnosis most of the time [33]. PITC acted as an alternative strategy that captured individuals who were unwilling to seek or unaware of seeking HIV testing independently [31, 34]. However, this "opt-out" testing for HIV following diagnosis of an indicator disease was an important strategy for HIV infection case finding [18]. To increase the efficacy of early diagnosis of HIV infection, researchers suggested that adjusting routine provider-initiated HIV testing for at-risk groups in settings such as sexually transmitted infection clinics, drug dependency programs, or antenatal care might be more effective [35].

Interestingly, mandatory HIV testing showed great potential in identifying cases early. In Guangzhou, mandatory testing was well targeted at drug users. The ongoing

Table 3: Comparison of different testing strategies on late presentation of HIV infection by multivariate logistic regression[+].

	Late presentation AOR (95% CI)	*P*	Presentation with advanced HIV disease AOR (95% CI)	*P*
VCT	Ref.		Ref.	
PITC	1.37 (1.09,1.73)	0.008	1.65 (1.29, 2.11)	<0.001
MHT	0.32 (0.23, 0.46)	<0.001	0.13 (0.08, 0.20)	<0.001
PITC versus MHT	3.09 (2.16, 4.42)	<0.001	13.14 (8.47, 20.39)	<0.001

AOR: adjusted odds ratio; CI: confidence intervals.
VCT: HIV voluntary counseling and testing; PITC: provider-initiated testing and counseling; MHT: mandatory HIV testing.
[+]Models were adjusted for year, age, sex, marital status, educational attainment, route of HIV infection, and place of living.

mandatory HIV testing among drug users increased early diagnosis of HIV infection among this population and significantly contributed to the control of HIV epidemic among intravenous drug users in Guangzhou, as well as across China [36]. This result also concurred with the finding of one study conducted in the Republic of Korea [32]. Furthermore, studies indicated that mandatory HIV testing allows healthcare providers to provide early intervention to the at-risk population and to provide opportunities for testing, substance abuse treatment, and ART, which played an important role in the control of HIV epidemic [37, 38].

China Nation Free Antiretroviral Therapy Program (NFATP) was piloted in 2002 and was implemented nationally in 2003. Free ART criteria were started at CD4 cell count of less than 200 cells/μL and increased to less than 350 cells/μL in 2008 [39]. Although the longitudinal trends of late presentation of HIV infection in this study had shown a declining gesture, persistently high prevalence of late presentation (about 60%) was observed among PITC. One important reason is that high level of HIV related stigma/discrimination and low HIV risk perception among at-risk populations hindered the initiative access to HIV services [40]. PITC was mainly applied for the general population in the medical settings, which presented a gap of demanding for intervention to raise HIV awareness among at-risk individuals in the general population. Another common reason for those presenting late was that patients have often been seen with indicator diseases in the recent past by healthcare professionals without prompt offering of an HIV test [41]. These represent missed opportunities for diagnosis. HIV testing should continue to expand across clinical settings to reduce the number of patients living with undiagnosed HIV infection [42].

The limitations of the current reported study include the following: first, more than half of the cases were excluded due to the lack of CD4 cell count results which may compose an unrepresentative sample. Nonetheless, we compared cases that had CD4 cell count result at first follow-up with those that did not; a minor difference of the proportion of cases that progressed to AIDS at the end of the observation year was found between these two groups (32.2% versus 29.1%). Second, the definition of late presentation of HIV infection used in this study was relatively conservative. The interval between HIV diagnosis and CD4 cell count test within two weeks could underestimate and make comparison difficult. However, evidence suggested that although the percentage of HIV-infected persons with late diagnosis increases with longer cut-offs, the general factors associated with late diagnosis may not vary substantially across the different cut-offs [43]. Third, stigma and social desirability may prevent drug users, sex workers, and homosexual men from reporting the transmission route correctly, and this may lead to information bias. Nonetheless, these three HIV testing strategies have been designed to target different population at risk of HIV infection. Understanding the efficiency of different testing strategies on early case finding would be useful for HIV prevention policy adjustment and resources allocation.

Even with these limitations, this study presented important data that pointed out a high prevalence of late presentation of HIV infection among HIV cases identified in Guangzhou. Furthermore, our data demonstrated that voluntary HIV counseling and testing strategies were more effective in early HIV diagnosis. Recently, the national antiretroviral therapy guideline updated initial ART to treat all patients once diagnosis of HIV infection is confirmed. The early identification of HIV infection cases enables treatment to be initiated at a time when responses are optimal, which in turn reduces the chance of transmission [39]. However, more efforts to better understand the optimal HIV testing practice to facilitate early HIV diagnosis are needed.

Abbreviations

HIV: Human immunodeficiency virus
AIDS: Acquired immune deficiency syndrome
VCT: HIV voluntary counseling and testing
PITC: Provider-initiated testing and counseling
MHC: Mandatory HIV testing
STI: Sex transmitted infection
CDC: Centers for Disease Control and Prevention
ART: Antiretroviral therapy
CRIMS: Chinese HIV/AIDS Comprehensive Response Information Management System
NCAIDS: National Centre for AIDS/STD Control and Prevention
STD: Sex transmitted disease
AOR: Adjusted odds ratio
CI: Confidence intervals
HR: Hazard ratio
NFATP: Nation Free Antiretroviral Therapy Program.

Disclosure

Funding organizations had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.

Competing Interests

The authors have no competing interests to report.

Authors' Contributions

Weibin Cheng developed study protocol, analyzed data, interpreted findings, and drafted and revised the manuscript. Weiming Tang and Thitikarn May Tangthanasup revised the manuscript and checked the language. Zhigang Han prepared the study proposal, supervised data collection, and interpreted the findings. Fei Zhong analyzed data and interpreted the findings. Faju Qin collected data and performed data management. Huifang Xu supervised the study and provided technical inputs to the manuscript. All authors critically reviewed and approved the final version of this paper for publication.

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References

[1] A. Antinori, T. Coenen, D. Costagiola et al., "Late presentation of HIV infection: a consensus definition," *HIV Medicine*, vol. 12, no. 1, pp. 61–64, 2011.

[2] M. Limb, "More routine HIV testing is needed to reduce late diagnoses, says public health agency," *British Medical Journal*, vol. 343, Article ID d7794, 2011.

[3] A. Mocroft, J. D. Lundgren, M. L. Sabin et al., "Risk factors and outcomes for late presentation for HIV-positive persons in Europe: results from the Collaboration of Observational HIV Epidemiological Research Europe Study (COHERE)," *PLoS Medicine*, vol. 10, no. 9, Article ID e1001510, 2013.

[4] R. O. Valdiserri, "Late HIV diagnosis: bad medicine and worse public health," *PLoS Medicine*, vol. 4, article e200, 2007.

[5] G. Marks, N. Crepaz, and R. S. Janssen, "Estimating sexual transmission of HIV from persons aware and unaware that they are infected with the virus in the USA," *AIDS*, vol. 20, no. 10, pp. 1447–1450, 2006.

[6] M. Fisher, "Late diagnosis of HIV infection: major consequences and missed opportunities," *Current Opinion in Infectious Diseases*, vol. 21, no. 1, pp. 1–3, 2008.

[7] G. D. Sanders, A. M. Bayoumi, V. Sundaram et al., "Cost-effectiveness of screening for HIV in the era of highly active antiretroviral therapy," *The New England Journal of Medicine*, vol. 352, no. 6, pp. 570–585, 2005.

[8] H. I. Hall, D. R. Holtgrave, and C. Maulsby, "HIV transmission rates from persons living with HIV who are aware and unaware of their infection," *AIDS*, vol. 26, no. 7, pp. 893–896, 2012.

[9] J. Brännström, B. Åkerlund, M. Arneborn, A. Blaxhult, and J. Giesecke, "Patients unaware of their HIV infection until AIDS diagnosis in Sweden 1996-2002—a remaining problem in the highly active antiretroviral therapy era," *International Journal of STD and AIDS*, vol. 16, no. 10, pp. 702–706, 2005.

[10] B. Castelnuovo, E. Chiesa, S. Rusconi et al., "Declining incidence of AIDS and increasing prevalence of AIDS presenters among AIDS patients in Italy," *European Journal of Clinical Microbiology and Infectious Diseases*, vol. 22, no. 11, pp. 663–669, 2003.

[11] A. K. Sullivan, H. Curtis, C. A. Sabin, and M. A. Johnson, "Newly diagnosed HIV infections: review in UK and Ireland," *British Medical Journal*, vol. 330, no. 7503, pp. 1301–1302, 2005.

[12] "Late versus early testing of HIV—16 sites, United States, 2000–2003," *Morbidity and Mortality Weekly Report*, vol. 52, no. 25, pp. 581–586, 2003.

[13] T. Shivaji, A. Diniz, and H. Cortes-Martins, "Characteristics of late presentation of HIV infection in MSM and heterosexual adults in Portugal 2011–2013," *Journal of the International AIDS Society*, vol. 17, no. 4, supplement 3, Article ID 19690, 2014.

[14] M. J. Trepka, K. P. Fennie, D. M. Sheehan, K. Lutfi, L. Maddox, and S. Lieb, "Late HIV diagnosis: differences by rural/urban residence, Florida, 2007–2011," *AIDS Patient Care and STDs*, vol. 28, no. 4, pp. 188–197, 2014.

[15] K. D. Wilson, R. Dray-Spira, C. Aubrière, C. Hamelin, B. Spire, and F. Lert, "Frequency and correlates of late presentation for HIV infection in France: older adults are a risk group-results from the ANRS-VESPA2 study, France," *AIDS Care*, vol. 26, supplement 1, pp. S83–S93, 2014.

[16] C. M. Carrizosa, E. J. Blumberg, M. F. Hovell et al., "Determinants and prevalence of late HIV testing in Tijuana, Mexico," *AIDS Patient Care and STDs*, vol. 24, no. 5, pp. 333–340, 2010.

[17] H. I. Hall, J. Halverson, D. P. Wilson et al., "Late diagnosis and entry to care after diagnosis of human immunodeficiency virus infection: a country comparison," *PLoS ONE*, vol. 8, no. 11, Article ID e77763, 2013.

[18] P. Scognamiglio, G. Chiaradia, G. De Carli et al., "The potential impact of routine testing of individuals with HIV indicator diseases in order to prevent late HIV diagnosis," *BMC Infectious Diseases*, vol. 13, article 473, 2013.

[19] D. Klein, L. B. Hurley, D. Merrill, and C. P. Quesenberry Jr., "Review of medical encounters in the 5 years before a diagnosis of HIV-1 infection: implications for early detection," *Journal of Acquired Immune Deficiency Syndromes*, vol. 32, no. 2, pp. 143–152, 2003.

[20] M. J. Mugavero, C. Castellano, D. Edelman, and C. Hicks, "Late diagnosis of HIV infection: the role of age and sex," *The American Journal of Medicine*, vol. 120, no. 4, pp. 370–373, 2007.

[21] C. Delpierre, R. Dray-Spira, L. Cuzin et al., "Correlates of late HIV diagnosis: implications for testing policy," *International Journal of STD and AIDS*, vol. 18, no. 5, pp. 312–317, 2007.

[22] K. Mojumdar, M. Vajpayee, N. K. Chauhan, and S. Mendiratta, "Late presenters to HIV care and treatment, identification of associated risk factors in HIV-1 infected Indian population," *BMC Public Health*, vol. 10, article 416, 2010.

[23] I. M. Kigozi, L. M. Dobkin, J. N. Martin et al., "Late-disease stage at presentation to an HIV clinic in the era of free antiretroviral therapy in Sub-Saharan Africa," *Journal of Acquired Immune Deficiency Syndromes*, vol. 52, no. 2, pp. 280–289, 2009.

[24] P. K. Drain, E. Losina, G. Parker et al., "Risk factors for late-stage HIV disease presentation at initial HIV diagnosis in Durban, South Africa," *PLoS ONE*, vol. 8, no. 1, Article ID e55305, 2013.

[25] B. Gazzard, N. Clumeck, A. d'Arminio Monforte, and J. D. Lundgren, "Indicator disease-guided testing for HIV—the next step for Europe?" *HIV Medicine*, vol. 9, supplement 2, pp. 34–40, 2008.

[26] WHO and UNAIDS, *Guidance on Provider-Initiated HIV Testing and Counselling in Health Facilities*, World Health Organization, Geneva, Switzerland, 2007.

[27] Z. Wu, X. Sun, S. G. Sullivan, and R. Detels, "Public health. HIV testing in China," *Science*, vol. 312, no. 5779, pp. 1475–1476, 2006.

[28] Y. Mao, Z. Wu, K. Poundstone et al., "Development of a unified web-based national HIV/AIDS information system in China," *International Journal of Epidemiology*, vol. 39, supplement 2, Article ID dyq213, pp. ii79–ii89, 2010.

[29] J. A. Sterne, M. May, D. Costagliola et al., "Timing of initiation of antiretroviral therapy in AIDS-free HIV-1-infected patients: a collaborative analysis of 18 HIV cohort studies," *The Lancet*, vol. 373, pp. 1352–1363, 2009.

[30] T. Coenen, J. Lundgren, J. V. Lazarus, and S. Matic, "Optimal HIV testing and earlier care: the way forward in Europe," *HIV Medicine*, vol. 9, supplement 2, pp. 1–5, 2008.

[31] S. M. Topp, M. S. Li, J. M. Chipukuma et al., "Does provider-initiated counselling and testing (PITC) strengthen early diagnosis and treatment initiation? Results from an analysis of an urban cohort of HIV-positive patients in Lusaka, Zambia," *Journal of the International AIDS Society*, vol. 15, no. 2, Article ID 17352, 2012.

[32] P. G. Choe, W. B. Park, J. S. Song et al., "Late presentation of HIV disease and its associated factors among newly diagnosed patients before and after abolition of a government policy of mass mandatory screening," *Journal of Infection*, vol. 63, no. 1, pp. 60–65, 2011.

[33] W. A. Duffus, K. Weis, L. Kettinger, T. Stephens, H. Albrecht, and J. J. Gibson, "Risk-based HIV testing in South Carolina health care settings failed to identify the majority of infected individuals," *AIDS Patient Care and STDs*, vol. 23, no. 5, pp. 339–345, 2009.

[34] D. R. Holtgrave, "Costs and consequences of the US centers for disease control and prevention's recommendations for opt-out HIV testing," *PLoS Medicine*, vol. 4, no. 6, pp. 1011–1018, 2007.

[35] Y. Yazdanpanah, J. Lange, J. Gerstoft, and G. Cairns, "Earlier testing for HIV—how do we prevent late presentation?" *Antiviral Therapy*, vol. 15, supplement 1, pp. 17–24, 2010.

[36] L. Wang, W. Guo, D. Li et al., "HIV epidemic among drug users in China: 1995–2011," *Addiction*, vol. 110, no. 1, pp. 20–28, 2015.

[37] S. Basu, D. Smith-Rohrberg, S. Hanck, and F. L. Altice, "HIV testing in correctional institutions: evaluating existing strategies, setting new standards," *AIDS and Public Policy Journal*, vol. 20, no. 1-2, pp. 3–24, 2005.

[38] A. A. Amankwaa, L. C. Amankwaa, and C. O. Ochie Sr., "Revisiting the debate of voluntary versus mandatory HIV/AIDS testing in U.S. prisons," *Journal of Health and Human Services Administration*, vol. 22, no. 2, pp. 220–236, 1999.

[39] F. Zhang, Z. Dou, Y. Ma et al., "Effect of earlier initiation of antiretroviral treatment and increased treatment coverage on HIV-related mortality in China: a national observational cohort study," *The Lancet Infectious Diseases*, vol. 11, no. 7, pp. 516–524, 2011.

[40] S. Arreola, G.-M. Santos, J. Beck et al., "Sexual stigma, criminalization, investment, and access to HIV services among men who have sex with men worldwide," *AIDS and Behavior*, vol. 19, no. 2, pp. 227–234, 2015.

[41] S. Ellis, H. Curtis, and E. L. C. Ong, "HIV diagnoses and missed opportunities. Results of the British HIV Association (BHIVA) National Audit 2010," *Clinical Medicine*, vol. 12, no. 5, pp. 430–434, 2012.

[42] A. Palfreeman, M. Fisher, E. Ong et al., "Testing for HIV: concise guidance," *Clinical Medicine*, vol. 9, no. 5, pp. 471–476, 2009.

[43] B. Yang, S. K. Chan, N. Mohammad et al., "Late HIV diagnosis in Houston/Harris County, Texas, 2000–2007," *AIDS Care*, vol. 22, no. 6, pp. 766–774, 2010.