HIV Transmission Pattern among Men who have Sex with Men in Shenzhen

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ABSTRACT (English)

Abstract of this thesis entitled:

HIV transmission pattern among men who have sex with men in Shenzhen

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For the degree of: Doctor of Philosophy in Public Health

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Background

Men who have sex with men (MSM) are at high risk of HIV infection. In China, relevant studies concerning HIV infection in MSMs have been reported since 2000s, which showed a dramatic increasing trend of the prevalence of HIV infection. However, most of the previous investigations were based on convenience sampling and none of them differentiated money boys (MB) from other MSMs. In addition, there was no study that combined molecular epidemiological data with population survey using probability sampling methods. The objectives of this study was to use two approximate probability sampling methods to determine the prevalence of HIV infection and its risk factors among MSMs in Shenzhen, China; and to provide information from molecular phylogenetical analysis that better interprets the transmission pattern of HIV among MSMs.

Subjects and Methods

A total of 2143 MSMs were recruited in Shenzhen, including 1651 persons from time-location sampling (TLS) recruited in either 2008 (728 persons) or 2009 (923 persons), and 492 from respondent-driven sampling (RDS) recruited in 2009. A separate recruitment of MBs and other MSMs was applied in TLS surveys and a mixed sample of MSMs was applied in RDS. All subjects were interviewed and had blood tests for HIV and syphilis. Chi-square test was used to compare characteristics between different subgroups and multivariate logistic regression analysis was applied to identify risk factors for HIV infection. HIV positive samples were further used for

laboratory analysis including phylogenetic analysis, drug resistant analysis and recent infection test.

Results

The prevalence of HIV infection was 5.5% among MSMs in Shenzhen. A significantly lower HIV infection was observed in MBs (4.5%) compared with other MSMs (7.0%). The HIV infection rate among MSMs was 5.7% by TLS and 4.6% by RDS, which were not significantly different. Factors found to be related to HIV infection in MSMs included syphilis infection, occupation, sexual orientation, venue for recruitment and hometown prevalence of HIV infection. HIV infection rate and social-behavioral characteristics were found to vary with venues, with significantly higher rates in family clubs, parks and saunas, than entertainment venue, such as bars and massage centers. Molecular phylogenetical analysis showed that genetic clusters were related to receptive anal intercourse, short stay in Shenzhen, early age of first sex, and hometown with high HIV prevalence. Venue-specific transmission chains were observed in 60% of the subjects.

Conclusions

Approximate probability sampling methods (TLS and RDS) applied in this study found that the prevalence of HIV infection in Shenzhen MSMs was similar to the national rate in MSMs. HIV infection and related characteristics in MBs and other MSMs were found to be different, indicating that a separate analysis was necessary. TLS could provide information on venue comparisons for venue-based MSMs and RDS could recruit more hidden subjects. Furthermore, a combination of molecular epidemiological study and population survey could provide more solid information on HIV transmission among MSMs at each specific venue. Results from this study suggest that venue-specific intervention approaches should be developed and provided to different venues.

中 文 摘 要

介紹

男男性接觸人群（MSM）是艾滋病（HIV）感染的高危人群。中國從2000年開始出現MSM人群HIV感染狀況的相關報導，發現HIV感染率在該人群呈迅速上升趨勢。既往研究主要基於方便抽樣方法，沒有區分男男性工作者（MB）和其他MSM，也沒有一項研究將基於概率抽樣方法的人群調查與分子流行病學調查結合起來進行分析。本文的目的在於通過兩種近似概率抽樣方法研究中國深圳 MSM 人群艾滋病感染率及相關危險因素，並結合分子流行病學研究進一步了解艾滋病在男男性接觸人中的傳播模式。

對象與方法

本研究調查了 2143 名 MSM，其中，2008 年及 2009 年通過時間空間抽樣方法（TLS）分別調查 MSM 728 人和 923 人，2009 年同伴推動抽樣方法（RDS）調查 492 人。 TLS 方法中 MB 與其它 MSM 採取分開招募形式，而 RDS 方法中採取混合招募方式。所有被訪者完成調查問卷及抽血檢測 HIV 和梅毒。不同亞群間人群特性的比較採用卡方檢驗，HIV 感染相關因素分析採用多重 logistic 回歸分析。對 HIV 陽性樣本進行了系統學分析，耐藥分析以及新近感染檢測。

結果

深圳 MSM 人群 HIV 感染率為 5.5%。MB 人群 HIV 感染率(4.5%)明顯低於其他 MSM(7.0%)。TLS 方法招募的 MSM 艾滋病感染率(5.7%)與 RDS 的感染率(4.6%)無顯著差異。HIV 感染相關因素包括梅毒感染，職業，性取向，招募場所以及家鄉的 HIV 感染率。不同場所 MSM 人群 HIV 感染率以及相關社會行為特性有顯著差異：家庭會所，公園及桑拿場所 HIV 感染率明顯高於酒吧及休閒中心。系統學分析結果顯示基因簇與接受性肛交，在深居住時間短，首次性交年齡小，以及家鄉 HIV 感染率高有關。60%樣本形成場所內傳播鏈。

結論

通過兩種近似概率抽樣方法(TLS 和 RDS)發現深圳 MSM 人群艾滋病感染率居於全國平均水平。MB 與其他 MSM 在 HIV 感染及相關特性上均存在顯著差異，

應該將他們分別進行研究。TLS 可以提供場所型 MSM 的場所比較信息而 RDS 可以招募更隱蔽人群。此外，將人群調查與分子流行病學結合起來可以為各個場所內 MSM 之間的 HIV 傳播提供更可靠的信息。本研究結果提示我們應該為不同 MSM 場所提供針對性的干預方法。

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LIST OF ABREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
ART	Anti-retroviral Treatment
ARV	Anti-retroviral
BSS	Behavioral Surveillance Surveys
CAPI	Computer-assisted personal-interview
CASI	Computer-assisted self-interview
CDC	Center for Disease Control and Prevention
CRF	Circulating recombinant form;
DR	Drug resistance
ELISA	Enzyme-linked immunosorbent assay
EMB	Money boy working in entertainment venue
EOMSM	Other MSMs in entertainment venue
FA	Formative research
FMB	Money boy working in family club (a kind of home-based brothel)
FOMSM	Other MSMs in family club
FSW	Female Sex Worker
HIV	Human Immuneodeficiency Virus
HIVDR	human immunodeficiency virus drug resistance
IDU	Injecting Drug User
MARP	Most At Risk Population
MB	Money boys, a term used in China for male sex worker
MDR	Multi-Drug resistance
ML	Maximum Likelihood method
MSM	Men Who Have Sex With Men
MSW	Male Sex Worker
NGO	Non-government organization
NJ	Neighbor joining
NNRTI	Non-nucleotide reverse transcriptase inhibitors

NRTI	nucleotide reverse transcriptase inhibitor
OMSM	Other MSM except MSW, MSM who have not sold sex to other men
OOMSM	Other MSMs in venue outside Shenzhen Special Economic Zone
OR	Odds Ratio
PLWHA	People Living with HIV and AIDS
PMB	Money boy working in park
POMSM	Other MSMs in park
RDS	Respondent Driven Sampling
RDSAT	Respondent Driven Sampling Analysis Tool
RMB	Renminbi, Chinese currency
RPR	Rapid Plasma Reagin test, a method for testing current syphilis infection
RT	Reverse transcriptase
SOMSM	Other MSMs in sauna
STIs	Sexually Transmitted Infections
TLS	Time-Location Sampling
TPPA	*T.pallidum* particle agglutination test, a confirmation test for syphilis
UAI	Unprotected Anal Intercourse
UAVI	Unprotected Anal and Vaginal Intercourse
URF	Unique recombinant forms
UNAIDS	Joint United Nations Programme on HIV/AIDS
VDT	Venue-date-time
WB	Western blot
WHO	The World Health Organization

Chapter 1 Introduction

1.1. Background

Reports from several Asian countries, including China, Thailand, India, Vietnam and Japan indicate a rapid spread of HIV among men who have sex with men (MSM) in recent years [1-6] Injection drug users and former plasma donors have been the major contributors to the HIV epidemic However, recent data suggested that the major transmission route might have been shifted from injection drug use to sexual transmission.

1.1.1. Global HIV epidemic and Asia aspect

HIV/AIDS continues to be a major global public health issue, despite improved accessibility to anti-retroviral treatment and increased life expectancy. The UNAIDS and WHO AIDS 2009 epidemic update reported that 33.4 million people were living with HIV and 2 5 million people were newly infected with HIV in 2008 [7]. Although the number of newly infected cases and the prevalence were steadily decreased by year from 1996 (Figure 1-1), the number of people living with HIV continues to increase. AIDS-related illnesses remain to be one of the leading causes of death globally in the coming decades [8]

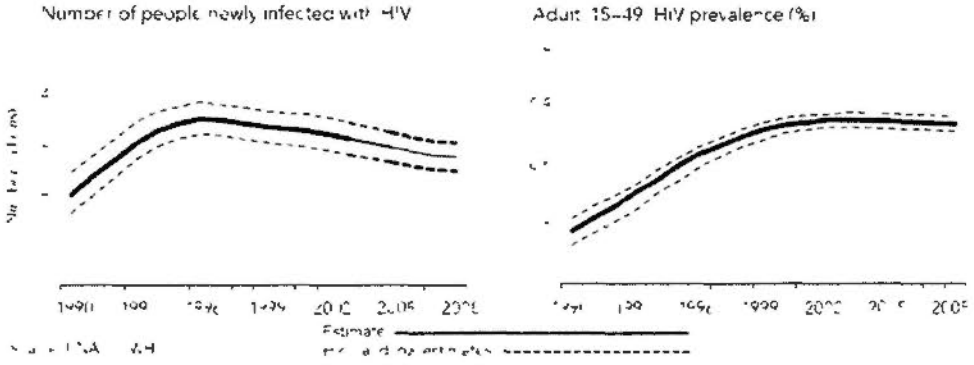

Figure 1-1 Global estimates 1990–2008 [8]

The nature of HIV epidemic varies considerably throughout the world The variations in prevalence of HIV infection and epidemiological patterns are not only

among countries and regions, but also within countries The distribution of HIV displays two main patterns generalized epidemics throughout the population in sub-Saharan African countries [9], and epidemics in other regions that are primarily concentrated among groups with high risk behaviors such as sex workers, men who have sex with men, injecting drug users, prisoners and mobile workers [7, 10] (Figure 1-2)

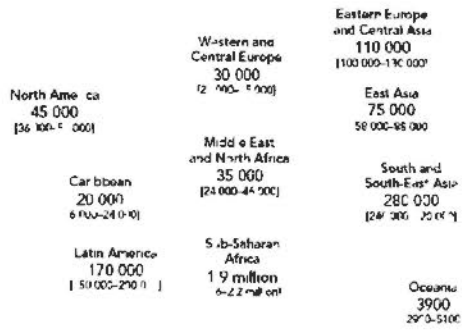

Figure 1-2 Estimated number of adults and children [7]
Total: 2.7 million (2.4 - 3.0 million)

Asia holds of 60% of the world's population and is the second to sub-Saharan Africa in terms of the number of people living with HIV It's estimated that about 4 7 million people in Asia were living with HIV, including 0 35 million who were newly infected in 2008 [7] India accounts for roughly half of Asia's HIV cases Thailand, Cambodia and Myanmar are considered to have generalized HIV epidemics, while epidemics in most of other Asian countries including Papua New Guinea (Oceania country), China, Indonesia, Vietnam and Malaysia are still regarded as concentrated in specific populations, namely injecting drug users [11], sex workers and their clients [12], and men who have sex with men [2-6, 13, 14] There are only a couple of countries with low level of HIV epidemics including Mongolia, Lao PDR, Philippines, et al However, the epidemics in many parts of Asia are steadily expanding into lower-risk populations through transmission from those most at risk to their sexual partners [15, 16], indicating that sexual transmission plays a crutial role in HIV epidemics The reasons of the variation in the spread of HIV in Asia

might be the behavioral factors and the timing of HIV introduction into the most at risk populations [15, 17] (Figure 1-3)

Figure 1-3 An expanding epidemic in Asia [18]

1.1.2. HIV Epidemic in China as well as in Shenzhen

Since the first case found among foreign tourists in 1985, HIV-1 has spread to all different places of mainland China By the end of October 2009, the cumulative reported HIV cases was 319,877, including 102,323 AIDS cases and 49,845 recorded deaths According to official estimates, there were about 740,000 HIV/AIDS cases in China in 2009 The estimated number of AIDS cases was 105,000 and the estimated newly infected HIV cases in 2009 was 48,000 [7, 19, 20] The annual reported HIV cases were continuously increasing in the recent years (Figure 1-4)

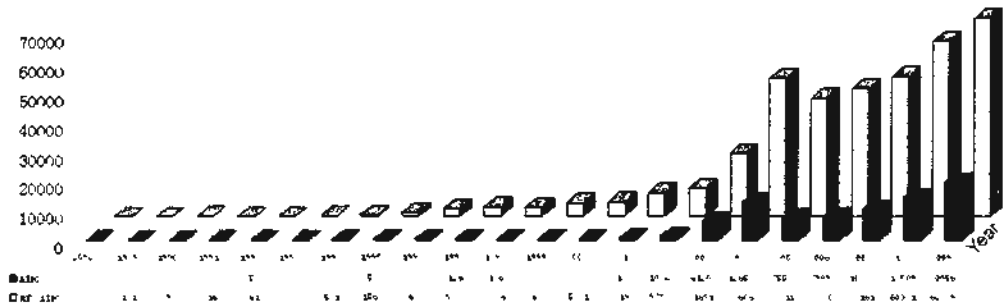

Figure 1-4 The Annual reported HIV positives and AIDS cases in China 1985-2009 [21]

The geographic distribution of HIV epidemics in China is unequal The cumulative number of people living with HIV in Yunnan, Henan, Guangxi, Xinjiang, Guangdong and Sichuan accounts for 80 5 per cent of the total reported cases in China (Figure 1-5) [19] The distribution of annual reported HIV cases by transmission routes showed that a trend of increasing sexual transmission including

homosexual transmission was observed in the past few years [21]

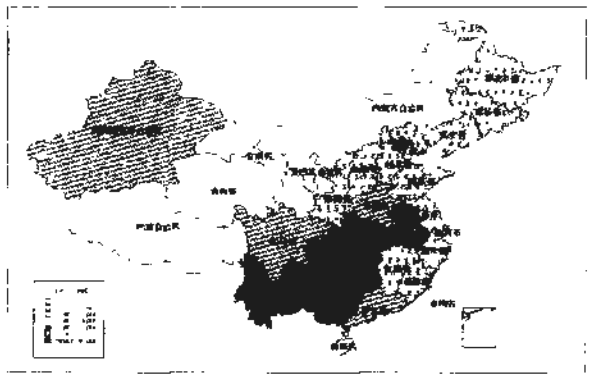

Figure 1-5 Geographic distribution of cumulative reported HIV positives in China (as of October 2007) [19]

Shenzhen is located in the southern tip of China, and shares a border with Hong Kong Shenzhen is China's first Special Economy Zone (SEZ) and is a pioneer and innovative city with a total area of 2,020 square kiolmeters. In its short history of 30 years, Shenzhen has developed from a small fishing village into an international metropolis with a premier gross domestic product (GDP) per capita ranking in China This is a remarkable achievement, notably in the annals of urbanization, industrialization and modernization across the globe. Shenzhen has the highest quality of life index in China. The population of Shenzhen excess 14 millions, among them, more than 85% are internal migrants from inland China, and most of them come from rural places Along with the national trend, the HIV epidemic is also increasing in Shenzhen by year (Figure 1-6)

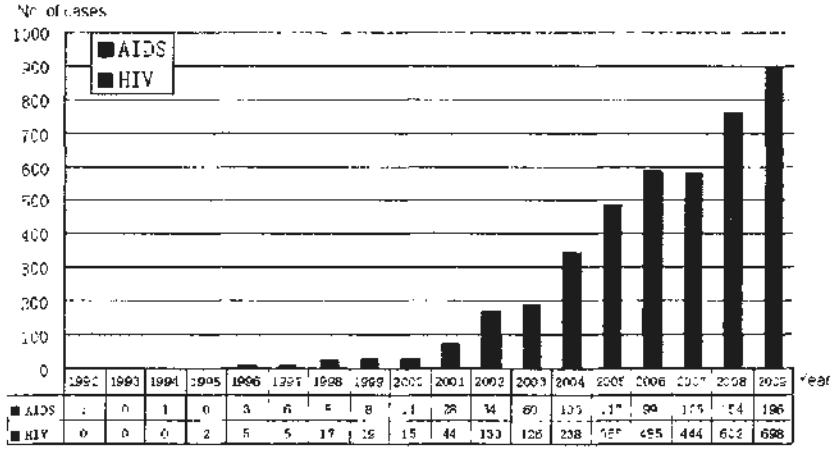

Figure 1-6 Number of people newly diagnosed of HIV positive in Shenzhen, 1992-2009 (data from Shenzhen CDC)

1.1.3. Men who have sex with men (MSM) is a major concern in China for HIV epidemics

Based on newly diagnosed HIV cases in 2009, a national report estimated that 42 2% of HIV infection was transmitted via heterosexual contact, and 32 5% via homosexual contact [20] While only 12 2% HIV infection was mediated via homosexual contact in 2007 and even lower percentage was reported in previous years [18] In some cities, the reported prevalence of HIV infection ranged from 0 5% to 12 5% among MSMs in the last two years [22-31] A dramatic increase in prevalence of HIV infection has been found among MSM in Beijing, from 0 4% in 2004 to 5 8% in 2006 [30] These studies also showed that the prevalence of other sexually transmitted infections (STIs) was high among MSM in China [22-31]

1.1.4. High proportion of migrants and male sex workers were the key characteristics of MSM population in Shenzhen

Shenzhen, a thriving city in southern Guangdong province, is located just north of Hong Kong Shenzhen was an ideal place for analyzing the HIV-related risk factors among MSMs in China because of its special geographic location, economic status and culture background As the first and the most successful Special Economic Zone (SEZ) of China, Shenzhen attracted more than 12 million internal migrants These migrants accounted for 87 0% of lotal population, and had a mean age of 26 [32] In addition, more than 210,000 travelers cross between Shenzhen and Hong Kong every day [32] The rapid development of market economy and the continuing influence of Western culture provided more personal freedom, privacy, and individuals' rights than any other cities in Mainland China The situation of Shenzhen obtained wide societal acceptance The populace of Shenzhen, especially the young, tends to be more open-minded than people from inland China in accepting new ideas and behaviors, including homosexuality

Consequently, the MSM population has grown rapidly over the past two decades in Shenzhen [24] According to the estimates conducted by the Shenzhen Center for

Disease Control and Prevention (CDC) in 2006, there were about 65,860 and 96,600 MSM lived in Shenzhen [33] Many of them frequently visited MSM venues like bars, massage centers, saunas, family clubs, parks and also parties The total number of MSM venues was more than 50 in 2006, when Shenzhen CDC started to estimate the MSM population size and venues [33] The number increased by year In saunas and bathhouses, MSM usually take a bath and contact others at the same time before engaging in sex In bars, MSM talk, drink, dance and watch shows performed by other MSM and then engage in sex with other MSMs or male sex workers (also called "money boy" in China, MB) in private rooms, toilets, hotels or at their homes Massage center is a kind of commercial sex clubs providing under-table commercial male-male sex services to clients besides massage or gym services Family club is the smallest unit of in-door commercial service agencies The managers or pimps rent apartments and recruited several MBs serving as call boy Park is a kind of public areas that provide MSMs a free opportunity to contact with other MSMs or MBs MSM generally search for information of MBs through Internet and call for commercial services at hotels or their homes [34] They also use Internet as a means of connection with other MSM

1.1.5. Money boys need to be stressed in MSM epidemics

Compared with other MSMs, MBs are more likely to have paid and unpaid sex with multiple partners, engage in risky sexual behaviors, frequently use illicit drugs, and thus are more likely to be infected by HIV [35-55] Their sexual partners include homosexual, heterosexual, and bisexual identified men and women MBs are more likely to self-identify as bisexual or even heterosexual besides homosexual/gay This characteristic makes them a core transmitter group for the MSM and the general population They may act as bridging population and are able to transmit HIV between the heterosexual and homosexual population, and from the risk population to the general population

It is estimated that there are around 4,000-5,000 MBs in Shenzhen (internal data

from Shenzhen CDC) Most of them work at venues like family clubs, bars and massage centers that controlled or managed by a boss or employer (pimps), while there is still a significant proportion of MBs who work on their own, finding clients in MSM activities or public areas such as parks or advertising themselves through Internet

Although one study had reported the behavioral characteristics of MBs in Shenzhen based on a community-based sampling strategy [29, 56], no serological data was collected and no specific question issued the relationship between Shenzhen MBs and Hong Kong MSMs was answered A well designed and carefully operated survey needs to be conducted including both the MBs and non-MB MSMs and the history of their sexual contact with Hong Kong MSMs

1.1.6. Probability sampling method should be considered in investigating the prevalence of HIV infection and HIV-related risk factors in MSMs

Annually MSM surveillance conducted by Shenzhen CDC has been started from 2002, and the surveillance found that the prevalence of HIV and syphilis among MSM has increased respectively from 0 9% and 9 4% in 2002 to 2 7% and 15 8% in 2005 [57], and further increased to 3 7% and 18 2% in 2008 [58] But all the surveillances were based on non-probability sampling methods including convenience sampling and snowball sampling, and no Hong Kong related data was obtained from the questionnaires Because of the high mobility of the whole population including MSMs, cohort study could hardly be conducted in the city It is accepted worldwide that time-location sampling (TLS) and respondent-driven sampling (RDS) are two approximate probability sampling methods suitable for sampling hard-to-reach populations For Shenzhen, a cross sectional strategy to recruit MSMs by using TLS and RDS should be considered In addition, most of previous studies on MSM mixed the MSMs with both MBs and other MSMs (OMSM) [27, 30, 59-61] Although many literatures reported significant differences between MBs and other MSMs in other countries [29, 39, 52, 62-64], no specific

differentiation and comparison of MBs with OMSMs was reported in China including Shenzhen

1.1.7. No cross-border HIV transmission was found by molecular epidemiology studies though frequent cross-border MSM activities were observed

Many venues in Shenzhen are famous in MSM communities in China including Hong Kong Lau's group found that 15 2% of the Hong Kong MSMs had engaged in sexual activity with men in Mainland China, most of them were Shenzhen residentes The "cross-border MSM" were more likely than others to practice risk behaviors such as patronizing male commercial sex workers, contracting a sexually transmitted infection, having more than 2 MSM partners, and having higher proportion of recent HIV testing [65] Although the active cross-border MSM behaviors have been observed, no HIV strain linkage has been found between Shenzhen and Hong Kong A Shenzhen-Hong Kong cooperation project on HIV molecular epidemiological study showed that HIV positive MSM groups were clustered into 3 clusters in both cities, but no overlap and crossover between the Shenzhen clusters and Hong Kong clusters was observed [66-68] The contradiction between the results from epidemiological survey and the molecular epidemiological analysis could hardly be explained only by data from Hong Kong The in-depth analysis of Shenzhen situation might be even more important for understanding the behavioral and molecular patterns of cross-border HIV transmission, since almost all cross-border sexual behaviors are practiced in Shenzhen This in-depth analysis should contain a detail clarification of the characteristics of both MBs and other MSMs by a scientific sampling method and a relevant molecular analysis with HIV positive samples

1.1.8. Rationale of the study, aim, and objectives

Knowing the epidemic among MSM and their response, as well as identifying the varieties in behaviors and other factors related to HIV infection among different subgroups of MSM can help health care providers to craft an optimal effective response [7] The aim of the study is to determine the characteristics of HIV infection

among MSMs in Shenzhen, China by approximate probability sampling methods, and to better interpret the transmission pattern of HIV among MSMs by molecular phylogenetical analysis

There are four main objectives of the study

To determine the characteristics of HIV and syphilis infections in MSMs in China using data collected from Shenzhen with time-location sampling (TLS) method and respondent-driven sampling (RDS) method

To compare the predictors and correlates for HIV infection among MBs and other MSM, and to identify the subgroups in MSM population that had higher HIV risks or had more contact with Hong Kong MSMs

To verify the representativeness of samples recruited from TLS by RDS recruitment, and to determine the sampling characteristics and coverage of the two methods

To describe the molecular evolution pattern of HIV and genetic clusters among HIV positive MSMs in Shenzhen, and to identify the HIV transmission pattern among MSM in combination with the epidemiological data

1.2. Layout of the dissertation

The thesis was consisted of an epidemiological survey and a molecular epidemiological study The epidemiological study was designed to investigate the biological, demographic, behavioral, and knowledge-related information of MSMs in Shenzhen by two different sampling methods Time-location sampling surveys on MSMs were conducted in year 2008 and 2009, respectively Each of the TLS survey was a combination of separate recruitments of male sex workers and other MSMs In addition, a respondent-driven sampling study on MSM was also performed in 2009 It combined the MBs with other MSMs in order to evaluate the proportion of MSMs visiting MSM venues as well as the percentage of MBs among MSMs All HIV positive samples collected from the epidemiological study were further prepared for a molecular epidemiology assay, including subtyping, drug resistance analysis, recent

infection and phylogenetic analysis

Chapter 1 is an introduction of the thesis It is an overview of the background, aim and thesis structure

Chapter 2 is a literature review on relevant topics The global HIV epidemic situations as well as the situation in China are firstly introduced Literatures on the prevalence of HIV infection and relevant risk factors among MSMs including MBs around the world and in China are summarized

Chapter 3 presents the epidemiological survey that was conducted in both 2008 and 2009 with two different sampling methods time-location sampling and respondent-driven sampling

Chapter 4 shows the molecular epidemiological analysis, recent HIV infection, HIV genotype and drug resistance determination

Chapter 2 Literature Review

2.1. HIV and Men who have sex with men

The term "men who have sex with men" or MSM in this thesis is refer to the extraordinarily varied communities of men found throughout the world MSM focuses on sexual behavior rather than on identity Men grouped under this term have different experiences, lifestyles, identities, and behaviors In Asian culture men do not tend to self identify as either gay or homosexual So the term MSM is the general term in attempt to be inclusive of all men who have sex with men, regardless of how they see themselves

It is estimated that at least 5-10% of HIV infections worldwide are the result of homosexual activities, although the proportion of cases attributed by this mode of transmission was massively varied among countries [7, 69] After years of intervention and education efforts focusing on high risk populations, the transmission of HIV among IDU, sex workers and blood recipients has been successfully controlled in many countries, while homosexual contact among MSM is still a prominent mode of HIV transmission in most of the developed countries, and it is becoming a big problem in some developing countries [70] The US CDC presented their data at the 2010 National STD Prevention Conference and showed that MSMs were at least 44 times more likely to be diagnosed with HIV infection than other men, and 40 times more likely than women [71] They were also 46 times more risky for syphilis than other men and 71 times more than women for sexually transmitted diseases According to the CDC report, gay men, bisexual men and other MSMs accounted for 53% of new HIV/AIDS cases in the United States in 2007, even though they only accounted for 2 8 to 5 3% of the U S male population [71] Recent data also showed that French gay men faced sixty-fold higher HIV risk than other men [72]

The social acceptance of MSM varies greatly worldwide In many countries, the

MSM practice remains hidden as it is taboo or even illegal Nowadays, although many years of HIV-related education and services has been provided to MSM population, countries with friendlier attitudes to MSM still have severe HIV epidemics among them (i e US, Western Europe) In homophobic countries (e g Africa and China), the HIV infections in gay men could be even worse [73] They have around 33% of new HIV infections being found in gay men, which was a significant increase during the past few years and indicated a potential risk of HIV pandemic among MSM population [73] The lack of acknowledgment of sex between men means preventive messages may focus largely on heterosexual sex With little or no accurate information about MSM and male-male sex, MSMs in these countries may believe that they are not at risk In addition, MSMs, especially commercial sex workers, may be reluctant to access healthcare services for HIV and STIs screening and treatment due to the fear of arrest, fines and/or humiliation and discrimination from insensitive health care workers Additionally, they may have difficulty in accessing lubricants and condoms, both of which are essential in reducing HIV transmission during anal sex, especially in settings where this practice is not acknowledged Male commercial sex workers may not have much power in negotiating condom use with their clients or they may make an economic choice to have unprotected sex for higher payment [74-76]

Some behaviors in MSM may increase their vulnerability to HIV Multiple sex partners and buying or selling sex increases the likelihood of coming into contact with HIV infected persons [30, 77-79] Lack of condom and lubricant use add to the likelihood of HIV infection [22, 50, 80, 81] Drug and alcohol use, was common in some settings where MSM socialize themselves, also influence decision-making about condom use and choice and number of partners[78, 82-84]

The Commission on AIDS used the Asian Epidemic Model (AEM) to construct a picture of HIV epidemic in different sub-populations in Asia over the years (Figure 2-1) The model shows that Asia is on the brink of a large increase of new HIV

infections among MSMs, if risk behavior stays at current levels with low condom use and many concurrent male partners. If efficient intervention strategy can be implemented to achieve a >80% condom use in anal sex among MSMs, the epidemic in this high risk population can be successfully controlled [69]. However, an efficient intervention strategy should be based on the clear image of the epidemic within this high risk population. The detail characteristics of each subgroup of MSMs as well as the preference of certain health services should be issued in behavior survey, and the transmission pattern of HIV should also be understood before a suitable intervention is effectively distributed.

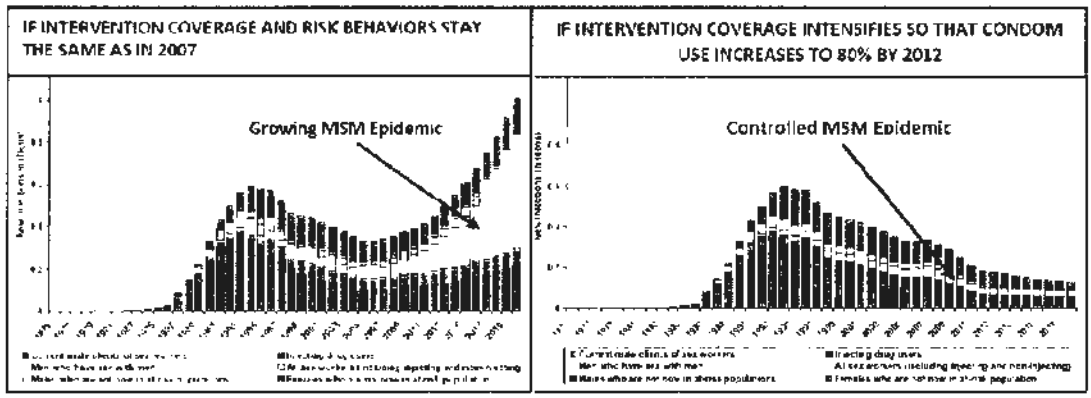

Figure 2-1 The Asian Epidemic Model (AEM) estimate the HIV epidemic in Asia with or without intervention among MSM [69]

2.1.1. MSM in China

The prevalence of HIV infection has been increasing in MSM in Asia, which raised the concern that this trend may prevail in China. The distribution of annual reported HIV cases by transmission routes showed that a trend of increasing sexual transmission including homosexual transmission was observed in the past few years [21] (Figure 2-2).

HIV positive rates among MSMs were ranged from 0.9% to 10.8% in different cities of China [23, 58, 85-87], and even higher in particular MSM subgroups (e.g. as high as 26.5% of the MSMs recruited from bathhouses and saunas were HIV positive in Chongqing) [60]. Until 2006, the homosexual transmission only accounted for 0.6% of the accumulated HIV cases. But the situation changed rapidly since 2007. Among

the estimated new infections during 2007, 12.2% was accounted for homosexual transmission; while in 2009, the percentage increased dramatically to 32.5 % [19, 20].

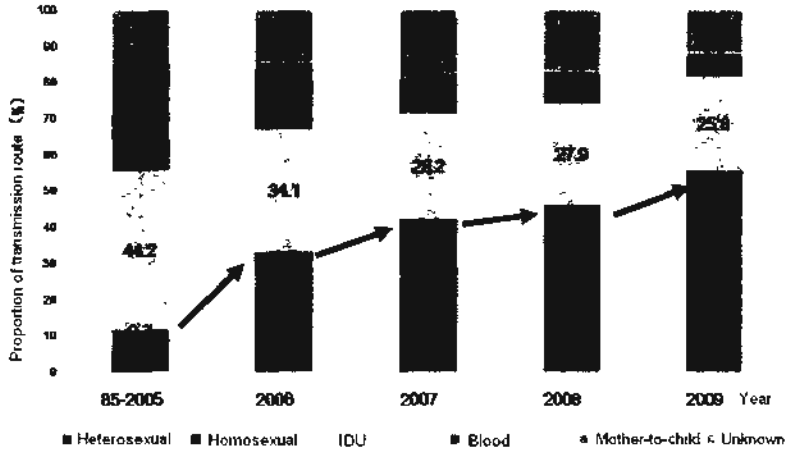

Figure 2-2 Proportion of transmission route in reported HIV positives in China by year [21]

In accordance with the variation of HIV prevalence in different areas in China, the distribution of HIV among MSM in China is also unequal. According to National MSM surveillance in 2008 covering 61 cities in China, the prevalence of HIV infection ranged from lower than 1% to higher than 10%. Although the average HIV prevalence in China was 4.9%, there are 4 provinces had a higher than 10% prevalence. All of these 4 provinces (Sichun, Chongqing, Guizhou and Yunnan) are located in southwest China, a poor region with very high population density[88].

Besides the unequal distribution of HIV infection, an increasing trend of prevalence of HIV infection was observed among MSM in both the high HIV prevalence areas and low prevalence areas in China within the past few years. It was found that the prevalence of HIV infection among MSMs increased from 0.4% to 5.8% between 2004 and 2006 in Beijing [30], 0.05% to 3.1% from 2006 to 2007 in Jinan [23], 19.7% to 26.5% from 2006 to 2007 in Chongqing bathhouse MSM [60], and 0.9% to 3.8% from 2004 to 2008 in Shenzhen [56-58].

Factors that contribute to the acquisition of HIV in MSMs were needed to be examined specifically in the context of China It includes biological, social behavioral and knowledge-related factors Besides the social-economic status which was well documented worldwide, a couple of behavioral factors were also found to be correlated with HIV infection For MSM in particular, unprotected anal intercourse (UAI) [30], multiple sexual partners [30, 89, 90] and receptive anal intercourse [90] were independently associated with HIV infection However, many reports documented high levels of above-mentioned risky sexual behavior, similar to those found in Western countries 54 7% of the MSMs in Guangzhou practiced unprotected anal intercourse with other men [28] The percentage was 46% in Jiangsu [91], 57% in Shanghai [5], 49%-55% in Beijing [59, 92], and 54 7% in Yunnan [93] In addition to unprotected sex, the number of male sexual partners among MSMs is also an important factor Ruan [94] and Zhang [90] both found that more than 10 lifetime male sex partners was associated with HIV sero-positivity, while Choi reported that sero-positivity was associated with having more than 20 male sexual partners [74]

Due to the stigmatization of homosexual and the culture in China that focused on procreation, most MSMs are forced to conceal their sexual orientation and marry with a woman Nearly one third of the MSMs have ever been married [23, 89, 93] This finding provides insight into the potential risk of HIV transmission from the high risk MSM population into the general population through heterosexual contact

2.1.2. MSM in Shenzhen and Hong Kong

MSM population in Shenzhen has grown rapidly over the past two decades [24] According to an estimate made by Shenzhen CDC in 2006, there were about 65,860 to 96,600 MSMs lived in Shenzhen [33], with many of them frequenting venues like bars, massage centers, saunas, family clubs, parks and also parties In saunas and bathhouses, MSMs take bath and contact with others before engaging in sex In bars, MSMs talk, drink, dance and watch shows performed by other MSMs and then

engage in sex with other MSMs or male sex workers (also called "money boy" in China, MB) in private rooms, toilets, hotels or at their homes Massage center is a kind of commercial sex clubs, providing under-table commercial male-male sex services to clients besides massage or gum services Family club is the smallest unit of in-door commercial service agencies The managers or pimps rent the apartments and recruited several MBs serving as call boy Park is a kind of public areas that provide MSMs a free opportunity to contact with other MSMs or MBs MSMs generally search MBs' information through Internet and then call for commercial services at hotels or at their homes [34] They also use Internet as a means of connecting with other MSM

The major differences about MSMs among Shenzhen and other cities in China including Hong Kong are (1) high proportion of internal migrants among Shenzhen MSMs who were coming from other places of inland China Most MSMs observed in Shenzhen have only lived in this city for no more than half a year [57, 58], and (2) high proportion of money boys (MBs) among Shenzhen MSMs, especially those observed in MSM venues [57, 95]

Along with the national trend, the HIV epidemic is also increasing in Shenzhen by year (Figure 2-3, data from Shenzhen CDC annual reports) Sexual transmission has become the major transmission route for HIV infection since 2007, which accounted for 51 8% of the newly reported cases in that year Among the 894 newly reported cases in 2009, 67 2% were infected through sexual transmission, with 27 5% infected through homosexual contact An increasing trend of HIV infection among MSMs compared with other risk groups was observed, the proportion of MSMs among annual newly reported cases was increased year by year from 1 4% in 2004 to 3 9% in 2005, 5 7% in 2006. and 15 7% in 2007, then 23 3% in 2008 and 27 5% in 2009 (Figure 2-3) The proportion of MSMs among annual HIV cases in Shenzhen was similar to the estimated proportion (12 2% in 2007 and 32 5% in 2009) of MSMs in all estimated HIV cases, and it was significantly higher than the proportions found in

other cities as well as the national level (3 4% in 2007 and 8 6% in 2009)

Shenzhen Center for Disease Control and Prevention (CDC) has started annual MSM surveillance from 2002, and found that the prevalences of HIV and syphilis among MSM have increased respectively from 0 9% and 9 4% in 2002 to 2 7% and 15 8% in 2005 [57], and to 3 7% and 18 2% in 2008 [58] (Figure 2-4) The increase in both HIV and syphilis prevalence was significant However, most of these data were come from convenience sampling methods

Shenzhen's neighbor, Hong Kong also reported a slightly increasing trend in HIV infection, with 4 05% in 2006 and 4 35% in 2008 among community-based MSMs [96-98] In addition, the number of annually diagnosed HIV positive MSMs was also significantly increased during the past 10 years according to the annual HIV reports from Center for Health Protection in Hong Kong [96] (Figure 2-5) Homosexual contact was continuously the major transmission route in Hong Kong from 2007

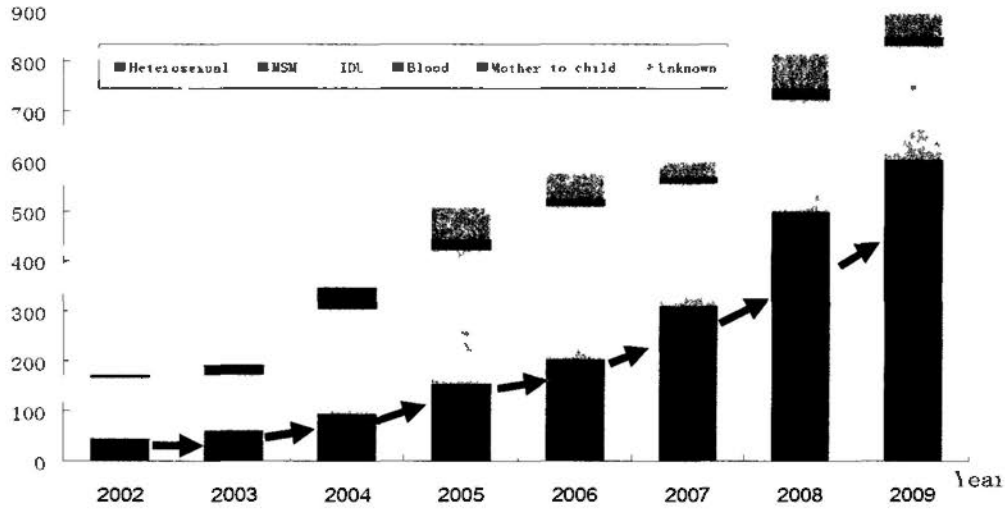

Figure 2-3 Distribution of annual new infections by mode of exposure in Shenzhen, 1992-2009 (data from Shenzhen CDC)

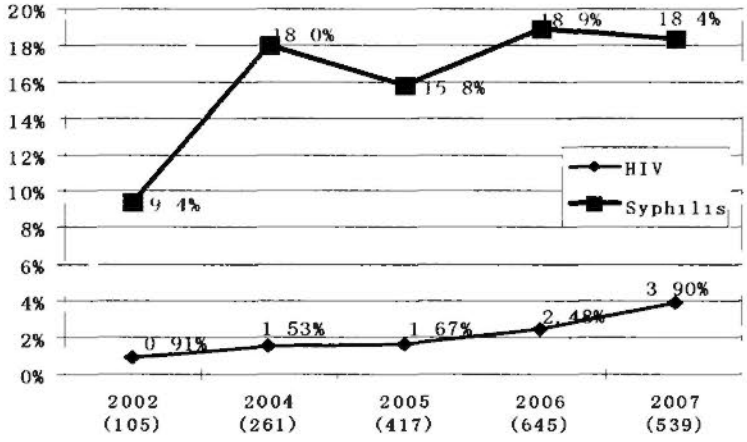

Figure 2-4 HIV and syphilis infection among MSM in Shenzhen, 2002-2007 [58]

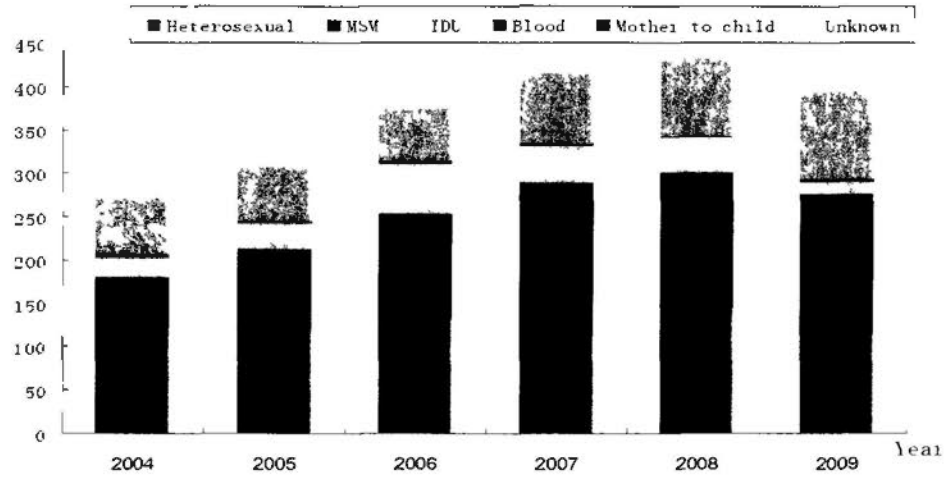

Figure 2-5 HIV reports in heterosexual men and MSM in Hong Kong, 1995-2007 [96]

2.1.3. MBs in China as well as in Shenzhen

The MSM studies provided some baseline information about the sexual risk behaviors of MSM in China, but they only reflected a certain subpopulation of MSM, namely well-educated urban men who access certain entertainment MSM venues like bars and saunas Our knowledge about MSMs is limited as they are difficult to reach and less visible, such as Internet-based MSM, MSM frequenting other MSM venues (like parks or small family clubs) and male sex workers (money boys) Male sex workers (MSW) as a subset of MSM have been studied for over 20 years since HIV/AIDS has become a major health concern in the MSM community Previous studies in some developing countries indicated that MSWs have risky behaviors and are vulnerable to HIV/STI infections [36, 39, 99, 100] A survey found an HIV infection rate of 33% among MSWs in Mumbai, India [100], which is significantly higher than the prevalence of 17% among MSMs [101] A longitudinal study reported an HIV infection rate of 16% at their baseline and an incidence rate of 4 8 per 100 person- years during a 6-month follow-up in Moscow, Russia [36] A 10-year follow-up study reported a 9% baseline HIV infection rate and an incidence rate of 3 7 per 100 person years in London, England [48] These literatures indicated that the main factors associated with MSWs' risk behaviors include age, education level, duration of sex work, migration patterns, financial status, drugs and alcohol intake, sexual orientation, being receptive during anal intercourse, and STIs history [64, 99, 100, 102-104]

In China, male sex workers who typically sell sex to MSM (and sometimes women) are called money boys (MB) In order to avoid confusion with the male sex workers who only sell sex to women, we use the term "money boys" in the thesis So far, there has been little information about the prevalence of HIV and risk factors among money boys in China Few reports have targeted MBs as study subjects [24, 26, 75, 105], while no biological data is available to address the issue However, MBs may

serve as a bridge for the HIV/STIs epidemic by spreading HIV to the general population [49]

Commercial sex trade in China is illegal, but sex services are available almost everywhere, especially in liberal cities such as Shenzhen [10] MBs, as a hidden and marginalized group, are very difficult to reach The Chinese government has recently launched a large-scale counseling and testing program targeting MSM, which involves many MSM non-governmental organizations (NGOs) Thus, more MBs are being reached for public health purposes than before A thorough search of the literature reported 3 quantitative surveys and one qualitative study about MBs [26, 75, 102, 105-107] They reported that MBs have limited knowledge of AIDS/STIs, have both male and female sex partners, have a high prevalence of risky sexual behaviors, and are in need of intervention

2.2. Sampling Method for Most at Risk Populations (MARPs)

In most countries outside of sub-Saharan Africa, the HIV infection is concentrated in three subpopulations men who have sex with men, injection drug users, and sex workers and their sexual partners [7] Consequently, it is commonly accepted that data of disease prevalence and risk behaviors within these most at risk populations (MARP) are critical for understanding and controlling the spread of the disease [108]

Unfortunately, due to the lack of appropriate sampling frames for these populations, relatively small number of cases and cases being anonymous, MARPs are difficult to study with standard sampling methods For this reason they are often called "hidden" or "hard-to-reach" population A variety of sampling approaches have been tried to study these hidden populations, but they produced estimates with unknown bias and variances in many cases [108, 109] The resulting uncertainty about key subpopulations has complicated public health efforts to evaluate prevention programs and effectively allocate resources

2.2.1. Types of Sampling Used among MARPs

Because most people are at very low level of HIV risks, general population surveys (e g household surveys or national censuses) typically do not obtain enough samples of interest Additionally, stigma related to high-risk populations inhibits individuals from disclosing their risk behaviors Alternative sampling methods designed to access these populations have been developed, which was applied by researchers and public health providers to collect useful data for approximate representative samples A number of sampling methods have been implemented for behavioral surveillances and researches among hard-to-reach populations

• Snowball Sampling It is a long-chain referral sampling started from a set of individuals (named "seeds") to recruit their peers, and then the recruited participants continue to recruit the next The chain of recruitment continues for several waves until the sample size is reached It is a non-probability sampling method

• Institution Based Sampling recruiting population members from a variety of facilities (e g drug treatment centers, STI clinics) frequented by members, which come with institutional bias [108]

• Key informant sampling the method to overcome response biases by selecting especially knowledgeable respondents and asking them about others' behavior, rather than their own The method reduces the tendency to exaggerate socially acceptable behavior and understate disreputable behavior, while add some other bias including professional orientated and institutional bias, lack of sufficiently detailed knowledge and lack of interaction with a random group of potential clients

• Respondent Driven Sampling the sampling method that relies on social network properties to sample hard-to-reach populations It is a modified long-chain referral sampling (or snowball) each individual recruits his or her peers into a study The recruitment links are tracked of who recruited whom as well as their numbers of

social contacts The data is analyzed by using statistical adjustments for network size to produce generalizable samples [110-112]

• Time Location Sampling This is a sampling method that recruits individuals from specific locations during specific time periods Time-location sampling can be a probability sampling based on the accuracy of the sampling frame to include all the venues and the size of the target population that frequents these venues Time location sampling is also referred as venue-based sampling, venue-time-based sampling, time-location cluster sampling, and time-space sampling [113]

• Targeted Sampling This sampling method recruits individuals from specific locations They map a target population and recruit a pre-specified number of subjects at sites identified by the ethnographic mapping, ensuring that subjects from different areas and subgroups will appear in the final sample Time-location sampling is an example of advanced targeted sampling

Among them, TLS and RDS were widely accepted as two approximate probability sampling methods for reaching hard-to-reach populations To any sampling method, the most important thing is the method's ability to potentially reach all members of the priority population In addition, it is crutial to follow a standardized protocol to help ensure reproducibility over time and comparability across locations [114]

2.2.2. Time-location sampling (TLS)

2.2.2.1. Overview

Sampling hard-to-reach populations has been a long and challenge topic, particularly in the AIDS era Venues where MARPs are known to congregate could help to increase the efficiency of obtaining a complete sample while retaining assurance that the target population was included Early efforts to efficiently identify and recruit MARPs using venues was made by Watters & Biernaki [109] Their research among IDU was designed to overcome limitations found in convenience sampling such as ensure inclusion of diverse subpopulations Enhanced targeted sampling was the

addition of estimation of density of targets in the target areas and the introduction of proportional sampling quotas [115]

TLS draws on this history in the use of venues and ethnographic understanding of the priority population but also adds venue-day-time (VDT) based random selection [116] VDT sampling takes advantage of the fact that MARPs attend a universe of venues at identifiable and specific days and times TLS combines the VDTs and a thorough formative research phase This ensures that a high proportion of venues attended by a priority population are included in the sampling frame Random selection of VDTs adds substantial rigor to the method by reducing selection bias Consecutive sampling VDT further reduces selection bias by not picking and choosing venues The TLS is currently the most representative sampling method for recruiting hard to reach populations, especially those subgroups who are frequenting venues

TLS is held to approximate random cluster sampling where everyone attending the cluster (venue) has an equal chance of inclusion and are sampled as a group Members of the target population have approximately equal or somewhat known chances of being sampled by randomly selecting venues, days and times where they can be found The equal chance is achieved by randomly sampling enough places and times (Table 2-1)

Table 2-1 Strengths and Limitations of TLS

Strengths	Limitations
• Assumed representativeness by approximating random cluster sampling	• Need complete "map" of venue-day-times
• Efficient for rare or hard to reach populations	• Somewhat hard to validate
• No need for a complete roster of individuals in a MARP	• Bias towards those who attend venues. leaves out those who never or rarely attend

However, the weakness of TLS is also obvious unless at least a very high percentage of venues are identified and a very high percentage of subgroup members visit such venues Another potential source of bias is the non-response bias rose from the

nature of recruitment sites Besides the above mentioned sampling bias, people in venues may not participate the survey at those times and locations [108] TLS is probably suitable for producing probability samples of the population that attend venue-day-time segments, if only they could not represent the whole population If frequent gay venue visitors were more likely to engage in high-risk sexual behaviors [117], HIV prevention research among MSM would better use TLS for the purpose of analyzing more risky subgroups [118]

TLS provides eligibility and response rates for the consideration of representativeness and for the adjustment of weighting in statistic analysis The statistical methods for TLS are well established - cluster sampling of venue - time clusters, and even without adjusting the cluster of venue and event, it is still reliable for representing the samples [116, 119-121]

2.2.2.2. Implementation of TLS

TLS typically includes 6 steps as (1) Formative assessment, (2) Venue Universe / Sampling Frame Construction, (3) Random selection / Sampling Calendar Creation, (4) Sampling events / recruitment, (5) Laboratory testing and results return/ Referrals, and (6) Data management and analysis

A complete venue universe is the fundamental key to the methodological rigor of TLS and the basis for TLS to be considered as a quasi-probability sampling method The venue universe is both a tool to form understanding of the geography and diversity of the priority population and serves as the sampling frame for TLS

2.2.2.3. Application of TLS

TLS was first used for behavioral surveillance among MSMs in the United States in the early 1990's [119, 122] Since then, TLS has been implemented to analyze a wide variety of MARPs with diverse settings in many countries over the world [104, 113, 123-130] A very successful application of TLS among MSMs is the HIV behavioral surveillance among MSMs in Bangkok, Thailand They detected a previously

unknown high level of HIV infection among Thai MSMs and showed an alarming trend in HIV infections (17% to 23% and to 31% over 2 years interval from 2003-2007) [120, 131] Currently TLS is the standard method for HIV behavioral surveillance among MSMs in the United States [132]

2.2.3. Respondent-driven sampling (RDS)

2.2.3.1. Overview

Respondent-driven sampling (RDS) represents an advance in sampling methodology because it resolves the previous intractable dilemma of the lack of coverage when a study was focused on the most accessible part of the target population Most location-based sampling surveys have no reliable way to determine the representativeness of their samples to the targets The other horn of the dilemma arises if priority is placed on coverage rather than statistical validity Network-based methods, also known as chain-referral and snowball sampling, can provide comprehensive coverage of the target population It asks individuals to refer those they know, these individuals in turn refer those they know and so on Based on the principle of "six degrees of separation", this approach could potentially reach any member of a population in only six waves, so total coverage is possible, at least theoretically [133] However, this approach is prey to a host of biases Well-connected individuals tend to be over-sampled as many recruitment paths lead to them, so network-based sampling is based on anything but random[134]

RDS is a chain-referral sampling technique which uses statistical adjustments for network size to produce generalizable samples It shows that the breadth of coverage of network-based methods can be combined with the statistical validity of standard probability sampling methods An RDS study begins by recruiting a small number of people in the target population to serve as seeds After participating, the seeds are asked to recruit other people that they know in the target population The sampling continues in this way with current sample members recruiting the next wave of sample members Researchers keep track of who recruited whom and their numbers

of social contacts (Figure 2-12) [112, 134] A mathematical model of the recruitment process then weights the sample to compensate for non-random recruitment patterns This model is based on a synthesis and extension of two areas of mathematics, Markov chain theory and biased network theory, which were not a part of the standard tool kit of mathematical sampling theory [111, 134, 135] The resulting statistical theory, termed RDS, enables researchers to provide both unbiased population estimates and measures of the precision of those estimates

Besides the adjustment process that calculates the selection probabilities, the new approach of RDS and the primary features that distinguish RDS from snowball sampling is the restriction of number of recruits from each recruiter Participants who also serve as recruiters are limited in the number of respondents they can recruit by the number of coupons they receive (e g 2-3), thereby minimizing the influence of each participant on the final sample composition and encourages longer recruitment chains to reach into more hidden pockets of the population

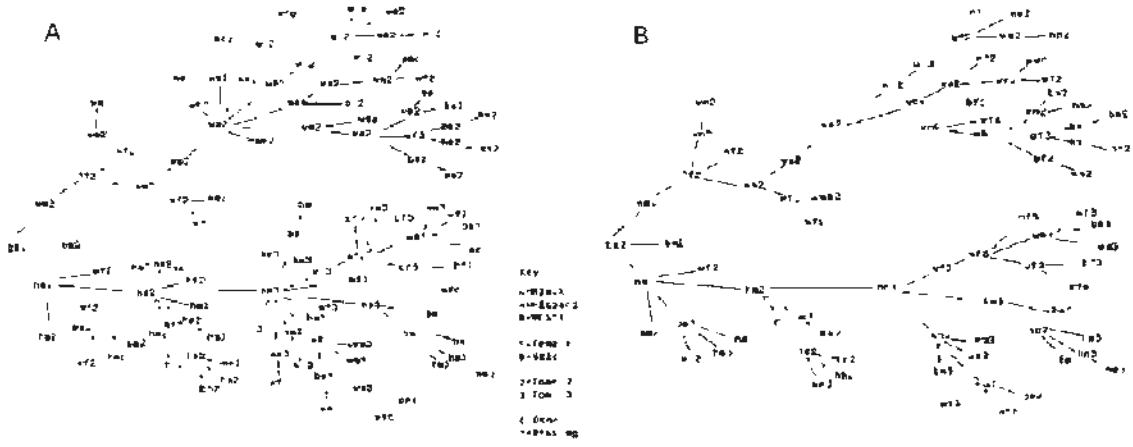

Figure 2-6 Recruitment network of snowball(A) and RDS(B), beginning from one "seed" [111]

However, there are some important sources of bias in RDS including (1) the difficulty in tracking refusal rates and the potential impact of non-response bias, (2) the assumption of random recruitment within personal networks, (3) unknown degree of overlap between networks, (4) the influence of initial seeds selection to the speed with which equilibrium can be reached, and (5) appropriate incentives to maximize

participation and minimize the refusal rate or the recruitment of ineligible respondents [112, 135].

2.2.3.2. Implementation of RDS

RDS combines "snowball sampling" with a mathematical model that weights the sample to compensate for the fact that the sample was collected in a non-random way. Although formative research is also needed in RDS, it is less dependent compared to TLS. The RDS recruitment process begins with a set number of individuals, or "seeds," selected purposefully from the target population. Seeds are trained to recruit a set number (generally no more than 3) of individuals from their social network of peers. The recruits are also asked to recruit a set number of peers from their social network. Both seeds and recruited participants typically receive incentives, both to be interviewed and to refer additional recruits. This recruitment process continues to produce long recruitment "chains" made up of several "waves" of recruits. Researchers record the link between recruiter and recruited peers for further network drawing. As the recruitment chains lengthen, the composition of the sample begins to reach a point of "equilibrium" whereby the composition of certain characteristics (e.g., age group, gender, ethnicity, prevalence of HIV infection) eventually stabilizes within the sample, indicating that the final sample is not biased by the purposeful selection of seeds [112]. It is generally understood that RDS can be applied only in populations that are socially networked and in which members of the network are willing to recruit from among their peers.

In addition to the recruitment process, RDS involves a complex analytical component that is crucial for generating representative estimates and confidence intervals. This is also the key point of RDS survey. It is done through adjustments of factors in the sizes of participants' social networks and the sample's different recruitment patterns. Fortunately, the software RDSAT can be used for data analysis in RDS survey [110-112, 136-138].

2.2.3.3. Application of RDS

Respondent-driven sampling (RDS) is a new approach for sampling hidden populations A recent review reported more than 120 RDS studies worldwide [139], including populations as diverse as men who have sex with men [28, 30, 136, 140-145], sex workers [130, 146], and injection drug users [127, 138, 146-148] Furthermore, the U S Centers for Disease Control and Prevention (CDC) recently selected RDS for a 25-city study of injection drug users as part of the National HIV Behavioral Surveillance System [149] RDS has also been used by the USCDC's Global AIDS Program to study injection drug users (IDUs) in Bangkok As CDC's decisions often influence global public health standards, RDS is likely to become increasingly common in the study of hidden populations

2.3. Molecular epidemiological study and transmission clusters

2.3.1. HIV genotype and molecular epidemiological study on HIV epidemics

Viruses, particularly RNA viruses, have an exceptionally high rate of evolution This extremely high evolutionary rate is an effective survival strategy for viruses to survive in facing host immune responses The natural history of virus including the transmission pattern can be reflected by viral population diversity The only way to trace back their ancestor and tract the distance among viruses is to study the imprints on their genome Molecular epidemiology is an incorporation of molecular phylogenetics in the study of epidemiology, the study of evolutionary relationships among organisms or genes on the basis of molecular data, i e DNA, RNA, and protein sequences

Phylogenetic analysis calculates and maps the genetic distance based on genetic sequence difference by constructing trees Clusters or lineages, i e branches in a phylogeny that fall together, can represent the particular viral genotypes or subtypes, or even small subgroups of evolutionarily close related viruses [150]

Circulating HIV isolates are classified into HIV-1 and HIV-2, while HIV-1 is the most prevalent and clinically relevant type of HIV HIV displays high genetic diversity due to the lack of proof reading capability of its reverse transcriptase Although the diversity of the virus is a major impediment to successful vaccination, it helps identify genotypes/subtypes and phylogenetic relationships between subgroups Currently, there are 9 recognized genetic subtypes of HIV-1 (A–K, excluding E and I) as well as 48 circulating recombinant forms (CRFs), which are created by frequent inter-subtype recombination (i e , genetic exchange among subtypes) The distribution of subtypes is largely dependent on geographic location, such as HIV-1 Subtype A in Africa, Subtype B in North and South Americas and Europe, Subtype C in India, China and South Africa However, there is very few evidence proving the correlations between subtypes and specific disease manifestations

In HIV molecular epidemiology studies, phylogenetic analysis of sequences can be used for tracing the history of epidemic and finding relationships among genetic clusters However, since recombination is common, it could be even more difficult to classify HIV-1 subtypes with clusters of sequences that were generated by a series of localized outbreaks in specific populations (45) Molecular epidemiology is also recognized as a powerful tool to study the origin of global transmission patterns among different risk groups It also provide supportive evidence to identify the possible epidemiological linkages among individuals transmitted HIV through a common route, e g infected healthcare worker, injecting drug users and men who have sex with men [66, 151, 152]

Phylogenetic analyses are broadly used to detect phylogenetic linkage, which could be used as evidence of epidemiological linkage Identifying broad clusters of genetically related viruses within large human populations is a new approach besides the evolutionary origin tracing in phylogenetic analyses Distinct clusters of epidemiologically linked viruses can be inferred by the presence of viruses from

distinct clades disproportionately represented in distinct subsets of the population On the other hand, genetic transmission chains were used for evidence of direct transmission between individuals [153-161] Moreover, Detection of genetic clusters could be used to infer the introduction of one or a small number of viruses into a population, and to explain outbreaks of HIV infections or close linkage within a certain transmission network [162-166] It was reported in both Shenzhen and Hong Kong that genetic clusters were observed in HIV strains from HIV positive MSMs [66-68]

2.3.2. Pol region for molecular epidemiological study and drug resistance analysis

HIV-1 phylogenetic analyses are determined by nucleotide sequences derived from multiple subgenomic regions, gag (gp40) and env (gp120) genes, of the same isolates or full-length genome sequence analysis (Figure 2-13) [167-179] In recent years, along with the drug resistance analysis that focus on mutations on pol region of the virus, a trend was found to include pol region for phylogenetic analysis [180-185] Pol region is a comparatively longer region than env and gag regions that is widely used in HIV molecular epidemiological studies in the early stage, and thereby contains more information for phylogenetic analysis [186]

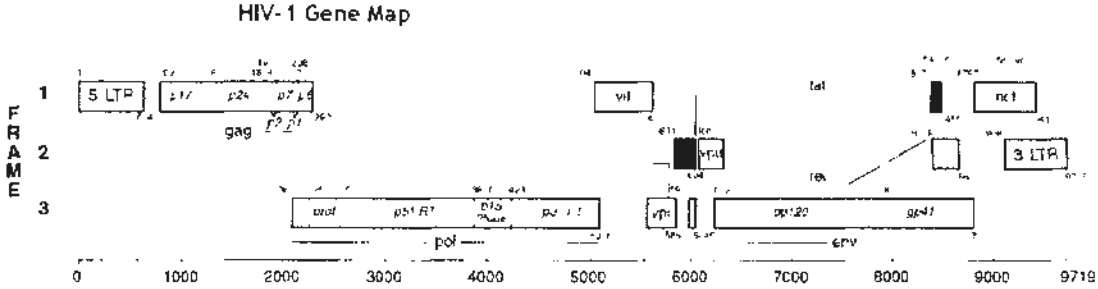

Figure 2-7 HIV-1 gene map.
Genomic regions of env, gag and pol were showed (www hiv.lanl.gov)

The combined use of various antiretroviral drugs for treating HIV-1 infection has been proven to be effective in reducing HIV-related morbidity and mortality The currently available arsenal of drugs for the treatment of HIV infection includes

agents that fall into three main classes the nucleoside analog reverse transcriptase (RT) inhibitors (NRTI), the non-nucleoside analog RT inhibitors (NNRTI) and the HIV protease inhibitors The aim of all three classes of drugs is to inhibit viral replication When a HIV-infected patient fails to respond, or stops responding to treatment with these therapeutic agents, the development of viral drug resistance is often the reason

Drug resistance arises from mutations in the viral genome, specifically in the regions that encode the molecular targets of therapy, HIV protease and RT enzymes HIV RT and protease mutations alter the viral enzymes in such a way that the enzymes' function is no longer inhibited by the drug, leaving the virus to replicate freely

HIV drug resistance results from the interplay of three factors (1) HIV diversity, (2) HIV replication, and (3) anti-HIV drug selection pressure HIV-1 genetic variability is an important factor to be considered in the management of antiretroviral (ARV) drug-treated patients, since it may determine the selection of viral populations with decreased susceptibility to currently used drugs The surveillance of ARV resistance mutations is necessary and has been widely provided to AIDS patients on ART treatment in developed countries, while it's still not widely available in developing countries The occurrence of drug resistant mutations in treatment experienced AIDS patients is not only a problem for the treatment of the patients, it also pushes the patients into a source for transmitting drug resistant strains to others

The transmission of drug resistant HIV-1 is constantly increasing in the past few years, which resulted in poor treatment outcome or failure in treatment naive patients Complete suppression of HIV-1 could be compromised if therapy-naive patients already harbor virus with mutations conferring resistance to antiretroviral drugs used for initial therapy This incomplete suppression in turn promotes the development of broader drug resistance and compromise subsequent treatment regimens [187, 188]

The primary drug resistance mutation rate ranged from 5% to 25% among treatment naive HIV patients in Western countries [189-191] The rate of drug resistant

transmission in China was relatively low (0 5-4 4%) as reported by some cities, yet presented high (17 5-27 8%) in some high risk population including former paid blood donors (FBDs) and gays [192-195] To the best of our knowledge, previous epidemiological studies mostly focused on the subtype B virus, therefore, investigations on non-B viruses would be invaluable

Drug-resistance mutations are traditionally identified during the pre-clinical and initial clinical evaluation of a new antiretroviral drug The genetic sequences of the resistant strains are compared with the sequences of the original HIV strain in phenotype assay Drug resistance mutations identified by this process acquire widespread acceptance as the predominant mutations responsible for resistance to the drug under evaluation, and are referred to as "canonical" resistance mutations

There is HIV drug resistance database (available at http //hivdb stanford edu/ index html) that allows establishment of correlations between certain drug treatments and the corresponding mutations in HIV strains isolated from patients receiving that treatment This approach can help to identify potential drug resistance mutant isolates which should be subjected to detailed in vitro studies

Overall, most reported transmitted mutations occur in the RT rather than in the PR gene though relative frequencies vary markedly in different studies and may depend on the dominant risk factors associated with transmission High rates of PR resistance have been observed in IDUs, however the sample sizes employed in these studies are too small to make any firm conclusion regarding whether the types of transmitted resistance mutations is related to the mode of transmission (e g mucosal vs blood borne transmission) [196, 197] Research also reported a consensus mutation site that was related to the transmission route of IDUs in China [198]

2.3.3. Testing for recent infection and its correlation to drug resistance transmission

Since the global incidence is still quite high, the prevention efforts will need to focus on new infections to prevent further transmission The changes in prevalence of HIV infection may not reflect trends in incidence, indicating the importance of continued monitoring for incidence Moreover, identifying HIV-1 subtype or pattern of drug resistance in newly infected persons can provide important information about the direction and dynamics of the epidemic Incidence is also important for vaccine design and selecting appropriate cohorts in vaccine trials

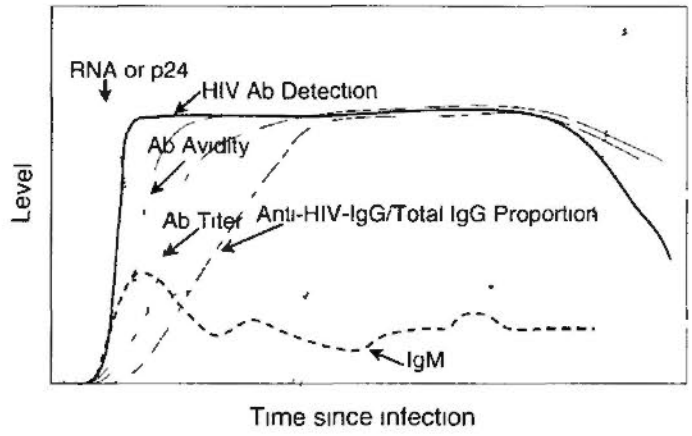

Figure 2-8 Schematic depicting changes in various parameters that define early and late HIV-1 infection [199]

A variety of methods are used to estimate incidence It includes the traditional epidemiological methods and newly emerged laboratory techniques The traditional method for detecting incident infections relies on the prospective testing or longitudinal monitoring of seronegative people for seroconversion, which is very expensive and cumbersome [200-202]

The laboratory test of incidence could be divided into detection of primary HIV-1 infection and recent infection Primary HIV-1 infection is defined as the window period between HIV infection and the development of detectable antibody to HIV (seroconversion) Two types of assays are used to detect primary HIV Infection (pre-seroconversion) RNA or DNA assay, and p24 antigen assay [200]

Recent infection is defined as recent seroconversion when tests are used to measure parameters of HIV antibody In population studies, it has been found that

characteristics of the initial HIV antibody response in recent infection differs from those of established or long-term infections either by antibody titer, proportion, specificity, isotype or avidity. Data demonstrated that this assay was useful for estimating seroincidence from a range of different HIV-1 subtypes using cross-sectional surveys.

Three methods are used in testing recent infection, they are: Vironostika-LS EIA (often known as STARHS – Serologic Testing Algorithm for Recent HIV-1 Seroconversion), BED Capture-EIA, and avidity measurements. As the development of antibodies vary greatly in each patient, the assays should be used only for population surveillance studies and should not be used for the diagnosis of individual patients.

Vironostika-LS (less-sensitive) EIA is based on the differential titer of HIV antibodies in recent versus long-term infection. However, since it was developed using HIV-1 subtype B antigens, its application is restriceted only to populations with subtype B infections.

Avidity measurement: Avidity index is a marker of recent seroconversion. Antibody avidity increases progressively with time after exposure to an immunogen. The avidity of the antibody can be measured in the presence or absence of a denaturing agent that will elute low-avidity antibody from the antigen-antibody complex.

BED Capture-EIA (Calypte), also named as the HIV-1 BED Incidence Assay, is an IgG-capture EIA using a multi-subtype gp41 peptide. It can be successfully used for both subtype B and non-subtype B population studies [203, 204]. A window period of 153 days after seroconversion has been validated for this assay. BED Capture-EIA measures the proportion of HIV-1 specific IgG in a given specimen with respect to total IgG. Early seroconverters have a lower proportion of HIV-specific IgG than those with long-term infection. HIV-specific IgG may continue to increase for more than 2 years after seroconversion when it is tested in this assay.

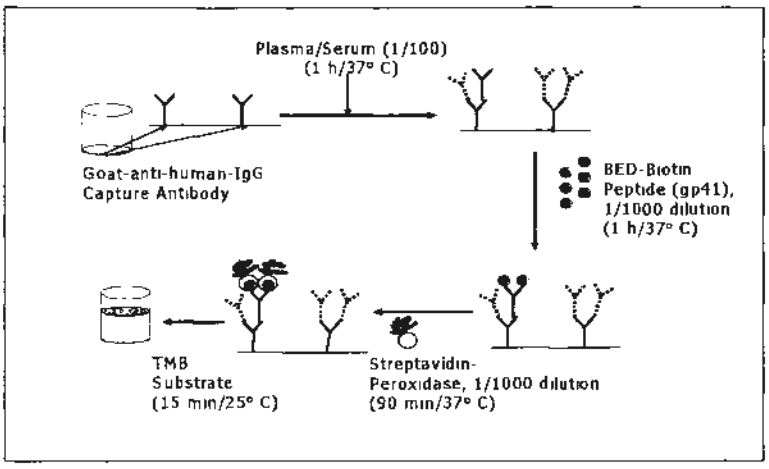

Figure 2-9 Principles of the BED assay (User manual from HIV-1 BED incidence EIA assay).

The confirmation of recent infection is not only an epidemiological issue for estimating HIV incidence, but also help to evaluate the reliability of the prevalence of primary drug resistance Because of the genetic variability of HIV, drug resistant mutations could revert back to wild types after relieving from drug selective pressure [205]. The sampling time for DR analysis excess 6 months after probable infection time, the transmitted virus may has time to adapt to the drug free host environment, meaning that the virus sampled at time points longer than several months from infection are unlikely to be a true representation of the transmitted virus [206, 207]. Therefore, it is crucial to test for primary drug resistance in the early stage of infection[40].

However, it is difficult to identify the exact infection time for those positive individuals only by epidemiological data. Testing for recent infection is an alternative for follow-up and record-based studies.

Chapter 3 Prevalence of HIV infection and its risk factors among MSM in Shenzhen

3.1. Background and objectives

The high risk of MSM population has been continually a major concern in HIV prevention and care since the very beginning of HIV research in 1980s Because of the cultural and other difference between Western and Eastern world, the risk factors addressed in Western world could not fully represent the situation in China A few literatures reported that the prevalence of HIV infection among MSM increased rapidly within the part few years in China Being the first Special Economic Zone (SEZ) and one of the most innovative cities in China, Shenzhen has one of the largest migrant populations (account for 87% of the total population) [32], most of which come from inland rural areas The prevalence of HIV infection among MSM was reported as 3 7-4 8% in Shenzhen [57, 58], which was similar to the national average of 4 9% in MSMs [88] The high percentage and wide variety of migrants among Shenzhen population and the similar prevalence of HIV infection compared with the average level of MSMs in the whole nation indicated that Shenzhen is an ideal place for analyzing the HIV prevalence and behavioral characteristics among MSM in China On the other hand, a significant proportion of money boys (MB) were observed among the MSM population in Shenzhen [24, 29] Based on this unique characteristic that was mainly found in big cities, the differentiation of MB with other MSMs (OMSM) should be considered when analyzing MSMs But the sampling population of most currently available studies mixed general MSMs with MBs [22, 28, 30, 58, 208] The influence of MBs in the whole population of MSMs and the different characteristics related to sex trading behaviors were not addressed In addition, the sampling methods for these studies mainly relied on non-probability sampling methods like snowball sampling method or convenience sampling method based on big entertainment venues or STI clinics, which might affect the

representativeness of the study and had greater risk of bias than probability sampling methods. Time-location sampling (TLS) and respondent-driven sampling (RDS) was by now the best two approximate probability sampling methods in sampling most at risk population (MARP). However, very limited researches used RDS in sampling MSMs [27, 28, 30, 92] and no study using TLS was reported in China.

The objectives of this part of study were to determine the prevalence of HIV along with syphilis among MSM in Shenzhen by TLS and RDS, and to compare the difference between money boys and other MSMs. In this chapter, we report the study results of 1651 MSMs who were recruited from time-location sampling surveys in 2008 and 2009, respectively, with a separate recruitment of MB and OMSM in each year. In addition, a respondent-driven sampling survey on a mixed sample of 492 MSMs was also applied in 2009 within the similar time period.

The chapter describes the distributions of HIV-risk related characteristics of MSMs, including: 1) homosexual and heterosexual risk behaviors, as well as the commercial sex behaviors, and 2) socio-demographic characteristics, sexual orientation, economic status, drug and alcohol use, personal HIV-related knowledge and service accessibility, and condom use. The chapter is divided into 4 parts: 1) Prevalence of HIV infection and HIV-related risk factors among MSM by TLS sampling, with a parallel comparison of data collected in 2008 and 2009. 2) A comparison of HIV-related risk factors between MBs and OMSMs with a combined data of 2008 and 2009 TLS. 3) A comparison of prevalence of HIV infection and HIV-related risk factors among MBs or OMSMs recruited from different venues with a combined data of 2008 and 2009 TLS. 4) RDS survey and the comparison between RDS and TLS survey with the OMSMs data collected in 2009.

3.2. Subjects and Methods

3.2.1. Flow diagram for the studies

A flow diagram was showed in Figure 3-1 to interpret the framework of the study.

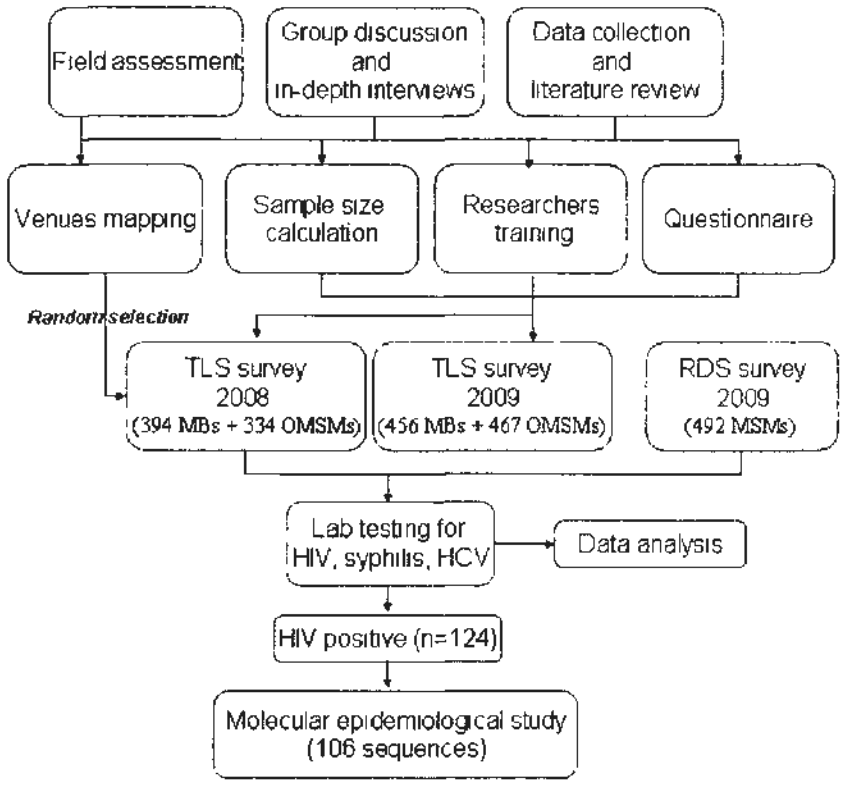

Figure 3-1 Flaw diagram of the study

3.2.2. Sampling population for TLS and RDS

The study tried to cover the spectrum of men who have sex with men - from those who self-identified as heterosexual and only engage in "situational sex" with other men to those who were gay-identified and had sex exclusively with other men. Therefore, the target population of the study included different subgroups of MSM, in which MBs and other MSMs were separately recruited in TLS A mixed sample of MSMs was recruited in RDS because it was difficult to differentiate MBs from other MSMs outside their working venues. In TLS survey, OMSM were eligible to participate if they were biologically male, aged 18 years or older; reported homosexual contact in Shenzhen in the previous 6 months of the survey; reported no paid male-male sex in the previous 6 months, and completed verbal informed consent to participate

Compared with OMSMs, MBs had a higher mobility, with an average of 3 months for changing working venues of sex trade [29, 56]. In addition, it was difficult for

MBs to retrieve the past 6 months' sexual activities because of the large number of commercial partners Therefore, the criteria of eligibility for MBs were changed to focus on commercial sex activities in the previous month MBs enrolled in the study were biologically male, aged 18 years or older, living in Shenzhen for more than 1 month at the time of survey, had ever sold sex (oral or anal) to another man in Shenzhen during the previous month, and completed verbal informed consent to participate

The criteria for eligibility of the participants in RDS was 1) biologically male with aged >18 years, 2) had male-male sex in the previous 6 months in Shenzhen, 3) were part of an extended network of MSM, and 4) completed verbal informed consent to participate

3.2.3. Sample Size Calculation

PASS (Power Analysis and Sample Size Software) 2008 was applied to calculate the sample size needed for risk factor analysis For binomially distributed independent variables, given a P0 (proportion of HIV infection in the reference group) = 0 04 to 0 05, and odds ratio (OR) = 2 0, the smallest sample size would range from 343 to 423 (α=0 05 and β=0 2) [209] Furthermore, according to the widely used recommendation of sample size calculation for multivariate analyses, additional 10-20 people were needed per variable [210] An additional sample of 400 would therefore allow us to add 20-40 independent variables in the model The number of variables used in the models of this study were smaller than 20, the sample size of 800 after combining the data of survey 2008 with 2009 or MBs with OMSMs was therefore adequate for the purpose The actual sample size for both MB and OMSMs was set to be 350-450 in sampling of MBs or OMSMs in each year of TLS survey Sample size for MSMs in RDS was set to be 450 since data for the adjusted analyses were sufficient after 4-5 waves of recruitment (sample size of 300-500) when the sample reaches "equilibrium", that was, when additional waves of recruitment do not

substantially change the composition of the sample with respect to key variables [211]

Since the estimated prevalence of HIV infection is 5%, from the above survey with a total of 2000 subjects, 100 HIV positive samples could be obtained for molecular epidemiological analysis For binomially distributed independent variables, given a sample size of 100 and P0 (proportion of non-clustered strains in the reference group) = 0 1 to 0 5, the odds ratio (OR) would range from 1 75 to 2 54 (α=0 05 and β=0 2)[209]

3.2.4. Sampling procedure

3.2.4.1. Study design

MBs or OMSMs in MSM venues were separately surveyed using the venue-day-time (VDT) sampling scheme, a specific method of time-location-sampling (TLS) Formative research and enumeration were conducted to identify venues to be included in the sampling frame VDTs were sampled systematically At sampled VDTs, eligible persons were asked to participate in a behavioral questionnaire and have their blood drawn for HIV and syphilis tests

In RDS survey, 10 MSM "seeds" were recruited purposively, ensuring that they meet the criteria of eligibility Each of the seeds and subsequent recruits was given three coupons to recruit other MSM they knew Participants received 50 RMB as incentive for their participation in both behavioral and biological data collection, and 10 RMB for each successful recruit The survey was terminated when the target sample size was reached

3.2.4.2. Formative assessment

Formative assessment (FA) was the process by which researchers or public health practitioners define the community of interest, ways of accessing the community and the attributes of the community relevant to the specific public health issue Typically the FA included 4 steps 1) Review of secondary data within geographic area of

interest/literature review, 2) Garnering community support, 3) Key informant interviews with local "experts in target population", 4) Focus groups with members of the priority population

The formative research conducted in the two years of TLS survey was consisted of 4 focus group discussions, 30 individual in-depth interviews and more than 10 field observations It was first conducted to select the MSM venues and to determine the most productive times for conducting the survey at these venues Key informants were approached from the community, leaders of MSM non-governmental organizations (NGO), MSM including MB representatives and local MSM experts, in order to include a diverse group of community members in key informant interviews so that venues catering to smaller subgroups within the MSM population were included CDC staff and volunteers from community were also trained about the operation of TLS survey Since no specific formative assessment was required in RDS, the information collected from the FA in TLS was used in RDS

3.2.4.2.1. Venue Universe / Sampling Frame Construction

After the formative assessment, a complete list of venues in Shenzhen was created There were two sampling frames that have been constructed the first sampling frame was a list of venues that meet the MSM attendance requirements The second sampling frame was a list of venue-specific sampling periods of 2-4 hours each, which could build the list of days and times of high attendance for each venue (VDTs)

Venue eligibility and identification

MSM venues eligible for the study were considered the universe venues we currently know as MSM venues in Shenzhen, including bars, family clubs, saunas, health centers, massage centers/parlors, parks and public toilets In 2008, venues with estimated > 5 eligible participants in formative research were considered as candidates and included in the venue list While in 2009, the minimum number of

samples collected in each event was decreased to 3, for the purpose of expanding the sampling coverage to the small venues, the venue that had never been touched before.

Monthly sampling frames changed over the course of data collection. For example, some venues were closed while some others opened, and some venues were closed temporarily for a few months and then reopened. At the start point of each sampling month, the status of venues was monitored.

Venue-day-time periods (VDTs) along with the venues

The entire day-time period was broken into smaller 2 hour VDTs for each venue. All of the venues were evaluated for the number of MSM regularly visiting them and the number of MBs in the venue by enumeration or inquiring the owner. The most productive time for each venue was decided by information from the gatekeeper or manager of the venue. In addition, the on site observation and enumeration was also conducted to confirm the best time for entry and intercept. Eligible venues were selected and their 1-2 peak two hour periods each day of the week were listed out in a chart (see Appendix II).

3.2.4.2.2. Random selection / Sampling Calendar Creation

When updated universe for monthly sampling frame was obtained, a monthly sampling calendar was created based on random selection principle by using the random number generator from www.random.org. Sixteen venues were randomly selected each month (based on the estimation of workload) and another two alternate sampling venues as replacements for refusals. These alternate venues were based on the "insurance policy" that valuable staff time was not wasted if the primary venue be inaccessible for some unforeseen reason. One 2-hour time slot for each venue was then randomly chosen using a random number table.

3.2.4.3. On field sampling events

Based on the list of VDTs sampled, a team of 2 CDC staff acting as counselor/quality controller and phlebotomist and 3-5 trained volunteers acting as enumerator and recruiters/interviewer went to the selected site on scheduled day and time to carry out the survey Starting at the beginning of the time specified for the VDT, enumerator enumerated every MB who started receiving clients and every MSM who walked into the venue Recruiters attempted to intercept every person enumerated, then introduced study, assessed interest, determined eligibility and enrolled subjects When the person agreed to participate in the interview, the interviewer brought him to a separate private room to administer the questionnaire and had their blood drawn for the testing of syphilis and HIV

3.2.5. Recruitment of MSMs

3.2.5.1. Recruitment of MSMs by TLS

Table 3-1 Enumeration and Recruitment count in MSM by TLS survey, Shenzhen, 2008-2009

	2008		2009	
	MB n(%*)	Other MSM n(%*)	MB n(%*)	Other MSM n(%*)
Enumeration count	494	785	521	844
Intercepted eligible count	456 (92 3)	546 (69 6)	496 (95 2)	724 (85 8)
Interview count	394 (79 8)	334 (42 5)	456 (87 8)	467 (55 2)

* number of intercepted eligible or interview / number of enumerated

Participants were recruited from April to July 2008 in 38 venue-date-times (VDTs) randomly selected from a total of 49 active venues, and from May to November 2009 in 64 VDTs randomly selected from a total of 85 active venues In 2008, a total of 1002 (77 4%) individuals were actually intercepted out of 1295 potentially eligible MSMs identified, and 728 (72 7%) of the intercepted completed the interview and biological test In 2009, 1220 (89 4%) among 1365 potentially eligible MSMs identified were intercepted and 923 (67 6%) of them completed the survey (Table 3-1) Reasons provided for unwillingness to participate in the study included having been

tested for HIV recently, not having enough time for the survey, being afraid of giving blood samples, and not feeling comfortable about participating in a study related to HIV risk and sexual activities

3.2.5.2. Recruitment of MSMs by RDS

The survey by RDS was carried out during May-November 2009 Survey data by TLS conducted in the same period were used for comparison CASI system was used for the purpose of improving self-reporting of sensitive behaviors The recruitment was started from 10 seeds at beginning, and a total of 1290 coupons were distributed with 492 (38 1%, including seeds) returned The seeds were selected for the diversity of demographic factors and key outcome variables [110] The background information of each "seed" was displayed in Table 3-2 1 seed failed to distribute coupons due to the unexpected reason of moving to other city All 492 eligible participants completed the questionnaire and 491 provided blood samples Participants with positive HIV or syphilis results were provided with post-test counseling and referred to appropriate treatment and care services

Table 3-2 The background information of the "seed" in RDS, 2009.

Seed	Age	Education	Occupation	Income (RMB)	HIV
0	26	Senior high school	Worker	1000-2000	Positive
1	19	College	MB	2000-3000	Negative
2	28	College	Clerk	3000-4000	Negative
3	25	Senior high school	Clerk	3000-4000	Negative
4	34	Senior high school	Soldier	2000-3000	Negative
5	30	College	Clerk	<1000	Negative
6	31	Junior high school	MB	>9000	Negative
7	29	College	MB	>9000	Negative
8	24	College	Business	5000-7000	Negative
9	28	Senior high school	Clerk	>9000	Negative

3.2.6. Questionnaire

The participants were interviewed by trained research assistants from MSM volunteer organization after informed consent was obtained Questionnaire used in the study was modified from the national survey on MSMs [27, 30] Data were

collected on demographics, commercial and non-commercial sex risk behaviors, drug addiction, STIs history, HIV-related knowledge and services, and clinical status (Questionnaire in Chinese is attached in Appendix I):

(a) Demographics: age, ethnicity, current resident place, officially registered resident place (location of *hukou*), hometown, education level, length of stay in Shenzhen, employment status, occupation, income level, marital status, and living status.

(b) Sexual behaviors: sexual orientation, age of first penetrative sexual experience, gender of first sexual partner, age of first male sex experience, gender of sex partners in the previous 6 months, major venue for seeking male partners, sex role in male anal intercourse. Venue for seeking male partners was defined as "venue where you always seek male partners", which included bar, massage center, family club, park, sauna, internet and others. In addition, venue-type for recruitment was also recorded as a variable in interpreting venue where participants were probably like to visit, which included bar, massage center, family club, park, sauna and venue in suburb. Sex role in male anal intercourse included insertive anal intercourse, receptive anal intercourse and both.

(c) Non-paid homosexual behaviors: regular sex partner and non-regular sex partner, sexual activities and sex role in the past six months, frequency of condom use in last six months for regular and non-regular sex partner, condom use and lubricant use in last sex activity for regular and non-regular sex partner, paying partner and condom use in paying sex in the past six months, male sex partner from Hong Kong or foreign countries. Number of sexual partners and condom use with partners in the past six months was reported according to partner type: a steady partner was defined as "somebody you have sex with regularly and to whom you feel an emotional bond or identify as boyfriend", a date partner was defined as "somebody you have sex for more than one encounter, you know some of the partner's background information and identify as friend", a one-night stand partner was defined as "somebody you have sex with without payment, and know mere background information about", a

paying partner or client was defined as "a partner from whom you received money, gifts or valuables in exchange for sex", and a paid partner was defined as "a partner to whom you gave money, gifts or valuables in exchange for sex" [212]

(d) Heterosexual behaviors regular and non-regular female sex partner in the past 6 months, condom use frequency in last six months for regular and non-regular sex partner

(e) Paid sexual behaviors age of first paid male and female sex, the percentage of male or female clients from Hong Kong, number of male and female clients in the past 1 month, average of payment per encounter with male clients, sex role in paid sex, frequency of condom use in paid sex, lubricant use in paid sex, mobility of sex trade activity A paid male sex partner was defined as "somebody you have sex with for payment"

(f) STI history STI related symptoms, attitude towards treatment choice, diagnosed STIs history Diagnosed STIs history was defined as ever been diagnosed with STIs in the past 1 year

(g) Drug and alcohol abuse drug use history, alcohol use before sexual behavior, frequency of substance use, substance use before sexual behavior, injecting drug use, and history of needle sharing Drug use included substance use (use of heroin, crystal methamphetamine, cocaine, ecstasy, marijuana, ketamine, and cough syrup) and popper/Viagra use

(h) HIV-related knowledge and services HIV testing history, perception of HIV infection, coverage of HIV-related public health services, HIV-related knowledge and condom use knowledge Coverage of HIV services was defined as receiving any services including condom distribution, lubricant distribution, peer education, STIs diagnosis or treatment, HIV counseling or testing, or AIDS/STIs educational materials in the past year Perceived risk of acquiring HIV was measured by a

question ("How much do you estimate your risk of getting HIV/AIDS?") using an integral scale from 1 (not at all) to 5 (a great deal) and to 6 (already infected)

3.2.7. Data collection

Because of the sensitivity of the questions in this study, a self-admistered questionnaire could capture the sensitive behaviors more reliably in high risk populations A paper based guided self-administered questionnaire was used in year 2008 for data collection Participants requiring assistance in reading or interpreting survey questions could consult volunteers However, the paper based questionnaire could hardly guarantee the quality of the interview without on-venue invading the privacy of the interviewees, since staff served as quality controller needed to check the questionnaire in front of the participants Therefore, computer-assisted self-interview (CASI) was used in 2009

Research on computerized interviewing in the USA showed that replacing the interviewer with a computer can provide conditions, including privacy and the perception of anonymity, that facilitate reliable and frank reporting, thereby increasing reports of sensitive behavior in surveys in sensitive populations [213]

In order to improve the self-reporting of sensitive behaviors, a computer-assisted personal interview system (ITACAPI®, Shanghai Nankang Technology CO , Ltd , China) was used to replace the face-to-face or guided self-administered interviews that performed in the previous year Questions were edited for use in ITACAPI® pilot-tested, and adjusted if necessary The ITACAPI® system was tested for performance and acceptability among MSM volunteers, key informants and MB representatives After that, five small venues (family clubs) and three big venues (one bar, one sauna and one park) were selected randomly for pilot study

A total of 8 notebooks with touch screen were provided on each VDT for the interview After providing verbal informed consent, participants completed self-administered questionnaires on hand-held computers, providing demographic, behavioral, and psychosocial information For those non-sensitive questions in the

beginning of the questionnaire, a trained volunteer from the local MSM NGO helped each participant to choose the answers and familiarized him with the touch screen of the notebook, and then left him to complete the rest of the questions on his own Participants requiring assistance in interpreting survey questions or layout could consult volunteers

Quality control measures, including close supervision, on-site observation, field and office editing were implemented Questions were tested for missing values and logic problems All key variables were examined The main method used to detect errors was to view the distributions of variables and construction of box plots If the original distributions were far from normal, we performed a logarithmic transformation to identify outliers There were two strategies for correcting unreasonable outliers One was to delete the whole record if it had more than 5 variables having unreasonable outliers or if it had signs of fabrications The other strategy was to designate the extreme outliers as "missing values"

A 5mL blood sample was collected from each participant with EDTA-K3 tube as anticoagulant The participants were given their negative test results over the phone Participants with positive results in screening test were asked to go to the Shenzhen CDC for a confirmatory test, and provided with post test counseling if the confirmation test was positive, and referred to additional STIs and HIV/AIDS services according to Chinese national guidelines (Ministry of Health of China) Field notes about the cooperation and refusals of establishments and participants were recorded in each VDT The entire length of the survey in each year lasted 4-6 months

The study was approved by the Medical Ethics Committee of Shenzhen CDC and the Chinese University of Hong Kong Informed consent was obtained from all participants

3.2.8. Laboratory assays

A 5mL blood sample was collected from each participant with EDTA-K3 tube as anticoagulant. All blood samples were centrifuged at 1000rpm for 5 minutes. Supernatant plasma from anti-coagulated blood was separated and aliquoted into 500uL per tube. Plasma was used for serological assays or stored at -70°C for the further extraction of HIV RNA. The HIV and syphilis tests were performed according to standard procedures of Shenzhen CDC laboratory.

3.2.8.1. HIV test

HIV screening - enzyme-linked immunosorbent assay (ELISA, Wantai Biotech Inc, Beijing)

Human serum or plasma were diluted in specimen diluent and incubated with the proteins of HIV-1 and HIV-2 coated microplate wells. If the HIV antibodies were present in the tested sample, they would bind with the proteins coated on the microwell. After washing off the unbound proteins, horse radish peroxidase conjugated with anti human IgG antibodies was added. Enzyme conjugate would bind through the antigen antibody complex if present. Unbound analyte was washed and substrate solution was added. Color would develop in proportion to the amount of HIV antibodies present in the specimen. The reaction was read by EIA reader.

The detail procedures of the experiments were listed as below:

- Add 100uL of the sample diluent to each of the plate well, set one blank control (A1), three negative controls (B1,C1,D1) and two positive controls (E1,F1).
- Add 50uL of each sample in each well, starting from G1 well; add controls to the corresponding well. Blank add diluent.
- Apply cover seal, incubate at 37°C ± 2°C for 30 min. +2 min. Wash the wells 5 times with working wash solution.
- Add 100uL of working conjugate solution in each well including A-1, incubate at 37°C ± 2°C for 30 min. + 2 min. Aspirate and wash the wells 5 times with working wash solution.
- Add 100uL of working substrate solution in each well including A-1 and incubate

at room temperature (20-30°C) for 30 min. in dark.

- Add 50uL of stop solution. Read absorbance at 450 nm within 30 minutes in Elisa Reader after blanking A1 well. Reactive is printed across that particular reactive sample number according to the O.D. value of the cutoff.

HIV screening – rapid test

Use anti-HIV-1/2/O rapid assay (determine, abbott) for a rapid test for on-venue testing.

HIV confirmation test - Western blot assay (WB)

Positive samples were confirmed by Western blot assay (MP Diagnostics HIV BLOT2.2, Singapore). The WB assay was used as the specific supplemental test on human serum or plasma specimens found reactive using ELISA. Specific HIV-1 viral antigens had been incorporated onto nitrocellulose strips via electrophoretic and electrotransblot procedures, in combination with a specific HIV-2 synthetic peptide on the same strip allowing for further delineation of the antibody responses to specific viral proteins. Antibodies that bind specifically to HIV proteins can be visualized using a series of reactions with goat anti-human IgG conjugated with alkaline phosphatase and the substrate BCIP/NBT. This method has good sensitivity to detect small amount of HIV specific antibodies in serum or plasma.

The standard procedure of the rapid assay was as follows:

- Add 2mL of DILUTED WASH BUFFER to each well.
- Using forceps, carefully remove required number of STRIPS from the tube and place numbered side up into each well. Include strips for Strong Reactive, Weak Reactive and Non-Reactive controls.
- Incubate the strips for 1 to 2 minutes a 25 ± 3°C on a rocking platform (speed of 12 to 16 cycles per minute). Remove buffer by aspiration.
- Add 2 mL of BLOTTING BUFFER to each well.
- Add 20uL each of patients' sera or controls to appropriate wells.

- Cover the tray with the cover provided and incubates for 1 hour at 25 ± 3°C on the rocking platform. Tilt the tray to aspirate the mixture from the wells.
- Wash each strip 3 times with 2 ml of DILUTED WASH BUFFER allowing 5 minutes soak on the rocking platform between each wash.
- Add 2 ml of WORKING CONJUGATE SOLUTION to each well.
- Cover tray and incubate for 1 hour at 25 ± 3°C on the rocking platform.
- Aspirate CONJUGATE from the wells. Wash again.
- Add 2 ml of SUBSTRATE SOLUTION to each well. Cover tray and incubate for 15 minutes on the rocking platform.
- Aspirate the SUBSTRATE and rinse the strips three times with reagent grade water to stop the reaction.
- Remove strips and dry. Observe the bands and grade the results.

3.2.8.2. Syphilis test

Syphilis screening – rapid test

Use anti-syphilis rapid assay (determine, abbott) for a rapid test for on-venue testing.

Syphilis screening –Rapid Plasma Reagin (RPR) test

RPR (Rongsheng Biotech Inc, Shanghai) was used to detect the presence of reagin, an antibody to cardiolipin. The principle of RPR is that patient sera mixed with a fine particle cardiolipin antigen which has been enhanced with cholesterol, lecithin, and charcoal will result in a macroscopically visible flocculation-type precipitation if the patient's sera contains reagin - an antibody formed against cardiolipin.

- Dispense one free-falling drop (50uL) of serum or plasma sample onto a circle on the test card. Repeat by adding one drop of REACTIVE, WEAK REACTIVE or NONREACTIVE control from the dropper vials supplied.
- Using the flat end of the stirrer pipette spread the sample over the entire area of the test circle. Do not scratch the surface of the test area.
- Dispense one drop of the CARBON ANTIGEN suspension onto each sample

while holding the bottle in a vertical position.

- Place the card on an automatic rotator and cover to maintain humidity. Rotate at 100 ± 5 rpm for 8 minutes. Following rotation, a brief hand rotation and tilting of the card (3–4 times) should be performed to aid in differentiating nonreactive from minimally reactive results. Immediately read results macroscopically in the "wet" state under a high intensity light source.

Syphilis confirmation –T. pallidum particle agglutination tests (TPPA)

The TPPA test was used to confirm a syphilis infection after another method tests positive for the syphilis bacteria. This test detected antibodies to the bacteria that cause syphilis and could be used to detect syphilis in all stages, except during the first 3 to 4 weeks. The TPPA test is performed by using Serodia TPPA kit (Fujirebio, Japan) according to the user manual.

The qualitative assay procedure was as below:

Four wells were required for each patient sample and controls run in this assay. Wells #1 &2 were for dilution of samples, well #3 for unsensitized particles and well #4 for sensitized particles. The Positive (Reactive) and Non-Reactive Controls were included in each assay run.

- Place 4 drops (100uL) of Sample Diluent in well #1, and 1 drop (25uL) in wells #2-#4 using a pipette.
- Using a micropipette, add 25uL of patient specimen or Positive or Non-Reactive Control Sera into wells #1.
- Mix the contents of well #1 and transfer 25uL of the diluted solution from well #1 into well #2. repeat the transfer steps to prepare diluete well #3 and #4.
- Place 1 drop (25uL) of Unsensitized Particles in well #3, and 1 drop of Sensitized Particles in well #4 using the droppers supplied in the kit.
- Mix the contents of the wells thoroughly (30 seconds) using a plate mixer. Cover the plate, stand at room temperature for 2 hours before reading. The incubation can be extended to overnight.

- Place the plate onto a flat surface, preferably with a white background, and visually observe the pattern of agglutination in each well

3.2.9. Data management and data analysis

3.2.9.1. Data cleaning and recoding

EPIData 3 1 was used to set up the questionnaire and enter the data in year 2008 Since data in 2009 was collected by using a computer-assisted self-interview system, the database was directly export from ITACAPI® All key variables (especially the variables needed to input numbers, such as numbers of different partners) were examined The main method used to detect errors was to view the distributions of variables and construction of box plots If the original distributions were far from normal, a logarithmic transformation was performed to identify outliers All upper extreme outliers (with a threshold of 75^{th} quartile plus 3 times of the inter-quartile range) were carefully examined But only unreasonable values were corrected There were two strategies for correcting unreasonable outliers One was to delete the whole record if it had more than 5 variables having unreasonable outliers or if it had signs of fabrications The other strategy was to designate the extreme outliers as "missing values" Based on these principles, 12 records were deleted, and 43 records had at least one variable corrected and set to "missing value"

For the variables with multiple categories, a category with just a few cases was recoded to its neighbor category Length of stay in Shenzhen was converted into three levels, "<= 3 months", "4-12 months" and "> 1 year" Income level was also converted into three levels in multivariate analysis, "< 1000 RMB", "1001-5000 RMB" and ">5000 RMB" Occupation was coverted into 3 categories "commercial services" (MBs and dancer or waiter in MSM venues), "student", "migrant worker" and "clerk or other" Perceived risk of acquiring HIV was converted into three levels of "no risk", "little risk" and "high risk" Hometown of the participants was recoded into binary variables of "high HIV prevalence areas" and "low HIV prevalence

areas" in consideration of the HIV prevalence of the hometown province according to the result in 2008 national MSM survey [88] The high HIV prevalence areas were located in Southwest China, including 4 provinces of Sichuan, Chongqing, Yunnan and Guizhou Venue-type of recruitment was recoded into 5 catergories "bar and massage center", "family club", "park", "sauna" and "small venue in suburb" Self-reported venue-type for seeking partners was recoded into 5 catergories "bar and massage center", "family club", "park", "sauna" and "internet and other" Count variables including the number of different partners and monthly sexual encounters, were recoded into categorical variables No risky anal sex was defined as no anal sex in the past 6 months or always using condoms in anal sex with men, any other responses were coded as unprotected anal intercourse (UAI) No risky vaginal or anal sex with female was defined as no vaginal or anal sex with women in the past 6 months or consistent use of condoms during sex with women, otherwise were coded as unprotected anal or vaginal intercourse (UAVI) There were nine core questions to evaluate HIV-related knowledge It was defined as the rate of correctly answering six or more out of the nine core questions [214] 5 questions were used to evaluate condom use knowledge [27] Correctly answering 4 or more out of the 5 questions was defined as "high" in the recoded variable of condom use knowledge

3.2.9.2. Statistical analysis

In RDS, a special weighting procedure was applied for the data analysis Information on the relationship between recruiters and recruits as well as the personal network size of each respondent was collected The information allowed weighted analysis through "post-stratification" to compensate for the over-sampling of respondents with larger social networks [108, 112, 134] Estimates of network size were based on the self-report network size in response to this question "In this city, how many MSM do you know? That means you could recognize the person's face and know his name or nickname, have his contact information, and could get in touch with him within one month" Respondent-Driven Sampling Analysis Tool (RDSAT 5 6 0,

available free at www respondentdrivensampling org) was used to generate adjusted point estimates and 95% confidence intervals (95% CI)

Since RDSAT could not perform multivariate analysis, SPSS (version 16 0, SPSS Inc , USA) was used after cases individual weights that were assigned for HIV and syphilis infection were exported from RDSAT The procedure of data analysis by SPSS for RDS and TLS data was similar and described as below

Descriptive analyses were carried out to examine the distribution/frequency of subjects' demographic characteristics, sexual behavior patterns, commercial sex work and condom use Pearson's chi square test or trend test was performed to identify the distribution of proportion of HIV positives within each category of variables Logistic regression analyses (univariate) were also performed to identify factors potentially associated with HIV infection Furthermore, multivariate logistic regression was applied with backward stepwise method to assess risk factors Variables in the multivariate analysis were selected based on the results of univariate analysis ($P < 0\ 05$) and prior knowledge

When candidate variables in multivariate analysis models were significantly correlated with each other, only those variables best explaining the relationships to outcome were included in the model The Hosmer and Lemeshow test was used to test the goodness-of-fit All reported *P* values were two-tailed, and a value <0 05 was considered statistically significant Adjusted odds ratios (OR) and 95% confidence intervals (CI) were obtained from logistic regression analyses

3.3. Results (Part I) – prevalence of HIV infection and related risk factors in MSM by TLS

In 2008, time-location sampling survey was conducted among male sex workers based on their working venues, and OMSMs who frequented MSMs venues Based on the collected data, we found that the MBs working in small family clubs and parks as well as OMSMs frequenting parks and saunas were at significantly higher

risk of HIV infection (9 9%) compared with the MSM recruited from bars and massage centers (2 0%) While bars and massage centers were the venues where most outreach activities were implemented After the 2008 TLS, specific venue targeting HIV education and intervention strategies were modified to meet the need of services among different types of MSM in the venues Moreover, a new TLS survey including both MBs and OMSMs was conducted in 2009 following the similar procedure that implemented in 2008, in order to identify the trend of changes in HIV infection and related risk factors

3.3.1. HIV/syphilis infections and demographic characteristics

Table 3-3 described the HIV/syphilis prevalence and demographic characteristics of the study participants Overall, the HIV and syphilis prevalence was 5 7% (95%CI 4 8-6 6) and 16 5% (95%CI 15 0-18 0), respectively, which was similar to the national average level in MSMs of 4 9% and 12 3%, respectively [88] Among the 1651 participants, 612 were recruited from bars and massage centers, 260 from Saunas, 321 from family clubs (small home-based brothels, where they worked as call boys for a pimp or manager who provided housing), 312 from public areas such as parks, and 146 from small venues located in suburb outside Shenzhen Special Economic Zone In total, 94 MSMs including 49 in 2008 and 45 in 2009 were detected HIV-seropositive, with prevalence of HIV infection of 6 7% (95% CI 4 9-8 5%) and 4 9% (95% CI 3 5-6 3%), respectively There were 272 (16 5%, 95% CI 14 7-18 3%) participants who were positive to syphilis, with 133 (18 3%, 95% CI 15 5-21 1%) in 2008 and 139 (15 1%, 95% CI 12 8-17 4%) in 2009 Among the HIV positive participants, the positive rate of syphilis increased to 44 9% in 2008 and 62 2% in 2009 Overall, the infection rate of both HIV and syphilis was slightly decreased from year 2008 to 2009

The median age of the participants was 24 years in the years of survey with a range of 18-51 years in 2008 and 18-62 years in 2009 Overall, 13 7% were younger than 20 years in age and 22 7% older than 30 years 97 8% MSMs received at least a

junior high school education (over 9 years) Only 6 8% held official residence cards (*hukou*) in Shenzhen and 10 1% came from other parts of Guangdong province Most of the MSMs originally came from other provinces, such as Hunan (13 2%), Hubei (9 2%), Sichuan (9 2%), Shandong (7 2%) and Jiangxi (6 8%) In consider the prevalence of HIV infection of the hometown province according to the result in 2008 national MSM survey, 14 5% of the MSMs came from high HIV prevalence areas including Sichun, Chongqing, Yunnan and Guizhou [88] The occupation of 66 8% of the participants belonged to commercial service industry, including sex trade and other normally accepted services like waiter, masseur or dancer in MSM venues 9 6% of the participants were migrant workers MSM involving in service industry had significantly lower HIV infection rate (4 2%) compared with migrant workers (7 6%) and MSM with other jobs (9 7%) About half of the participants lived in Shenzhen for less than 1 year 82 9% lived within the SEZ of Shenzhen, the city area of Shenzhen, and 17 1% lived in Shenzhen suburb area outside SEZ 62 0% of the participants lived in Luohu district, one of the most economically vibrant districts of Shenzhen and a commercially developed area that was attractive to people from Hong Kong for business and entertainment 44 8% of the MSMs reported having male sex partners from Hong Kong in the previous 6 months Over 85% of the MSMs earned >1,000 RMB (approximately 147 US Dollars) per month Only 42 6% of the participants self-identified as gay or homosexual, while 33 9% described themselves as bisexual A quarter of the participants identified themselves as heterosexual

Compared with MSMs in 2008, a greater proportion of MSMs in 2009 survey were married, older in age, had high income, self-identified as gay or homosexual, and were recruited from venues outside Shenzhen SEZ

Among the biological and demographic characteristics, MSMs with syphilis infection, being recruited from venues other than bars and massage centers and self-identifying as homosexual/gay were more likely to be HIV positives in both

2008 and 2009 surveys MSMs being older in age, hometown in high HIV prevalence areas and occupation as migrant worker, clerks or others were found to have higher HIV infection rate only in 2008 data

Two variables related to venues were analyzed in the study, including venue where participants were recruited and self-reported venue for seeking male sex partners Although significant difference in HIV infection was found among MSMs being recruited in different venues in the two years of survey, no such difference was observed in self-reported venues for seeking sex partners

3.3.2. HIV-related sexual behaviors in MSMs

Table 3-4 showed the HIV-related sexual practices and behavioral patterns among MSMs Since the sampling strategy was to parallel recruit MBs and OMSMs until a pre-decided sample size reached for each subgroup, the proportion of MBs among the whole samples was 51 5% (54 1% in 2008 and 49 4% in 2009) However, the prevalence of HIV infection was lower among MB than among OMSM in both surveys In total, 394 MBs and 334 OMSMs were enrolled in the first survey in 2008, and 456 MBs and 467 OMSMs were enrolled in the second survey in 2009 The HIV infection rate in MBs (4 5%) was lower than that in OMSMs (7 0%) in a combined data of 2008 and 2009 survey Prevalence of HIV infection was decreased from 5 3% to 3 9% in MB group and from 8 4% to 5 8% in OMSM group after one year In accordance with the drop of HIV positive rate, syphilis positive rate was also slightly decreased from 14 2% to 11 8% in MBs and from 23 1% to 18 3% in OMSMs

42 2% of the MSM reported that the gender of their first sex partner was male The average age at first sex was 20 0 years and first male-male sex was 21 6 years Among the 1651 participants recruited in both 2008 and 2009 TLS surveys, 26 7% had stable male partner and 69 4% had more than 1 sex partner in the previous 6 months of the interview On the other hand, more than 40% had sexual contact with female partners in the past 6 months One third of these MSMs self-reported of having sex with Hong Kong MSM 40 9% of the MSMs reported that they engaged

in unprotected male-male anal sex within the past 6 months, including 35 6% who reported unprotected non-commercial male sex In contrast, condom use rate was higher in heterosexual contact, with only 25 6% self-reported of having unprotected vaginal or anal intercourse with women

In accordance with the greater proportion of homosexual/gay among MSMs in 2009 than 2008, the proportion of MSM reported of no female partner in the previous 6 months was also higher in 2009 In contrast, a greater proportion of MSM in 2009 reported no or only one anal male sex partners in the previous 6 months compared with MSM in 2008 In addition, condom use rates among sexual contact with both male and female partners were significantly higher among MSM in 2009 (63 4% in male sex and 79 2% in female sex) than those in 2008 (53 7% and 68 4%, respectively), indicating an overall improvement in reducing sexual risk behaviors taking among MSMs in 2009

MSMs with only one or no female partner in the previous 6 months had significantly higher HIV infection rate compared with those who had multiple female partners in both 2008 and 2009 survey In 2008 survey, MSMs having no male sex partner from Hong Kong were found twice as likely to be infected by HIV (9 6% vs 4 9%) In addition, MSMs having unprotected anal intercourse with men including non-commercial male partners, and having unprotected anal or vaginal intercourse with male or female sex partner also showed a significantly higher likelihood to acquire HIV in 2008 MSMs having first sex with man, having initial male-male sex before the age of 20, and having unprotected anal or vaginal sex with women in the previous 6 months were found to have higher HIV rate in 2009 data

3.3.3. Self-reported STIs, drug use, accessible services and knowledge

Table 3-5 showed that approximately 20% of the participants reported having used illegal drug in their life time 14 5% reported that they had used drugs in the previous 6 months, and 4 2% used them before having sex However, soft drug use was much more common in bars and massage centers (30 7%), and family clubs (26 8%)

compared with other venues (9.9%). A lower proportion of MSMs ever used drugs in 2009 (17.0%) than in 2008 (25.8%).

Nearly 12% of the MSMs self-reported ever been diagnosed with STIs in the previous 1 year. This subgroup of MSMs had significantly higher HIV positive rate. About half of the participants reported that they ever tested for HIV, and one quarter of them received a HIV test within 6 months prior to the survey.

There was a significantly greater proportion of MSMs in 2009 reporting that ever received HIV test, as well as HIV-related education and services, while a lower proportion reported having diagnosed with STIs and having taken illegal drugs. Although the HIV-related knowledge level was decreased in 2009, the condom use knowledge level was significantly improved (Table 3-5).

MSMs receiving HIV test and self percepting of high HIV risk had significantly higher risk in HIV infection in 2008 data, while MSMs having diagnosed with STIs were more likely to be infected by HIV in 2009.

Table 3-3 Prevalence of HIV infection and related risk factors for MSM by 2008 and 2009 (TLS)

	2008				2009			
	Total (n =728) n (%[a])	HIV positive (n=49) n (%[b])	X^{2*}	P	Total (n =923) n (%[a])	HIV positive (n =45) n (%[b])	X^{2*}	P
Overall	728 (100)	49 (6.7)			923 (100)	45 (4.9)		
Tested Syphilis positive								
Yes	133 (18.3)	22 (16.5)	24.949	**<0.001**	139 (15.1)	28 (20.1)	82.260	**<0.001**
No	595 (81.7)	27 (4.5)			784 (84.9)	17 (2.1)		
Venue type of recruitment								
Bar and massage center	295 (40.5)	6 (2.0)	18.472	**<0.001**	317 (34.3)	3 (0.9)	20.983	**<0.001**
Family club	120 (16.5)	14 (11.7)			201 (21.8)	11 (5.5)		
Park	166 (22.8)	15 (9.0)			146 (15.8)	15 (10.3)		
Small venue in suburb	38 (5.2)	3 (7.9)			108 (11.7)	6 (5.6)		
Sauna	109 (15.0)	11 (10.1)			151 (16.4)	10 (6.6)		
Self-reported frequenting venue for finding sex partners								
Bar and massage center	166 (22.8)	6 (3.6)	6.700	0.244	225 (24.4)	5 (2.2)	10.102	0.072
Family club	134 (18.4)	9 (6.7)			177 (19.2)	12 (6.8)		
Sauna	127 (17.4)	12 (9.4)			162. (17.6)	7 (4.3)		
Park	82 (11.3)	8 (9.8)			40 (4.3)	5 (12.5)		
Internet and other	219 (30.1)	14 (6.4)			319 (34.6)	16 (5.0)		
Age（Years）								
18-20	113 (15.5)	5 (4.4)	5.729[#]	**0.017**	113 (12.2)	4 (3.5)	3.331[#]	0.068
21-25	355 (48.8)	19 (5.4)			397 (43.0)	14 (3.5)		
26-30	141 (19.4)	12 (8.5)			211 (22.9)	14 (6.6)		
>30	119 (16.3)	13 (10.9)			202 (21.9)	13 (6.4)		

Table 3-3 Prevalence of HIV infection and related risk factors for MSM by 2008 and 2009 (TLS). Cont.

	2008				2009			
	Total (n =728) n (%[a])	HIV positive (n=49) n (%[b])	X^{2*}	P	Total (n =923) n (%[a])	HIV positive (n =45) n (%[b])	X^{2*}	*P*
Education level								
Primary or less	13 (1.8)	0 (0.0)	0.254[#]	0.614	23 (2.5)	1 (4.3)	2.259[#]	0.133
Junior high school	172 (23.6)	11 (6.4)			237 (25.7)	8 (3.4)		
Senior high school	358 (49.2)	26 (7.3)			427 (46.3)	20 (4.7)		
College or above	185 (25.4)	12 (6.5)			236 (25.6)	16 (6.8)		
Residency								
Shenzhen	47 (6.5)	3 (6.4)	2.397	0.302	65 (7.0)	3 (4.6)	0.069	0.966
Guangdong	96 (13.2)	10 (10.4)			112 (12.1)	6 (5.4)		
Others	585 (80.4)	36 (6.2)			746 (80.8)	36 (4.8)		
Length of stay in Shenzhen								
<=3 months	201 (27.6)	14 (7.0)	1.248[#]	0.536	289 (31.3)	15 (5.2)	1.494[#]	0.474
4-12 months	181 (24.9)	9 (5.0)			141 (15.3)	4 (2.8)		
>1 year	346 (47.5)	26 (7.5)			493 (53.4)	26 (5.3)		
Hometown								
Low HIV prevalence area	631 (86.7)	36 (5.7)	7.934	**0.005**	780 (84.5)	38 (4.9)	0.000	0.991
High HIV prevalence area	97 (13.3)	13 (13.4)			143 (15.5)	7 (4.9)		
Occupations								
Commercial services	488 (67.0)	23 (4.7)	12.591	**0.006**	615 (66.6)	23 (3.7)	6.417	0.093
Student	15 (2.1)	0 (0.0)			12 (1.3)	1 (8.3)		
Migrant worker	61 (8.4)	7 (11.5)			98 (10.6)	5 (5.1)		
Clerk or other	164 (22.5)	19 (11.6)			198 (21.5)	16 (8.1)		

Table 3-3 Prevalence of HIV infection and related risk factors for MSM by 2008 and 2009 (TLS). Cont.

	2008				2009			
	Total (n =728) n (%[a])	HIV positive (n=49) n (%[b])	X^{2*}	P	Total (n =923) n (%[a])	HIV positive (n =45) n (%[b])	X^{2*}	P
Monthly income								
<=1000	130 (17.9)	13 (10.0)	1.046[#]	0.306	69 (7.5)	4 (5.8)	1.110[#]	0.292
1001-3000	327 (44.9)	20 (6.1)			397 (43.0)	24 (6.0)		
3001-5000	151 (20.7)	9 (6.0)			280 (30.3)	9 (3.2)		
5000-9000	77 (10.6)	3 (3.9)			120 (13.0)	5 (4.2)		
>9000	43 (5.9)	4 (9.3)			57 (6.2)	3 (5.3)		
Marital status								
Unmarried	642 (88.2)	41 (6.4)	1.232	0.540	731 (79.2)	36 (4.9)	1.071	0.585
Married	59 (8.1)	5 (8.5)			142 (15.4)	8 (5.6)		
Divorced or other	27 (3.7)	3 (11.1)			50 (5.4)	1 (2.0)		
Self-identified sexual orientation								
Homosexual/gay	275 (37.8)	31 (11.3)	15.001	**0.001**	428 (46.4)	31 (7.2)	13.208	**0.001**
Bisexual	256 (35.2)	12 (4.7)			304 (32.9)	13 (4.3)		
Heterosexual or unsure	197 (27.1)	6 (3.0)			191 (20.7)	1 (0.5)		

[a]: percentage of the total N; [b]: percentage of each category; *: chi square test was based on distribution of HIV infection; [#]: using a trend test; UAI: unprotected anal intercourse; STIs: sexually transmitted infections.

Table 3-4 HIV infection and sexual behavioral factors in MSM by 2008 and 2009 (TLS)

	2008				2009			
	Total (n =728) n (%[a])	HIV positive (n=49) n (%[b])	X^{2*}	*P*	Total (n =923) n (%[a])	HIV positive (n =45) n (%[b])	X^{2*}	*P*
Sold sex to men								
Yes	394 (54.1)	21 (5.3)	2.684	0.101	456 (49.4)	17 (3.7)	2.558	0.110
No	334 (45.9)	28 (8.4)			467 (50.6)	28 (6.0)		
Gender of first sex partner								
Male	292 (40.1)	25 (8.6)	2.603	0.107	405 (43.9)	30 (7.4)	9.976	**0.002**
Female	436 (59.9)	24 (5.5)			518 (56.1)	15 (2.9)		
Number of anal sex partners								
0	11 (1.5)	0 (0.0)	0.026#	0.871	51 (5.5)	1 (2.0)	0.083#	0.774
1	140 (19.2)	11 (7.9)			303 (32.8)	16 (5.3)		
>1	577 (79.3)	38 (6.6)			569 (61.6)	28 (4.9)		
Hong Kong male sex partners								
Yes	371 (51.0)	19 (5.1)	6.054	0.048	368 (39.9)	12 (3.3)	4.004	0.135
No	282 (38.7)	27 (9.6)			464 (50.3)	29 (6.3)		
Unknown	75 (10.3)	3 (4.0)			91 (9.9)	4 (4.4)		
Paid male sex								
Yes	394 (54.3)	21 (5.3)	2.752	0.097	456 (49.4)	17 (3.7)	2.558	0.110
No	334 (45.7)	28 (8.4)			467 (50.6)	28 (6.0)		
Sex role in male anal intercourse								
Insertive only	218 (29.9)	16 (7.3)	2.860	0.239	311 (33.7)	11 (3.5)	1.883	0.390
Both	327 (44.9)	19 (5.8)			462 (50.1)	26 (5.6)		
Receptive only	114 (15.7)	12 (10.5)			126 (13.7)	7 (5.6)		

Table 3-4 HIV infection and sexual behavioral factors in MSM by 2008 and 2009 (TLS) Cont

	2008				2009			
	Total (n =728) n (%[a])	HIV positive (n=49) n (%[b])	X^{2*}	*P*	Total (n =923) n (%[a])	HIV positive (n =45) n (%[b])	X^{2*}	*P*
Female sex partner								
0	371 (51 0)	33 (8 9)	11 453	**0.003**	582 (63 1)	38 (6 5)	11 729	**0.003**
1	143 (19 6)	12 (8 4)			143 (15 5)	6 (4 2)		
>1	214 (29 4)	4 (1 9)			198 (21 5)	1 (0 5)		
Age of first male sex experience								
<=20	259 (35 6)	21 (8 1)	0 397	0 529	387 (41 9)	26 (6 7)	4 881	0 027
>20	227 (31 2)	15 (6 6)			536 (58 1)	19 (3 5)		
UAI in male sex								
No	391 (53 7)	16 (4 1)	9 368	**0.002**	585 (63 4)	28 (4 8)	0 027	0 869
Yes	337 (46 3)	33 (9 8)			338 (36 6)	17 (5 0)		
UAI in non-commercial male sex								
No	449 (61 7)	21 (4 7)	7 871	**0.005**	615 (66 6)	29 (4 7)	0 102	0 750
Yes	279 (38 3)	28 (10 0)			308 (33 4)	16 (5 2)		
UAVI in female sex								
No	498 (68 4)	35 (7 0)	0 222	0 638	731 (79 2)	44 (6 0)	9 912	**0.002**
Yes	230 (31 6)	14 (6 1)			192 (20 8)	1 (0 5)		
Condom use in all sex								
Always	270 (37 1)	11 (4 1)	4 825	**0.028**	481 (52 1)	27 (5 6)	1 179	0 278
Not always	458 (62 9)	38 (8 3)			442 (47 9)	18 (4 1)		

[a] percentage of the total N, [b] percentage of each category, * chi square test was based on distribution of HIV infection, # using a trend test, UAI unprotected anal intercourse, UAVI unprotected anal or vaginal intercourse, STIs sexually transmitted infections

Table 3-5 HIV infection and STIs, knowledge and services factors in MSM by 2008 and 2009 (TLS)

	2008				2009			
	Total (n =728) n (%[a])	HIV positive (n=49) n (%[b])	X^{2*}	*P*	Total (n =923) n (%[a])	HIV positive (n =45) n (%[b])	X^{2*}	*P*
Ever diagnosed with STIs								
Yes	129 (17.7)	12 (9.3)	0.86	0.199	76 (8.2)	9 (11.8)	8.667	**0.003**
No	599 (82.3)	37 (6.2)			847 (91.8)	36 (4.3)		
Ever took drugs								
No	540 (74.2)	36 (6.7)	0.014	0.907	766 (83.0)	40 (5.2)	1.166	0.280
Yes	188 (25.8)	13 (6.9)			157 (17.0)	5 (3.2)		
Ever drunk alcohol before sex								
Never	314 (43.1)	22 (7.0)	0.067	0.796	563 (61.0)	28 (5.0)	728.00	0.863
Ever	414 (56.9)	27 (6.5)			360 (39.0)	17 (4.7)		
History of HIV test								
Within 6 months	172 (23.6)	19 (11.0)	6.917[#]	**0.009**	248 (26.9)	11 (4.4)	0.011[#]	0.918
Before 6 months	160 (22.0)	11 (6.9)			272 (29.5)	16 (5.9)		
No	389 (53.4)	19 (4.9)			403 (43.7)	18 (4.5)		
Any HIV education and service								
No	167 (22.9)	10 (6.0)	0.190	0.663	112 (12.1)	4 (3.6)	0.467	0.494
Yes	561 (77.1)	39 (7.0)			811 (87.9)	41 (5.1)		
HIV-related knowledge								
Low	107 (14.7)	7 (6.5)	0.007	0.933	255 (27.6)	8 (3.1)	2.295	0.130
High	621 (85.3)	42 (6.8)			668 (72.4)	36 (5.5)		

Table 3-5 HIV infection and STIs, knowledge and services factors in MSM by 2008 and 2009 (TLS). Cont.

	2008				2009			
	Total (n =728) n (%[a])	HIV positive (n=49) n (%[b])	X^{2*}	*P*	Total (n =923) n (%[a])	HIV positive (n =45) n (%[b])	X^{2*}	*P*
Condom use knowledge								
Low	252 (34.6)	15 (6.0)	0.372	0.542	273 (29.6)	10 (3.7)	1.229	0.268
High	476 (65.4)	34 (7.1)			650 (70.4)	35 (5.4)		
Self-perception of HIV infection								
No risk	609 (83.7)	35 (5.7)	15.397	**<0.001**	822 (89.1)	40 (4.9)	0.654	0.721
Little risk	103 (14.1)	11 (10.7)			90 (9.8)	5 (5.6)		
High risk	8 (1.1)	3 (37.5)			11 (1.2)	0 (0.0)		

[a]: percentage of the total N; [b]: percentage of each category; *: chi square test was based on distribution of HIV infection; [#]: using a trend test; UAI: unprotected anal intercourse; STIs: sexually transmitted infections.

3.3.4. Risk factors associated with HIV infection

Multivariate analysis was performed to further evaluate the determinants for HIV risk from different candidate variables among MSM in 2008 and 2009. In addition, a combined data with both 2008 and 2009 survey was used for the multivariate analysis in consideration of small number of positive cases in each year of survey (Table 3-6). Age, occupation, sexual orientation, venue type of the recruitment conducted, number of male anal sex partners, hometown HIV prevalence, gender of first sex partner, age in first male sex, male sex partner from Hong Kong, condom use in male sex, condom use in female sex, HIV-related knowledge, condom use knowledge, HIV test history, HIV-related services, STIs diagnosed history, and syphilis infection were considered to enter into the logistic regression model.

Table 3-6 Risk factors for HIV infection among MSM from TLS survey, 2008 (n=728), 2009 (n =923) and combined

	2008	2009	Total
	AOR#	AOR#	AOR#
Syphilis infection	2.62 (1.32-5.19)*	8.59 (4.45-16.56)*	5.46 (3.50-8.53)*
Occupation			
Commercial services	1	—	1
Clerk or other	2.25 (1.06-4.78)*		1.76 (1.06-2.90)*
Migrant worker	2.36 (0.83-6.75)		1.47 (0.72-2.99)
Venue-type for recruitment			
Bars and massage centers	1	1	1
Family clubs	6.06 (2.12-17.30)*	3.96 (1.05-14.86)*	4.11 (1.85-9.14)*
Parks	2.02 (0.70-5.83)	5.24 (1.42-19.30)*	3.26 (1.46-7.31)*
Venue in suburb	1.78 (0.37-8.47)	3.43 (0.80-14.67)	2.06 (0.77-5.56)
Sauna	2.26 (0.74-6.87)	3.14 (0.81-12.22)	2.79 (1.19-6.54)*
Hometown in high HIV prevalence areas			1.77 (1.01-3.07)*
Sexual orientation			
Homosexual/gay	2.94 (1.02-8.49)*	—	2.54 (1.10-5.84)*
Bisexual	1.22 (0.42-3.53)		1.52 (0.63-3.67)
Heterosexual	1		1
Condom use in female sex	0.42 (0.19-0.95)*	0.11 (0.01-0.86)*	
Self-perception of HIV infection			
No risk	1	—	—
Little risk	1.92 (0.87-4.25)		
High risk	5.62 (1.02-30.83)*		
History of HIV test			
Within 6 months	1	—	—
Before 6 months	0.52 (0.22-1.24)		
Never	0.39 (0.18-0.82)*		
History of diagnosed STIs	—	2.54 (1.05-6.15)*	—

#AOR: adjusted odds ratio; STIs: sexually transmitted infections.

In addition, the Hosmer and Lemeshow test was non-significant (*P* value was 0.934, 0.356 and 0.585, respective for the 3 analysis), which indicated that the model fit was good. After going through backward stepwise selection, only variables of syphilis infection, venue-type for recruitment and condom use in female sex were found to be associated with HIV infection in both surveys of 2008 and 2009. No HIV test experience and self-perception of higher HIV risk were related to HIV infection in 2008 TLS, while having been diagnosed with STIs was related to HIV infection only in 2009 data.

For the combined data, syphilis infection, occupation as clerk or other, being recruited from family clubs, parks or saunas, hometown in high HIV prevalence areas, and self-identifying as homosexual or gay, were significantly associated with HIV infection.

3.3.5. Summary

This part mainly focused on the prevalence of HIV infection and risk factors for HIV infection in MSM through time-location surveys that were conducted in year 2008 and 2009. The HIV and syphilis prevalence was 5.7% and 16.5%, respectively, which was similar to the national average level of 4.9% in MSMs [88] .

The prevalence of HIV infection in MBs (4.5%) was lower than in OMSMs (7.0%). Further comparison of MBs with OMSMs was presented in result part II.

Consistent with the literature targeting MSMs, sexual orientation was found associated with HIV infection, with a significantly higher prevalence of HIV infection among MSMs self-identified as gay/homosexual. However, it was good to find that not all MSMs self-identified as gay or homosexual (42.6%), and around one quarter of the MSMs in TLS survey self-reported as heterosexual orientation. This situation could be explained by the large proportion of MBs in the TLS study.

Significantly lower HIV risk was observed in MSMs frequenting bars and massage centers compared with MSMs in family clubs, parks and saunas. This finding was the first report in China showing the correlation of HIV risk with venue based on the venues where MSMs were recruited. Detailed comparison of different venue types that were related to HIV risk in MBs and OMSMs were reported in the result part III.

Although more MSMs in 2009 self-identified as gay/homosexual, a smaller proportion of them reported of high risky behaviors compared with MSMs in 2008, including multiple male sex partners and unprotected anal intercourse In addition, a greater proportion of MSM in 2009 reported that they had received HIV test and other HIV-related education and services

HIV infection was associated with the hometown of the participants MSMs from high HIV prevalence areas had significantly higher risk in HIV infection than those coming from low HIV prevalence areas

3.4. Results PART II: A comparison between MBs and OMSMs

Higher prevalence of HIV infection, sexual risk behaviors and social vulnerabilities of MBs were reported in most of the literatures [39, 40, 215], while data from the TLS survey conducted in 2008 showed that equal or even lower prevalence of HIV and syphilis among MBs compared with other MSMs in Shenzhen Therefore, the detailed variation in factors of social economics background and sexual behavioral characteristics should be investigated to find the key determinants for the difference between MBs and OMSMs

Although some differences in HIV-related risk factors in MSMs between 2008 and 2009 data were observed, the distributions of the majority of variables in both years were similar Given a small number of HIV infections included in the data, MB and OMSM data from both 2008 and 2009 were combined and compared in order to enhance study power in the analysis Separate data of each year was attached in appendix III

3.4.1. Demographic Characteristics and HIV/syphilis infections

Among the 1651 MSMs enrolled in the study, 94 MSMs were tested as HIV-positive [4 6% (95%CI 3 2-6 1) among MBs and 7 0% (95%CI 5 4-9 0) among OMSMs] with HIV positive rate of 5 7% (95%CI 4 6-7 0) The difference of HIV infection among MBs and OMSMs was statistically significant with a chi square value of 4 08 and $P = 0\ 043$ In addition, 272 were found to be syphilis positive, with a prevalence of 16 5% (95%CI 15 1-18 8) While the prevalence of syphilis among MBs (12 9%, 95%CI 10 8-15 4) was significantly lower than that among OMSMs (20 3%, 95%CI 17 5-23 2) ($X^2 = 16\ 25$, $P < 0\ 001$) In addition, syphilis positive MSMs had

significantly higher HIV infection rate than those negatives [18 4% (95%CI 14 1-23 6) vs 3 2% (95%CI 2 4-4 3), $X^2 = 97\ 65$, $P < 0\ 001$] (Table 3-7)

HIV risk was highly related to the venues where the participants were recruited, no matter they sold sex or not to another man Bars and massage centers were classified as a type of MSM venues having the biggest MB organization and a type of venues where most HIV-related services were provided Both MBs and OMSMs frequenting these venues had the lowest HIV positive rate Another variable of self-reported venue for seeking male sex partners was also collected and analyzed Although significant difference in HIV infection was found among MBs or OMSMs being recruited in different venues, no such difference was observed in self-reported venues for seeking sex partners

As showed in Table 3-7, MSMs being syphilis positive, being recruited from family clubs, parks and saunas, and self-identifying as gay or homosexual were more likely to be tested HIV positive among both MBs and OMSMs A greater proportion of HIV positive was observed in MBs who were older, and came from high HIV prevalence areas A trend of increasing HIV infection with decreasing income level was found among OMSMs

Nearly all demographic characteristics except for monthly income were significantly different between MBs and OMSMs MBs were younger and less educated compared with OMSMs A smaller proportion of MBs held Shenzhen or Guangdong resident cards (Hukou), had fulltime jobs or were married A smaller number in MBs self-reported homosexual/gay orientation Instead, a bigger proportion of them self-identified heterosexual (34 8%) compared with OMSMs

3.4.2. HIV risk and sexual behaviors

Table 3- 8 showed that HIV infection rate was significantly different among different categories of sexual behavioral variables in both MBs and OMSMs However, the sexual behavioral variables with unequal HIV distribution among MBs were totally different from those among OMSMs Higher HIV infection rates were observed in MBs reporting first sex partner as male, less male partner from Hong Kong, no or only one female sex partners and lower condom use rate in all sex While higher HIV infection rates were observed in OMSMs being younger in first sex or male-male sex, and with exclusive receptive anal intercourse in male-male sex

A significantly bigger proportion of MBs reported having sex with women in the previous 6 months and having multiple female sexual partners within the same time period than OMSMs In addition, MBs were more likely to have multiple male anal sexual partners However, a greater proportion of MBs consistently used condoms and lubricant in male sex, though they were less likely to use condoms in sex intercourse with women The inconsistency of condom use in male and female sex among MBs and OMSMs resulted in a similar rate of condom use overall Furthermore, MBs were four times more likely to have sex with MSMs from Hong Kong than OMSMs (Table 3-8)

MBs having more than 4 male clients in the previous 1 month, having no clients from Hong Kong and having no female clients were more likely to be infected with HIV However, no significant increase of HIV rate was observed in MBs practicing receptive anal intercourse, being paid less in each sex encounter and having longer experience in selling sex

3.4.3. Self-reported STIs, drug use, accessible services and knowledge

A trend of increasing HIV risk with the decreasing of time interval from the most recent HIV test to the time of survey was found among MBs However no such finding was observed in OMSMs Instead, a trend of higher HIV infection rate was observed among OMSMs with increasing self-perception of HIV risk

A significantly greater proportion of MBs reported ever using illegal drugs or drinking alcohol before having sex compared with OMSMs The HIV-related knowledge and condom use knowledge level were significantly lower in MBs However, they were more likely to receive HIV-related education and services, and receiving a HIV test within 6 months than OMSMs (Table 3-8)

Table 3-7 Prevalence of HIV infection and demographic characteristics for MB and OMSM using a combination data of 2008 and 2009 (TLS)

	MB				OMSM			
	Total (n=850) n (%[a])	HIV positive (n=38) n (%[b])	X^{2*}	*P*	Total (n=801) n (%[a])	HIV positive (n=56) n (%[b])	X^{2*}	*P*
Overall	850 (100)	38 (4 5)			801 (100)	56 (7 0)		
Tested Syphilis positive								
No	740 (87 1)	19 (2 6)	48 489	**<0.001**	639 (79 8)	25 (3 9)	46 061	**<0.001**
Yes	110 (12 9)	19 (17 3)			162 (20 2)	31 (19 1)		
Venue type of recruitment								
Bar / massage center	497 (58 5)	8 (1 6)	28 465	**<0.001**	115 (14 4)	1 (0 9)	12 380	**0.015**
Family club	252 (29 6)	18 (7 1)			69 (8 6)	7 (10 1)		
Park	68 (8 0)	7 (10 6)			246 (30 7)	23 (9 3)		
Small venue in suburb	33 (3 9)	5 (15 2)			113 (14 1)	4 (3 5)		
Sauna	/	/			258 (32 2)	21 (8 1)		
Self-reported venue for seeking sex partners								
Bar and massage center	292 (34 4)	8 (2 7)	6 493	0 261	99 (12 4)	3 (3 0)	6 047	0 302
Family club	206 (24 2)	13 (6 3)			105 (13 1)	8 (7 6)		
Sauna	79 (9 3)	3 (3 8)			210 (26 2)	16 (7 6)		
Park	25 (2 9)	2 (8 0)			97 (12 1)	11 (11 3)		
Internet	164 (19 3)	10 (6 1)			221 (27 6)	14 (6 3)		
Other	84 (9 9)	2 (2 4)			69 (8 6)	4 (5 8)		
Age（Years）								
18-20	183 (21 5)	4 (2 2)	5 526[#]	**0.019**	43 (5 4)	5 (11 6)	0 639[#]	0 424
21-25	501 (58 9)	23 (4 6)			251 (31 3)	10 (4 0)		
26-30	133 (15 6)	7 (5 3)			219 (27 3)	19 (8 7)		
>30	33 (3 9)	4 (12 1)			288 (36 0)	22 (7 6)		

Table 3-7 Prevalence of HIV infection and demographic characteristics for MB and OMSM using a combination data of 2008 and 2009 (TLS). Cont.

	MB				OMSM			
	Total (n=850) n (%[a])	HIV positive (n=38) n (%[b])	X^{2*}	*P*	Total (n=801) n (%[a])	HIV positive (n=56) n (%[b])	X^{2*}	*P*
Education level								
Primary school or lower	20 (2.4)	1 (5.0)	1.359[#]	0.244	16 (2.0)	0 (0.0)	0.156[#]	0.693
Junior high school	256 (30.1)	8 (3.1)			153 (19.1)	11 (7.2)		
Senior high school	448 (52.7)	21 (4.7)			337 (42.1)	25 (7.4)		
College or above	126 (14.8)	8 (6.3)			295 (36.8)	20 (6.8)		
Residence card holding								
Shenzhen	37 (4.4)	2 (5.4)	2.438	0.295	75 (9.4)	4 (5.3)	0.392	0.822
Guangdong	76 (8.9)	6 (7.9)			132 (16.5)	10 (7.6)		
Others	737 (86.7)	30 (4.1)			594 (74.2)	42 (7.1)		
Length of stay in Shenzhen								
<=3 months	323 (38.0)	17 (5.3)	1.069[#]	0.301	167 (20.8)	12 (7.2)	0.378[#]	0.538
4-12 months	218 (25.6)	10 (4.6)			104 (13.0)	3 (2.9)		
>1 year	309 (36.4)	11 (3.6)			530 (66.2)	41 (7.7)		
Hometown								
Low HIV prevalence areas	715 (84.1)	23 (3.2)	16.571	**<0.001**	696 (86.9)	51 (7.3)	0.924	0.337
High HIV prevalence areas	135 (15.9)	15 (11.1)			105 (13.1)	5 (4.8)		
Occupational status								
Commercial services	723 (85.1)	27 (3.7)	7.086	0.069	380 (47.4)	19 (5.0)	7.155	0.067
Student	18 (2.1)	1 (5.6)			9 (1.1)	0 (0.0)		
Migrant worker	36 (4.2)	4 (11.1)			123 (15.4)	8 (6.5)		
Clerk or other	73 (8.6)	6 (8.2)			289 (36.1)	29 (10.0)		

Table 3-7 Prevalence of HIV infection and demographic characteristics for MB and OMSM using a combination data of 2008 and 2009 (TLS). Cont.

	MB				OMSM			
	Total (n=850) n (%[a])	HIV positive (n=38) n (%[b])	X^{2*}	*P*	Total (n=801) n (%[a])	HIV positive (n=56) n (%[b])	X^{2*}	*P*
Monthly income								
<=1000	111 (13.1)	8 (7.2)	0.057[#]	0.811	88 (11.0)	9 (10.2)	3.874[#]	**0.049**
1001-3000	350 (41.2)	13 (3.7)			374 (46.7)	31 (8.3)		
3001-5000	227 (26.7)	8 (3.5)			204 (25.5)	10 (4.9)		
5000-9000	105 (12.4)	6 (5.7)			92 (11.5)	2 (2.2)		
>9000	57 (6.7)	3 (5.3)			43 (5.4)	4 (9.3)		
Marital status								
Unmarried	791 (93.1)	37 (4.7)	1.436	0.488	582 (72.7)	40 (6.9)	0.062	0.970
Married	34 (4.0)	1 (2.9)			167 (20.8)	12 (7.2)		
Divorced or other	25 (2.9)	0 (0.0)			52 (6.5)	4 (7.7)		
Self-identified sexual orientation								
Homosexual/gay	253 (29.8)	22 (8.7)	17.484	**<0.001**	450 (56.2)	40 (8.9)	6.010	**0.050**
Bisexual	301 (35.4)	12 (4.0)			259 (32.3)	13 (5.0)		
Heterosexual or unsure	296 (34.8)	4 (1.4)			92 (11.5)	3 (3.3)		

[a]: percentage of the total N; [b]: percentage of each category; *: chi square test was based on distribution of HIV infection; [#]: using a trend test.

Table 3-8 HIV infection and sexual behavioral factors for MB and OMSM using a combination data of 2008 and 2009 (TLS)

	MB				OMSM			
	Total (n=850) n (%[a])	HIV positive (n=38) n (%[b])	X^{2*}	*P*	Total (n=801) n (%[a])	HIV positive (n=56) n (%[b])	X^{2*}	*P*
Gender of first sex partner								
Male	252 (29.6)	19 (7.5)	7.900	**0.005**	445 (55.6)	36 (8.1)	1.859	0.173
Female	598 (70.4)	19 (3.2)			356 (44.4)	20 (5.6)		
Number of anal sex partners								
0	19 (2.2)	1 (5.3)	0.066[#]	0.797	43 (5.4)	0 (0.0)	1.606[#]	0.205
1	108 (12.7)	4 (3.7)			335 (41.8)	23 (6.9)		
>1	723 (85.1)	33 (4.6)			423 (52.8)	33 (7.8)		
Hong Kong male sex partners								
Yes	597 (70.2)	22 (3.7)	13.655	**0.001**	142 (17.7)	9 (6.3)	0.242	0.886
No	174 (20.5)	16 (9.2)			572 (71.4)	40 (7.0)		
Unknown	79 (9.3)	0 (0.0)			87 (10.9)	7 (8.0)		
Sex role in male anal intercourse								
Insertive only	220 (25.9)	10 (4.5)	0.060	0.971	309 (38.6)	17 (5.5)	6.666	**0.036**
Both	467 (54.9)	22 (4.7)			322 (40.2)	23 (7.1)		
Receptive only	142 (16.7)	6 (4.2)			98 (12.2)	13 (13.3)		
Female sex partner								
0	352 (41.4)	27 (7.7)	20.729	**<0.001**	601 (75.0)	44 (7.3)	0.619	0.734
1	131 (15.4)	8 (6.1)			155 (19.4)	10 (6.5)		
>1	367 (43.2)	3 (0.8)			45 (5.6)	2 (4.4)		
Age of first male sex experience								
<=20	361 (42.5)	20 (5.5)	1.194	0.274	285 (35.6)	27 (9.5)	5.653	**0.017**
>20	319 (37.5)	12 (3.8)			444 (55.4)	22 (5.0)		

Table 3-8 HIV infection and sexual behavioral factors for MB and OMSM using a combination data of 2008 and 2009 (TLS). Cont.

	MB				OMSM			
	Total (n=850) n (%[a])	HIV positive (n=38) n (%[b])	X^{2*}	*P*	Total (n=801) n (%[a])	HIV positive (n=56) N (%[b])	X^{2*}	*P*
Age of first sex experience								
<=18	402 (47.3)	16 (4.0)	0.766[#]	0.382	209 (26.1)	20 (9.6)	**4.041[#]**	**0.044**
19-20	253 (29.8)	12 (4.7)			207 (25.8)	17 (8.2)		
21-22	124 (14.6)	5 (4.0)			165 (20.6)	7 (4.2)		
>22	71 (8.4)	5 (7.0)			220 (27.5)	12 (5.5)		
No. of commercial male partners								
<=4	445(52.4)	12(2.7)	6.882	**0.009**	/			
>4	405(47.6)	26(6.4)						
Sold sex to women								
Yes	254(29.9)	4(1.6)	7.113	**0.008**	/			
No	596(70.1)	34(5.7)						
Male clients from Hong Kong								
No	208(24.5)	17(8.2)	8.840	**0.003**	/			
Yes	642(75.5)	21(3.3)						
Per encounter payment for selling sex to male clients								
<=500 RMB	528(62.1)	26(4.9)	1.007	0.316	/			
>500 RMB	292(34.4)	10(3.4)						
Experience in paid sex								
<=1 year	376(44.2)	15(4.0)	0.729	0.694	/			
>1 year	472(55.5)	23(4.9)						
UAI in male sex								
No	556 (65.4)	21 (3.8)	1.811	0.178	420 (52.4)	23 (5.5)	3.117	0.077
Yes	294 (34.6)	17 (5.8)			381 (47.6)	33 (8.7)		

Table 3-8 HIV infection and sexual behavioral factors for MB and OMSM using a combination data of 2008 and 2009 (TLS). Cont.

	MB				OMSM			
	Total (n=850) n (%[a])	HIV positive (n=38) n (%[b])	X^{2*}	*P*	Total (n=801) n (%[a])	HIV positive (n=56) n (%[b])	X^{2*}	*P*
UAI in non-commercial male sex								
No	644 (75.8)	27 (4.2)	0.481	0.488	420 (52.4)	23 (5.5)	3.117	0.077
Yes	206 (24.2)	11 (5.3)			381 (47.6)	33 (8.7)		
UAVI with female partners								
No	556 (65.4)	30 (5.4)	3.221	0.073	673 (84.0)	49 (7.3)	0.543	0.461
Yes	294 (34.6)	8 (2.7)			128 (16.0)	7 (5.5)		
UAVI with male or female partners								
Always	270 (37.1)	11 (4.1)	4.825	**0.028**	481 (52.1)	27 (5.6)	1.179	0.278
Not always	458 (62.9)	38 (8.3)			442 (47.9)	18 (4.1)		

[a]: percentage of the total N; [b]: percentage of each category; *: chi square test was based on distribution of HIV infection; [#]: using a trend test; UAI: unprotected anal intercourse; UAVI: unprotected anal or vaginal intercourse.

Table 3-8 HIV infection and STIs, drug use, HIV knowledge and services for MB and OMSM using a combination data of 2008 and 2009 (TLS)

	MB				OMSM			
	Total (n=850) n (%[a])	HIV positive (n=38) n (%[b])	X^{2*}	*P*	Total (n=801) n (%[a])	HIV positive (n=56) n (%[b])	X^{2*}	*P*
Diagnosed with STIs								
Yes	101 (11.9)	6 (5.9)	0.580	0.446	104 (13.0)	15 (14.4)	10.152	**0.001**
No	749 (88.1)	32 (4.3)			697 (87.0)	41 (5.9)		
Ever took drugs								
No	602 (70.8)	28 (4.7)	0.158	0.691	704 (87.9)	48 (6.8)	0.268	0.605
Yes	248 (29.2)	10 (4.0)			97 (12.1)	8 (8.2)		

Table 3-9 HIV infection and STIs, drug use, HIV knowledge and services for MB and OMSM using a combination data of 2008 and 2009 (TLS). Cont.

	MB				OMSM			
	Total (n=850) n (%[a])	HIV positive (n=38) n (%[b])	X^{2*}	*P*	Total (n=801) n (%[a])	HIV positive (n=56) n (%[b])	X^{2*}	*P*
Alcohol use before sex								
Never	355 (41.8)	15 (4.2)	0.086	0.770	522 (65.2)	35 (6.7)	0.189	0.863
Ever	495 (58.2)	23 (4.6)			279 (34.8)	21 (7.5)		
History of HIV test								
Within 6 months	242 (28.5)	19 (7.9)	9.097[#]	**0.003**	178 (22.2)	11 (6.2)	0.015[#]	0.903
Before 6 months	193 (22.7)	8 (4.1)			239 (29.8)	19 (7.9)		
No	410 (48.2)	11 (2.7)			382 (47.7)	26 (6.8)		
Any HIV education and service								
No	130 (15.3)	5 (3.8)	0.140	0.708	149 (18.6)	9 (6.0)	0.255	0.614
Yes	720 (84.7)	33 (4.6)			652 (81.4)	47 (7.2)		
HIV-related knowledge								
Low	235 (27.6)	9 (3.8)	0.312	0.576	127 (15.9)	6 (4.7)	1.193	0.275
High	615 (72.4)	29 (4.7)			674 (84.1)	50 (7.4)		
Condom use knowledge								
Low	293 (34.5)	11 (3.8)	0.537	0.464	232 (29.0)	14 (6.0)	0.460	0.498
High	557 (65.5)	27 (4.8)			569 (71.0)	42 (7.4)		
Self-perception of HIV risk								
No risk	741 (87.2)	33 (4.5)	0.613	0.736	690 (86.1)	42 (6.1)	11.372	**0.003**
Little risk	92 (10.8)	5 (5.4)			101 (12.6)	11 (10.9)		
High risk	9 (1.1)	0 (0.0)			10 (1.2)	3 (30.0)		

[a]: percentage of the total N; [b]: percentage of each category; *: chi square test was based on distribution of HIV infection; [#]: using a trend test

3.4.4. HIV-related risk factors for MB in comparison with OMSM

To further evaluate determining factors for HIV infection from different candidate variables in MBs and OMSMs by TLS, logistic regression model was used for multivariate analysis (Table 3-9) Considered variables included syphilis infection, age, occupation, sexual orientation, venue type of the recruitment conducted, hometown HIV prevalence, gender of first sex partner, age of first sex, number of male anal sex partners, male sex partner from Hong Kong, condom use in male sex, condom use in female sex, HIV-related knowledge, condom use knowledge, HIV test history, coverage of HIV-related services, STIs diagnosed history, and perception of HIV risk For MB, two extra variables including number of male clients and selling sex to women were added in the model The Hosmer and Lemeshow test was non-significant (*P* value = 0 936 and 0 708, respective, for the MB and OMSM analysis), which indicated that the model fit was good

Table 3-9 Risk factors related to HIV infection among MBs and OMSM, 2008-2009

	MB (n=850)		OMSM (n=801)	
	AOR	*P*	AOR	*P*
Syphilis infection	**5.22(2.48-10.98)**	**<0.001**	**4.93(2.77-8.79)**	**<0.001**
Venue-type for recruitment				
Bars and massage centers	**1**		**1**	
Family clubs	**3.36(1.38-8.17)**	**0.007**	**13.39(1.57-114.10)**	**0.018**
Parks	**3.57(1.13-11.17)**	**0.030**	**8.98(1.18-68.38)**	**0.034**
Venue in suburb	**5.33(1.45-19.52)**	**0.012**	3.31(0.36-30.79)	0.293
Sauna	/		**8.04(1.05-61.75)**	**0.045**
Hometown with high HIV prevalence	**2.77(1.29-5.95)**	**0.009**	—	
No male partner from HK	**2.40(1.15-5.00)**	**0.019**	—	
>4 clients in previous 1 month	**2.19(1.02-4.73)**	**0.046**		
Early age of first sex	—		**1.82(1.00-3.32)**	**0.049**

AOR: adjusted odds ratio.

Variables including syphilis infection, venue type of the recruitment conducted, hometown HIV prevalence, Hong Kong male partner and number of male clients were found significantly associated with HIV infection in MBs While syphilis infection, venue of recruitment, and age of initial male sexual practice less than 20, were related to HIV infection in OMSMs (Table 3-10)

Table 3-10 HIV-related characteristics, OMSM (n = 801) vs. MB (n = 850), 2008-2009

	AOR (95%CI)	*P*
HIV infection	2.00 (1.00-4.08)	0.050
Age >25	4.48 (3.15-6.37)	<0.001
Venue-type for recruitment		
Bar / massage center	1	
Family club	0.75 (0.49-1.16)	0.193
Park	10.50 (6.75-16.34)	<0.001
Venue in suburb	4.85 (2.78-8.46)	<0.001
Gender of 1st sex partner for man	1.88 (1.32-2.67)	<0.001
Number of male anal sex partners		
0	1	
1	0.62 (0.25-1.50)	0.287
>1	0.17 (0.07-0.39)	<0.001
Male sex partner from HK		
Yes	1	
No	10.13 (6.94-14.81)	<0.001
Unknown	6.59 (3.71-11.71)	<0.001
Condom use knowledge	1.50 (1.05-2.13)	0.026
Condom use in female sex	1.59 (1.05-2.42)	0.029

AOR: adjusted odds ratio.

A direct comparison between MBs and OMSMs were also performed with the above common candidate variables in combined with HIV infection by logistic regression model. Variables including HIV infection, older in age, being recruited in sauna, park and suburb, having less anal sex partner, having higher condom use knowledge, more frequent using condoms in female sex, first sex partner being man and having no male sex partner from Hong Kong, were the independent correlates of OMSMs compared with MBs.

3.4.5. Summary

This part mainly focused on the prevalence and risk factors for HIV infection in MBs compared with OMSMs using a combined data of two time-location surveys that conducted in year 2008 and 2009. The HIV and syphilis prevalence in MBs (4.5% and 12.9%, respectively) was significantly lower than that in OMSMs (7.0% and 20.2%, respectively). The result was inconsistent with the studies conducted in most

Western countries that indicated a higher prevalence of HIV infection and related higher risky behaviors in MBs

Syphilis infection and venue for recruitment were the variables associated with HIV infection in both MBs and OMSMs While hometown HIV prevalence and self-reported Hong Kong male partner were correlated with HIV infection only in MBs, and early age of first sex was related to HIV infection in OMSMs

A smaller proportion of OMSMs reported encountering male sex partner from Hong Kong compared with MBs Combined with the finding that a greater proportion of OMSMs were infected with HIV, this implies that Hong Kong MSMs coming to Shenzhen mainly contacted with MBs with lower HIV infection This finding could partially explain the previous finding that no similar genetic clustered HIV strain was observed among Shenzhen and Hong Kong HIV positive MSMs A molecular epidemiological study on HIV positive cases collected from our TLS and RDS surveys was conducted and the results were showed in Chapter 4

3.5. Results Part III: Different characteristics were found in MBs and OMSMs frequenting different venues

From the result of HIV infection and related risk factors of the time-location sampling survey, venue-type where MSMs were recruited was always a factor related to HIV risk among both MBs and OMSMs (Table 3-9) Very few previous reports discussed venue-based prevalence of HIV infection among MSM, though most health education and interventions relied on outreach efforts that were fully based on venues Up to this point, most HIV-related health education and intervention strategies have mainly focused on big entertainment venues in Shenzhen for the purpose of covering more target populations through limited outreach effort Health education and condom distribution were only occasionally provided in parks and almost no such service was offered in small venues like family clubs The variance of services offered in different venues might influence the HIV risks To better understand the possible correlation of working venues with HIV risk and

syphilis infection, and to identify risky sexual behaviors among MSMs in different types of venues, the characteristics in MBs or OMSMs by different venue types were compared with a combination of 2008 and 2009 TLS data

The total number of MBs in Shenzhen exceeded 4,000 (documented data from Shenzhen CDC) Most of them were agency-based and controlled or managed by a boss or manager (pimps) They could be divided into two subgroups 1) MBs working at big entertainment venues like bars and massage centers (EMB), acting like waiters or masseurs but providing sex services under the table They usually worked in large groups (16-80 MBs in a venue) 2) MBs acting like call boys in family clubs, a kind of small home-based call boy brothel (FMB) There was a smaller number of MBs in each family club (between 3 and 15 and averaged 8), usually with no license for entertainment services MBs working in small venues outside Shenzhen Special Economic Zone were in the similar situation like FMBs 3) Besides the agency-based MBs, there was a significant proportion of MBs who were self-employed and solicited clients by going to public MSM venues like parks (PMB), thus acting as street MBs Nearly all types of MBs also solicited clients over the Internet

The estimated number of MSMs in Shenzhen was between 65,860 and 96,600 in 2006 [33] Most of them frequented MSM venues including bars, massage centers, saunas, parks, small family clubs, and multi-functional clubs in suburb 1) OMSM visited bars to meet and chat with other MSMs including MBs, drink beverages and watch MSM shows in bars 2) OMSM ordinarily visited massage centers to take massage and sometimes seek MBs Most OMSM visiting bars and massage centers were not fully focus on sex, but had other entertainment purposes besides seeking sex partner Therefore, these two types of OMSMs were combined together in the part as entertainment OMSM (EOMSM) 3) OMSM visiting saunas (SOMSM) could have casual sex within saunas with any known or unknown partners besides having bath there with a very low cost of entry ticket Occasional sex and even group sex

were frequently happened in sauna 4) Park was a kind of public area that provides MSM a free opportunity to contact with other MSMs or even have sex in some dark area in parks OMSM in park (POMSM) normally represented a subgroup of MSM of lower social-economic status 5) OMSMs recruited in family clubs (FOMSM) were ordinary the pimps or clients of MBs who were engaging in paying sex and sex with multiple partners A small group of MBs that were at the starting point of their sex work or had no client in the previous month were not eligible for the MB criteria in the study Thereby, these MBs were also included in the subgroup of FOMSMs if they were eligible for the criteria of OMSMs 6) Venues in suburb that located outside Shenzhen SEZ were different from venues in downtown, with a combination of bar, sauna, video house and Mahjongg room The size of venues in suburb was comparatively small, which result in a small sample size of OMSM outside SEZ (OOMSM)

3.5.1. Comparison of prevalence of HIV infection and HIV related risk factors among MBs recruited from different venues

The venues where MBs were recruited by time-location sampling were recorded and divided into three venue-types (EMBs, PMBs and FMBs) Characteristics of EMB, PMB and FMB were compared based on combined data of 2008 and 2009 TLS surveys

3.5.1.1. HIV/syphilis infections

Of the 850 participants, 38 MBs were found to be HIV-seropositive, with a HIV positive rate of 4 5% (95% CI 3 1-5 9) The rate was 10 3% (95%CI 4 6-20 7) in PMBs, 8 1% (95%CI 5 3-12 0) in FMBs and 1 6% (95%CI 0 8-3 3) in EMBs However, none of the 196 participants from bars was found to infect with HIV The difference in HIV rate was statistically significant among different groups In addition, 110 of the participants were positive for syphilis [21/68 (30 9%, 95%CI 20 5-43 4) from parks, 44/285 (15 4%, 95%CI 11 6-20 3) from family clubs, and

45/497 (9 1%, 95%CI 6 7-12 0) from licensed entertainment venues] The differences were statistically significant among these venues (Table 3-12)

3.5.1.2. Demographic Characteristics

Average age of the 850 MBs enrolled in the study was 23 3 years, with only 19 5% older than 25 years (Table 3-12) Independent PMBs were significantly older and a greater number of them held Shenzhen Hukou compared with those in group venues In addition, PMBs generally had lived in Shenzhen for a longer time, were more likely to live with sexual partners and have other jobs besides engaging in commercially selling of sex A slightly greater proportion of PMBs and FMBs came from regions with high HIV prevalence, such as Sichuan, Chongqing, Yunnan and Guizhou [88] MBs working in larger MB groups were less likely to self-identify as gay or homosexual, and tend to have higher incomes, including both higher monthly incomes and higher payments from each encounter

3.5.1.3. Sexual Practices

Sexual behaviors of these MBs were consistent with their sexual orientation, with a trend of increasing in MBs reporting partner in first sex being male was found with the decreasing of MB's working organization In addition, a greater proportion of EMBs having sex with women and having multiple female sexual partners in the previous 6 months In contrast, 94% of PMBs reported having sex with non-commercial male partners, which was significantly higher than FMBs and EMBs (64%) Furthermore, compared with agency-based MBs, PMBs were more than twice as likely to practice unprotected anal intercourse with both commercial and non-commercial male partners Although all of these MBs had similar number of commercial male partners in the previous 1 month and had anal intercourse with such partners, EMBs encountered more male clients from Hong Kong and had more female clients, but had less experience in sex trade (Table 3-13)

Table 3-11 HIV/syphilis prevalence and HIV-related behaviors in money boys by venue type, 2008-2009

	Participants by Venue Type n (%)					
	EMB (n = 497)	*FMB (n =285)*	*PMB (n = 68)*	*Total n (%) (n = 850)*	X^2	*P*
Test result						
HIV positive	8 (1.6)	23 (8.1)	7 (10.3)	38 (4.5)	23.57	<0.001
Syphilis positive	45 (9.1)	44 (15.4)	21 (30.9)	110 (12.9)	27.67	<0.001
Age（Years）						
18-21	98 (19.7)	73 (25.6)	12 (17.6)	183 (21.5)	10.48	0.033
22-25	311 (62.6)	154 (54.0)	36 (52.9)	501 (58.9)		
>25	88 (17.7)	58 (20.4)	20 (29.4)	166 (19.5)		
Education level						
Junior high school or lower	158 (31.8)	88 (30.9)	30 (44.1)	276 (32 5)	7.29	0.121
Senior high school	271 (54.5)	151 (53.0)	26 (38.2)	448 (52.7)		
College or above	68 (13.7)	46 (16.1)	12 (17.6)	126 (14.8)		
Holding residence card						
Yes	14 (2.8)	16 (5.6)	7 (10.3)	37 (4.4)	11.79	0.019
No	483 (97.2)	269 (94.4)	61 (89.7)	813 (95.6)		
Length of stay in Shenzhen						
<=3 months	165 (33.2)	151 (53.0)	7 (10.3)	323 (38.0)	54 41	<0.001
4-12 months	140 (28.2)	53 (18.6)	25 (36.8)	218 (25.6)		
>1 year	192 (38.6)	81 (28.4)	36 (52.9)	309 (36.4)		
Occupational status						
Migrant worker	13 (2.6)	12 (4.2)	11 (16.2)	36 (4.2)	70.39	<0.001
Commercial service (MB)	446 (89.7)	242 (84.9)	35 (51.5)	723 (85.1)		
Clerk or other	38 (7.6)	31 (10.9)	22 (32.4)	91 (10.7)		
Hometown						
High HIV risk area	70 (14 1)	53 (18 6)	12 (17 6)	135 (15 9)	2.93	0 231
Low HIV risk area	427 (85 9)	232 (81 4)	56 (82 4)	715 (84 1)		
Monthly income						
<=1000	48 (9.7)	41 (14.4)	22 (32.4)	111 (13.1)	31.89	<0.001
1001-5000	345 (69.4)	190 (66.7)	42 (61.8)	577 (67.9)		
>5000	104 (20.9)	54 (18.9)	4 (5.9)	162 (19.1)		
Self-identified sexual orientation						
Homosexual/gay	99 (19.9)	116 (40.7)	38 (55.9)	253 (29.8)	78.89	<0.001
Bisexual	184 (37.0)	89 (31.2)	28 (41.2)	301 (35.4)		
Heterosexual or unsure	214 (43.1)	80 (28.1)	2 (2.9)	296 (34.8)		

EMB MB in entertainment venue. PMB MB in park. FMB MB in family club

Table 3-12 Sexual behavior of money boys by venue type, Shenzhen, China, 2008-2009

	Participants by Venue Type n (%)					
	EMB (n = 497)	*FMB (n =285)*	*PMB (n = 68)*	*Total n (%) (n = 850)*	X^2	*P*
Gender of first sex partner						
Male	99 (19.9)	108 (37.9)	45 (66.2)	252 (29.6)	75.35	<0.001
Female	398 (80.1)	177 (62.1)	23 (33.8)	598 (70.4)		
Female sex partner#						
0	143 (28.8)	157 (55.1)	52 (76.5)	352 (41.4)	95.11	<0.001
1	83 (16.7)	45 (15.8)	3 (4.4)	131 (15.4)		
>1	271 (54.5)	83 (29.1)	13 (19.1)	367 (43.2)		
Number of non-commercial male partners#						
0	179 (36.0)	102 (35.8)	4 (5.9)	285 (33.5)	42.54	<0.001
1-2	130 (26.2)	67 (23.5)	11 (16.2)	208 (24.5)		
>2	188 (37.8)	116 (40.7)	53 (77.9)	357 (42.0)		
Number of commercial male partners, past 1 month						
<=4	261 (52.5)	151 (53.0)	33 (48.5)	445 (52.4)	0.45	0.799
>4	236 (47.5)	134 (47.0)	35 (51.5)	405 (47.6)		
Payment for selling sex to male clients#						
<=500	281 (58.5)	190 (69.3)	57 (86.4)	528 (64.4)	23.99	<0.001
>500	199 (41.5)	84 (30.7)	9 (13.6)	292 (35.6)		
Percentage of HK clients#						
<50%	371 (74.6)	252 (88.4)	64 (94.1)	687 (80.8)	30.60	<0.001
>50%	126 (25.4)	33 (11.6)	4 (5.9)	163 (19.2)		
Commercial female sex#						
Yes	191 (38.4)	57 (20.0)	6 (8.8)	254 (29.9)	45.01	<0.001
No	306 (61.6)	228 (80.0)	62 (91.2)	596 (70.1)		
Sex role in male anal sex#						
Insertive	139 (28.3)	67 (24.4)	14 (22.2)	220 (26.5)	2.94	0.568
Both	271 (55.2)	161 (58.5)	35 (55.6)	467 (56.3)		
Receptive only	81 (16.5)	47 (17.1)	14 (22.2)	142 (17.1)		
Year of selling sex						
<1 year	237 (47.8)	114 (40.0)	25 (37.3)	376 (44.3)	22.24	<0.001
1-3 years	211 (42.5)	152 (53.3)	27 (40.3)	390 (46.0)		
>3 years	48 (9.7)	19 (6.7)	15 (22.4)	82 (9.7)		
UAI in commercial male sex#						
No	386 (77.7)	223 (78.2)	30 (44.1)	639 (75.2)	38.24	<0.001
Yes	111 (22.3)	62 (21.8)	38 (55.9)	211 (24.8)		
UAI in non-commercial male sex#						
No	392 (78.9)	221 (77.5)	31 (45.6)	644 (75.8)	36.83	<0.001
Yes	105 (21.1)	64 (22.5)	37 (54.4)	206 (24.2)		
UAI in male sex#						
No	345 (69.4)	191 (67.0)	20 (29.4)	556 (65.4)	42.80	<0.001

Table 3-13 Sexual behavior of money boys by venue type. Shenzhen, China, 2008-2009 Cont

	Participants by Venue Type n (%)					
	EMB (n = 497)	FMB (n =285)	PMB (n = 68)	Total n (%) (n = 850)	X^2	P
Yes	152 (30 6)	94 (33 0)	48 (70 6)	294 (34 6)		
UAVI with female partners#						
No	287 (57 7)	214 (75 1)	55 (80 9)	556 (65 4)	31 89	<0 001
Yes	210 (42 3)	71 (24 9)	13 (19 1)	294 (34 6)		
UAVI in all sex#						
No	219(44 1)	146(51 2)	16(23 5)	381(44 8)	17 31	<0 001
Yes	278(55 9)	139(48 8)	52(76 5)	469(55 2)		

in the past 6 months, UAI unprotected anal intercourse, UAVI unprotected anal or vaginal intercourse, EMB MB in entertainment venue PMB MB in park, FMB MB in family club

Table 3-13 STIs, drug use, HIV knowledge and services of MB by venue, 2008-2009

	Participants by Venue Type n (%)					
	EMB (n = 497)	FMB (n =285)	PMB (n = 68)	Total n (%) (n = 850)	X^2	P
Ever diagnosed with STIs						
No	57 (11 5)	31 (10 9)	13 (19 1)	101 (11 9)	3 76	0 153
Yes	440 (88 5)	254 (89 1)	55 (80 9)	749 (88 1)		
History of HIV test						
Within 6 months	117 (23 6)	102 (36 2)	23 (33 8)	242 (28 6)	22 08	<0 001
Before 6 months	110 (22 2)	61 (21 6)	22 (32 4)	193 (22 8)		
No	268 (54 1)	119 (42 2)	23 (33 8)	410 (48 5)		
Ever took drugs						
No	341 (68 6)	206 (72 3)	55 (80 9)	602 (70 8)	4 80	0 091
Yes	156 (31 4)	79 (27 7)	13 (19 1)	248 (29 2)		
Ever drunk alcohol before sex#						
No	192 (38 6)	138 (48 4)	25 (36 8)	355 (41 8)	7 90	0 019
Yes	305 (61 4)	147 (51 6)	43 (63 2)	495 (58 2)		
Any kind of HIV education and service#						
No	74 (14 9)	48 (16 8)	8 (11 8)	130 (15 3)	1 24	0 537
Yes	423 (85 1)	237 (83 2)	60 (88 2)	720 (84 7)		
HIV-related knowledge						
Low	129 (26 0)	93 (32 6)	13 (19 1)	235 (27 6)	6 72	0 035
High	368 (74 0)	192 (67 4)	55 (80 9)	615 (72 4)		
Self-perception of HIV infection						
No risk	435 (89 0)	254 (89 1)	52 (76 5)	741 (88 0)	21 02	<0 001
Little risk	52 (10 6)	28 (9 8)	12 (17 6)	92 (10 9)		
High risk	2 (0 4)	3 (1 1)	4 (5 9)	9 (1 1)		

in the past 6 months, STIs sexually transmitted infections EMB MB in entertainment venue, PMB MB in park, FMB MB in family club

3.5.1.4. Self-reported STIs, drug use, accessible services and knowledge

There was a significantly greater proportion of PMBs reporting ever having symptoms of sexually transmitted infections, and being tested for HIV PMBs were less likely to use illegal drugs, while they were more prone to drink alcohol before having sex (Table 3-13)

3.5.1.5. Comparison of HIV-risk related characteristics for PMB vs. EMB and FMB vs. EMB

To further evaluate the determinants for HIV risk from different candidate variables among the different groups, multivariate analysis was conducted and results were shown in Table 3-15. Binary logistic regression model with backward stepwise method was used for the analysis The *P* value for Hosmer and Lemeshow test was >0 05, which indicated a good model fit

Variables were considered in the model including age, length of stay in Shenzhen, occupational status, monthly income, sexual orientation, number of commercial and non-commercial male partners, payment for each encounter with male clients, percentage of male clients from Hong Kong, UAI in commercial and non-commercial male sex, paid sex with women, UAVI in female sex, history of HIV test, drug use, alcohol use before sex, HIV-related knowledge and self-perception of HIV risk

Variables determining the MBs' likelihood of soliciting their clients in a park involved staying in Shenzhen for a longer time, having less monthly income, being migrant worker or having other jobs in addition to the sex trade, self-identifying as gay/homosexual, having a larger number of non-commercial male partners in the past 6 months, receiving lower payment in each encounter with a male clients, selling sex to women, and having been tested for HIV

Being older in age, staying in Shenzhen for a shorter time, self-identified gay/homosexual, receiving lower payment in each encounter with male clients, selling sex to women, having a longer experience of selling sex, and having been tested for HIV were found to be the predicting variables for working in family clubs (Table 3-15).

Table 3-14 Comparison of characteristics for PMB vs. EMB and FMB vs. EMB (n =497) [§]

	PMB (n=68)	FMB (n=252)
	AOR[$] (95% CI)	AOR[$](95% CI)
Age		
18-20	—	1.00 (0.59-1 71)
21-25		0.62 (0.39-0.98)*
>25		1
Length of staying in Shenzhen		
<= 3months	1	1
4-12 months	4.74 (1.39-16.12)*	0.42 (0.27-0.64)*
>12 months	4.38 (1.39-13.86)*	0.45 (0.31-0.66)*
Income level		
<1000	5.88 (1.49-23.20)*	
1001-5000	2.38 (0.69-8.22)	
>5000	1	—
Occupation		
Migrant worker	5.44 (1.07-27.76) *	
Commercial services (MB)	0.21 (0.08-0.52) *	
Cleark or other	1	—
Sexual orientation		
Homosexual	30.80 (6.03-157.16)*	2.24 (1.48-3.39)*
Bisexual	16.10 (3.23-80.30)*	1.08 (0.73-1.62)
Heterosexual/unsure	1	1
No. of non-commercial male partners[#]		
0	1	—
1-2	1.84 (0.45-7.51)	
>2	4.74 (1.33-16.93) *	
Average payment per encounter[#] <500RMB	3.35 (1.35-8.34) *	1.67 (1.17-2.38)*
<50% male clients from Hong Kong[#]	—	2.05 (1 28-3.29)*
Sold sex to women[#]	3.72 (1.32-10 47) *	2.03 (1.38-2.97)*
Year of selling sex		
<1 year	—	1
1-3 years		1 68 (1.19-2.39)*
>3 years		0.85 (0.43-1.68)
History of HIV test		
Never tested	1	1
Within 6 months	3.02 (1.26-7 27) *	1.70 (1.16-2.49)*
Before 6 months	4.32 (1.66-11.20) *	1.07 (0.69-1.64)

[§]: EMB served as reference group. [$] adjusted odds ratio. adjusted for other variables listed in the text; * P < 0.05; [#]: in the past 6 months

EMB MB in entertainment venue. PMB MB in park. FMB MB in family club

3.5.2. Different characteristics among the OMSMs frequenting different venues

The venues where OMSMs were recruited by time-location sampling were recorded and divided into 5 venue-types (EOMSM, POMSM, SOMSM, FOMSM and OOMSM) Characteristics of these 5 subgroups of OMSMs were compared based on a combined data of 2008 and 2009 TLS surveys Since sample sizes of FOMSM and OOMSM were comparatively small, only comparisons of POMSM vs EOMSM and SOMSM vs EOMSM were conducted for multivariate analysis

3.5.2.1. HIV/syphilis infections

Of the 801 participants, 56 OMSMs were found to be HIV-seropositive with a rate of 7 0% (95% CI 5 5-8 5) The rate was 0 9% (95%CI 0 04-5 5) in EOMSMs who frequented entertainment venues including bars and massage centers, 9 3% (95%CI 6 1-13 9) in those in parks (POMSM), 8 1% (95%CI 5 2-12 3) in Sauna OMSMs, 10 1% (95%CI 4 5-20 4) in FOMSM, and 3 5% (95%CI 1 1-9 4) in OOMSM The difference in HIV rate was statistically significant among different groups In addition, 162 were positive for syphilis [11/115 (9 5%, 95%CI 5 1-16 8) from entertainment venues, 55/246 (22 3%, 95%CI 17 4-28 1) from parks, 58/258 (22 4%, 95%CI 17 6-28 2) from saunas, 12/69 (17 3%, 9 7-28 8) from family clubs, and 26/113 (23 0%, 95%CI 15 8-32 1) from venues outside Shenzhen SEZ] The differences were statistically significant among these venues (Table 3-15)

3.5.2.2. Demographic characteristics

Average age among the 801 OMSMs enrolled in the study was 29 5 years, with 37% younger than 25 years and 36% older than 30 (Table 3-15) SOMSMs were significantly older and well educated compared with OMSMs frequenting other venues EOMSMs (13 0%) and SOMSMs (12 8%) were nearly 4 times more likely to hold Shenzhen residence card (hukou) than OOMSM (3 5%) EOMSMs generally had lived in Shenzhen for a shorter time, and were more likely to work in service industry In addition, the income level of EOMSMs and SOMSMs was

comparatively higher than others A greater proportion of EOMSMs self-identified as heterosexual or unsure, while a significantly greater proportion of POMSMs and SOMSMs self-identified as gay or homosexual

3.5.2.3. Sexual Practices

Sexual behaviors of these OMSMs were consistent with their sexual orientation, with a greater proportion of EOMSMs having sex with women, and having multiple female sexual partners within the same time period (Table 3-16) In contrast, 64% of SOMSMs had multiple non-commercial male partners, which was significantly higher than POMSMs (50%) and others (45%) Furthermore, a significantly greater proportion of EOMSMs and SOMSMs encountered male partners from Hong Kong (26%) than POMSMs (14%) and other OMSMs (5%) In addition, POMSMs and OOMSMs were more likely to practice unprotected anal intercourse in male-male sex, while EOMSMs and FOMSMs were more likely to use condoms consistently in male anal sex Condom use rate in sexual contact with women was high in all subgroups (Table 3-16)

3.5.2.4. Self-reported STIs, drug use, accessible services and knowledge

There were a significantly greater proportion of POMSMs and SOMSMs reporting being diagnosed of sexually transmitted infections in the previous year In addition, SOMSM were more likely to receive HIV test EOMSM were more prone to use illegal drugs (28%) or drink alcohol before having sex (60%) In contrast, OOMSMs (3%) had the smallest proportion of drug use with only 3 out of 110 ever tried drugs (Table 3-17)

The accessibility of HIV-related services among OMSMs in different venues were different, with a better coverage in saunas and family clubs (88%), moderate coverage in bars (80%), and comparatively low coverage in parks (77%) and venues in suburb (71%) HIV-related knowledge was high among all groups with an average

of 84%, while a significantly lower knowledge level was observed among FOMSMs (68 1%) and OOMSMs (75 2%)

Table 3-15 HIV/syphilis prevalence and demographic characteristics in OMSMs by venue type, 2008-2009

	Participants by Venue Type n (%)							
	EOMSM (n = *115*)	*POMSM* (n = *246*)	*SOMSM* (*n=258*)	*FOMSM* (n =*69*)	*OOMSM* (*n=113*)	*Total n (%)* (n = *801*)	X^2	*P*
Test results								
HIV positive	1 (0.9)	23 (9.3)	21 (8.1)	7 (10.1)	4 (3.5)	56 (7.0)	12.38	0.015
Syphilis positive	11 (9.6)	55 (22.4)	58 (22.5)	12 (17.4)	26 (23.0)	162 (20.2)	10.49	0.033
Self-identified sexual orientation								
Homosexual/gay	55 (47.8)	149 (60.6)	157 (60.9)	34 (49.3)	55 (48.7)	450 (56.2)	29.11	<0.001
Bisexual	33 (28.7)	78 (31.7)	77 (29.8)	24 (34.8)	47 (41.6)	259 (32.3)		
Heterosexual or unsure	27 (23.5)	19 (7.7)	24 (9.3)	11 (15.9)	11 (9.7)	92 (11.5)		
Age（Years）								
<25	73 (63.5)	115 (46.7)	42 (16.3)	32 (46.4)	32 (28.3)	294 (36.7)	101.46	<0.001
25-30	22 (19.1)	61 (24.8)	86 (33.3)	15 (21.7)	35 (31.0)	219 (27.3)		
>30	20 (17.4)	70 (28.5)	130 (50.4)	22 (31.9)	46 (40.7)	288 (36.0)		
Education level								
Junior high school or lower	19 (16.5)	57 (23.2)	35 (13.6)	26 (37.7)	32 (28.3)	169 (21.1)	56.85	<0.001
Senior high school	57 (49.6)	109 (44.3)	88 (34.1)	26 (37.7)	57 (50.4)	337 (42.1)		
College or above	39 (33.9)	80 (32.5)	135 (52.3)	17 (24.6)	24 (21.2)	295 (36.8)		
Length of stay in Shenzhen								
<=3 months	35 (30.4)	37 (15.0)	64 (24.8)	20 (29.0)	11 (9.7)	167 (20.8)	41.55	<0.001
4-12 months	24 (20.9)	39 (15.9)	20 (7.8)	8 (11.6)	13 (11.5)	104 (13.0)		
>1 year	56 (48.7)	170 (69.1)	174 (67.4)	41 (59.4)	89 (78.8)	530 (66.2)		

Table 3-16 HIV/syphilis prevalence and demographic characteristics in OMSMs by venue type, 2008-2009, Cont

	Participants by Venue Type n (%)							
	EOMSM (n = *115*)	*POMSM* (n = *246*)	*SOMSM* (*n=258*)	*FOMSM* (n =*69*)	*OOMSM* (*n=113*)	*Total n (%)* (n = *801*)	X^2	*P*
Residence card holding								
Yes	15 (13 0)	17 (6 9)	33 (12 8)	6 (8 7)	4 (3 5)	75 (9 4)	39 22	<0 001
No	100 (87 0)	229 (93 1)	225 (87 2)	63 (91 3)	109 (96 5)	726 (90 6)		
Marital status								
Unmarried	106 (92 2)	189 (76 8)	174 (67 4)	49 (71 0)	64 (56 6)	582 (72 7)	45 49	<0 001
Married	7 (6 1)	43 (17 5)	68 (26 4)	13 (18 8)	36 (31 9)	167 (20 8)		
Divorced or other	2 (1 7)	14 (5 7)	16 (6 2)	7 (10 1)	13 (11 5)	52 (6 5)		
Occupational status								
Migrant worker	10 (8 7)	34 (13 8)	33 (12 8)	10 (14 5)	36 (31 9)	123 (15 4)	41 09	<0 001
Commercial service	69 (60 0)	121 (49 2)	108 (41 9)	37 (53 6)	45 (39 8)	380 (47 4)		
Clerk or other	36 (31 3)	91 (37 0)	117 (45 3)	22 (31 9)	32 (28 3)	298 (37 2)		
Monthly income								
<=1000	12 (10 4)	30 (12 2)	25 (9 7)	12 (17 4)	9 (8 0)	88 (11 0)	76 47	<0 001
1001-3000	52 (45 2)	142 (57 7)	87 (33 7)	32 (46 4)	61 (54 0)	374 (46 7)		
3001-5000	28 (24 3)	53 (21 5)	69 (26 7)	20 (29 0)	34 (30 1)	204 (25 5)		
>5000	23 (20 0)	21 (8 5)	77 (29 8)	5 (7 2)	9 (8 0)	135 (16 9)		

* adjusted by recruitment rate at each event considering homogeneity of persons sampled in each VDT

EOMSM OMSM in entertainment venue, POMSM OMSM in park, SOMSM OMSM in sauna, FOMSM OMSM in family club, OOMSM OMSM in other venue

Table 3-16 Sexual behavior of OMSMs by venue type, Shenzhen, China, 2008-2009

	Participants by Venue Type n (%)							
	EOMSM (n = *115*)	*POMSM* (n = *246*)	*SOMSM* (*n=258*)	*FOMSM* (n =*69*)	*OOMSM* (*n=113*)	*Total n (%)* (n = *801*)	X^2	*P*
Gender of first sex partner								
Male	62 (53.9)	149 (60.6)	141 (54.7)	37 (53.6)	56 (49.6)	445 (55.6)	4.47	0.347
Female	53 (46.1)	97 (39.4)	117 (45.3)	32 (46.4)	57 (50.4)	356 (44.4)		
Female sex partner#								
0	80 (69.6)	196 (79.7)	192 (74.4)	52 (75.4)	81 (71.7)	601 (75.0)	19.77	0.011
1	20 (17.4)	38 (15.4)	56 (21.7)	13 (18.8)	28 (24.8)	155 (19.4)		
>1	15 (13.0)	12 (4.9)	10 (3.9)	4 (5.8)	4 (3.5)	45 (5.6)		
No. of male anal sex partners#								
0	10 (8.7)	9 (3.7)	12 (4.7)	6 (8.7)	6 (5.3)	43 (5.4)	28.08	<0.001
1	51 (44.3)	114 (46.3)	80 (31.0)	30 (43.5)	60 (53.1)	335 (41.8)		
>1	54 (47.0)	123 (50.0)	166 (64.3)	33 (47.8)	47 (41.6)	423 (52.8)		
UAI in male sex#								
No	74 (64.3)	109 (44.3)	138 (53.5)	43 (62.3)	56 (49.6)	420 (52.4)	16.25	0.003
Yes	41 (35.7)	137 (55.7)	120 (46.5)	26 (37.7)	57 (50.4)	381 (47.6)		
UAVI with female partners								
No	94 (81.7)	215 (87.4)	215 (83.3)	58 (84.1)	91 (80.5)	673 (84.0)	3.65	0.455
Yes	21 (18.3)	31 (12.6)	43 (16.7)	11 (15.9)	22 (19.5)	128 (16.0)		

*: adjusted by recruitment rate at each event considering homogeneity of persons sampled in each VDT; #: in the past 6 months; UAI: unprotected anal intercourse; UAVI: unprotected anal or vaginal intercourse.

EOMSM: OMSM in entertainment venue; POMSM: OMSM in park; SOMSM: OMSM in sauna; FOMSM: OMSM in family club; OOMSM: OMSM in other venue.

Table 3-17 STIs, drug use, HIV knowledge and services of OMSMs by venue type, Shenzhen, China, 2008-2009

	Participants by Venue Type n (%)							
	EOMSM (n = 115)	*POMSM (n = 246)*	*SOMSM (n=258)*	*FOMSM (n =69)*	*OOMSM (n=113)*	*Total n (%) (n = 801)*	X^2	*P*
Ever diagnosed with STIs								
No	107 (93.0)	210 (85.4)	210 (81.4)	66 (95.7)	104 (92.0)	697 (87.0)	18.58	0.001
Yes	8 (7.0)	36 (14.6)	48 (18.6)	3 (4.3)	9 (8.0)	104 (13.0)		
History of HIV test								
Within 6 months	26 (22.6)	55 (22.4)	66 (25.7)	12 (17.6)	19 (16.8)	178 (22.3)	17.00	0.030
Before 6 months	32 (27.8)	62 (25.2)	84 (32.7)	30 (44.1)	31 (27.4)	239 (29.9)		
No	57 (49.6)	129 (52.4)	107 (41.6)	26 (38.2)	63 (55.8)	382 (47.8)		
Ever took drugs								
No	83 (72.2)	220 (89.4)	230 (89.1)	61 (88.4)	110 (97.3)	704 (87.9)	37.13	<0.001
Yes	32 (27.8)	26 (10.6)	28 (10.9)	8 (11.6)	3 (2.7)	97 (12.1)		
Alcohol use before sex								
No	46 (40.0)	163 (66.3)	184 (71.3)	50 (72.5)	79 (69.9)	522 (65.2)	39.26	<0.001
Yes	69 (60.0)	83 (33.7)	74 (28.7)	19 (27.5)	34 (30.1)	279 (34.8)		
Any kind of HIV education and service								
No	21 (18.3)	57 (23.2)	30 (11.6)	8 (11.6)	33 (29.2)	149 (18.6)	22.31	<0.001
Yes	94 (81.7)	189 (76.8)	228 (88.4)	61 (88.4)	80 (70.8)	652 (81.4)		
HIV-related knowledge								
Low	23 (20.0)	26 (10.6)	28 (10.9)	22 (31.9)	28 (24.8)	127 (15.9)	31.51	<0.001
High	92 (80.0)	220 (89.4)	230 (89.1)	47 (68.1)	85 (75.2)	674 (84.1)		
Condom use knowledge								
Low	30 (26.1)	69 (28.0)	68 (26.4)	22 (31.9)	43 (38.1)	232 (29.0)	6.24	0.182
High	85 (73.9)	177 (72.0)	190 (73.6)	47 (68.1)	70 (61.9)	569 (71.0)		

*: adjusted by recruitment rate at each event considering homogeneity of persons sampled in each VDT.

EOMSM: OMSM in entertainment venue; POMSM: OMSM in park; SOMSM: OMSM in sauna; FOMSM: OMSM in family club; OOMSM: OMSM in other venue.

3.5.2.5. Comparison of characteristics of OMSM visiting different venues (POMSM vs. EOMSM and FOMSM vs. EOMSM)

To further evaluate the predicting characteristics for OMSMs visiting different venues, multivariate analysis was conducted in comparing POMSM and EOMSM, FOMSM and EOMSM (result was shown in Table 3-19) FOMSM and OOMSM were not analyzed because their sample sizes were too small

Table 3-18 Comparison of characteristics for OMSMs visiting different venues [POMSM vs EOMSM and SOMSM vs EOMSM (n = 115)] [$]

	POMSM (n = 246)	SOMSM (n = 258)
	AOR[$] (95% CI)	AOR[$](95% CI)
Age		
<=25	—	1
26-30		7 42 (3 65-15 07)*
>30		6 74 (3 19-14 22)*
Education		
Junior high school or lower	—	0 78 (0 33-1 82)
Senior high school		0 48 (0 26-0 89)*
College above		1
Length of stay in Shenzhen		
<= 3months	1	—
4-12 months	1 55 (0 69-3 48)	
>12 months	2 64 (1 38-5 05)*	
Income		
<1000	4 30 (1 46-12 61)*	
1001-5000	3 17 (1 45-6 94)*	
>5000	1	—
Being married	1 59 (1 06-2 40)*	1 79 (1 13-2 84)*
Sexual orientation		
Homosexual	4 25 (1 87-9 63)*	2 97 (1 31-6 70)*
Bisexual	3 26 (1 36-7 81)*	2 08 (0 85-5 04)
Heterosexual/unsure	1	1
>1 male sex partners[#]	—	1 41 (1 06-1 87)*
Drug use[#]	0 42 (0 21-0 84)*	—
Drunk alcohol before having sex[#]	0 41 (0 24-0 70)*	0 20 (0 11-0 36)*
UAI in male sex[#]	2 41 (1 43-4 08)*	—

[$] EOMSM served as reference group [$] adjusted odds ratio, adjusted for other variables listed in the text * P<0 05, # in the past 6 months. UAI unprotected anal intercourse EOMSM OMSM in entertainment venue, POMSM OMSM in park, SOMSM OMSM in sauna

Variables were considered in the models including age, education, length of stay in Shenzhen, occupational status, monthly income, marital status, sexual orientation, number of male anal sex partners, UAI in male sex, UAVI in female sex, history of HIV test, drug use, alcohol use and HIV-related knowledge Backward stepwise approach was used to select significant variables Hosmer and Lemeshow test was non-significant with P >0 05, indicating that the model fit was good

Variables associated with OMSMs frequenting parks were staying in Shenzhen for a longer time, having less income, being married, self-identifying as gay/homosexual or bisexual, no drug use, no alcohol use before having sex with another man, and practicing unprotected anal intercourse with men

Increasing age, lower education level, being married, self-identified gay/homosexual, having multiple male sexual partners, and no alcohol use before having sex with another man were found to the determinants for OMSMs frequenting saunas

3.5.3. Summary

Since HIV infection was significantly different in MBs or OMSMs in different venues, this part mainly focused on the comparison of characteristics in MBs and OMSMs recruited from different venues based on venue type where participants were recruited Venues with high HIV infection rate including parks, family clubs and saunas were compared with venue with low HIV infection rate (entertainment venues including both bars and massage centers)

PMBs and FMBs were at higher HIV infection rate, were more likely to self-identify as gay or homosexual and earned less in each sexual encounter with clients compared with EMBs However, EMB were more likely to sell sex to women and became a bridge population for transmitting HIV to general population Intervention efforts should be targeted toward all these subgroups of MBs and different strategies for different subgroups were recommended

Being married and self-identifying as homosexual or gay were the predictors for OMSMs frequenting parks and saunas compared with OMSMs in bars and massage centers The large proportion of married MSMs frequenting MSM venues with higher HIV infection rate indicated that the intervention efforts should be strengthened on this risky bridge population

Although the overall prevalence of HIV in MSMs was still on the average level of China MSM, the infection rate in some venues was similar to the level in high prevalence areas However, MSMs in high risk venues had significantly higher likelihood of having sexual contact with women

3.6. PART IV: RDS survey and the comparison between RDS and TLS survey

3.6.1. Background

Because of the stigmatization of homosexuality in contemporary China, social taboos and discrimination lead MSM to be discreet about their sexual behavior and thus are hard-to-reach [216] Most studies of MSM in China recruited participants by convenience sampling from key volunteers or venues [29, 74, 217] While convenience sampling is not a statistically valid sampling approach and leads to biased selection of subjects Time-location sampling (TLS) is a venue based probability sampling method, and widely accepted, while the representativeness of TLS needs to concern the impact of non-venue visitors to the whole population Respondent-driven sampling (RDS), on the other hand, has the highest potential in the coverage of the target population, though it is not a method that based on random selection A typical RDS combines network-based sampling and a mathematical model of the recruitment process that weights the sample to compensate for non-random recruitment patterns With careful recording and tracing of the recruitment track, as well as the complicated statistical analysis for weighting and homogenization, the RDS could also acquired a quasi-probability sample [139]

The RDS survey was tried as an alternative to TLS in the similar time period to assess its advantages in reaching more "hidden" MSM This section described the distributions of the studied variables among MSMs from an RDS survey The main objective of the section was to provide information on the comparison between RDS and TLS survey The results could be used to assess the coverage of TLS for MSM in Shenzhen and guide the selection of sampling methods for MSM in future survey

3.6.2. Prevalence of HIV infection and related risk factors of MSM by RDS survey

3.6.2.1. Description of RDS recruitment

Figure 3-2 and Figure 3-3 showed procedure diagram of RDS recruitment of the 492 MSM The number of waves per initial respondent ranged from 0 to 18, and the highest recruitment was achieved in the fourth wave The reported social network sizes ranged from 1 to 1000 (median, 10, interquartile range [IQR], 4–18) The homophily index provided information about the tendency among study participants to recruit others with characteristics like or unlike their own [111] Homophily index values of greater than ±0 7 were thought to be problematic in RDS studies because design effects were estimated at 4 or more, with steep increased in design effect as homophily values rise [112, 218] While in practice, a figure of 0 4 was commonly used as the hard line of demarcation defines low homophily The homophily scores of -0 14 to 0 37 were observed in this study with HIV status (-0 016 to -0 002), syphilis status (-0 02 to 0 076), venue group (-0 101 to 0 368), and risk behavioral group categorized by ever reported unprotected anal intercourse in male sex (-0 144 to 0 225) This result demonstrated that the recruitment patterns were generally consistent with random mixing

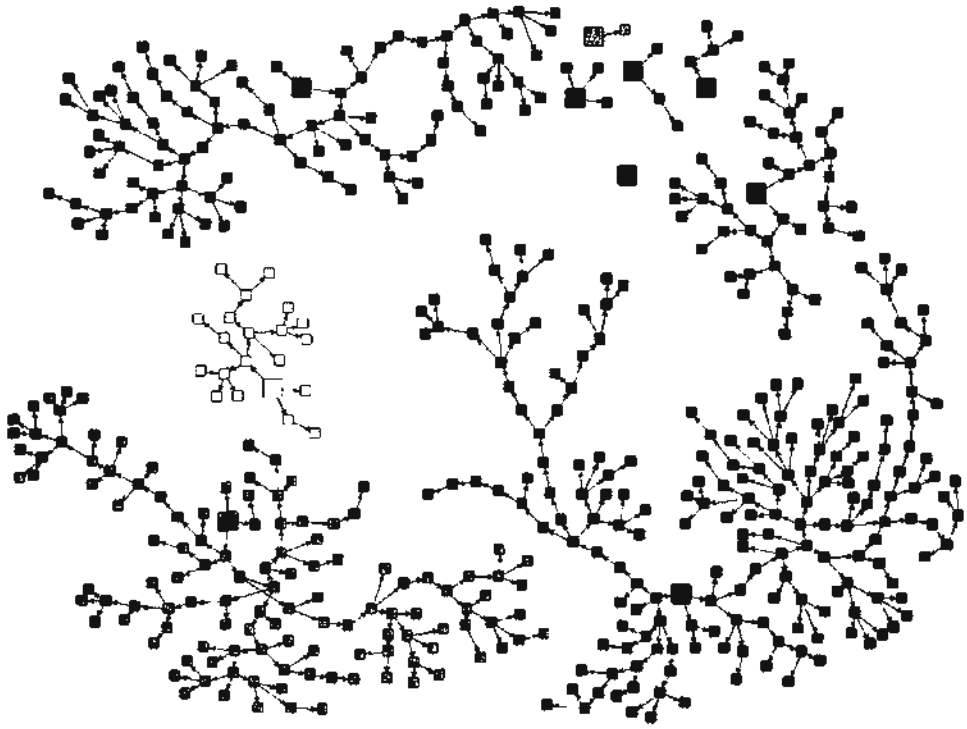

Figure 3-2 Diagram of RDS sampling procedure among MSM (n=492) in Shenzhen, 2009
Small: recruit; big: seeds

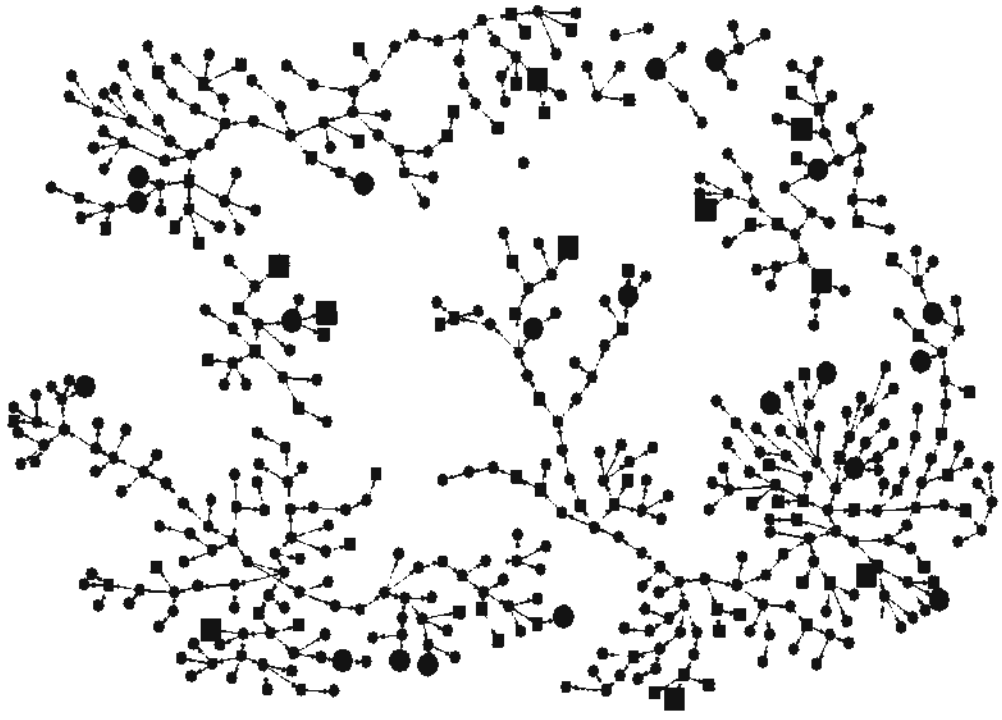

Figure 3-3 The recruitment of HIV/syphilis positive compared to condom use
Small: HIV negative; big: HIV positive
Circle: syphilis negative; Square: syphilis positive
Blue: use condom with both male and female partner; red: not always use condom in male sex; green: use condom in male sex but not use in female sex

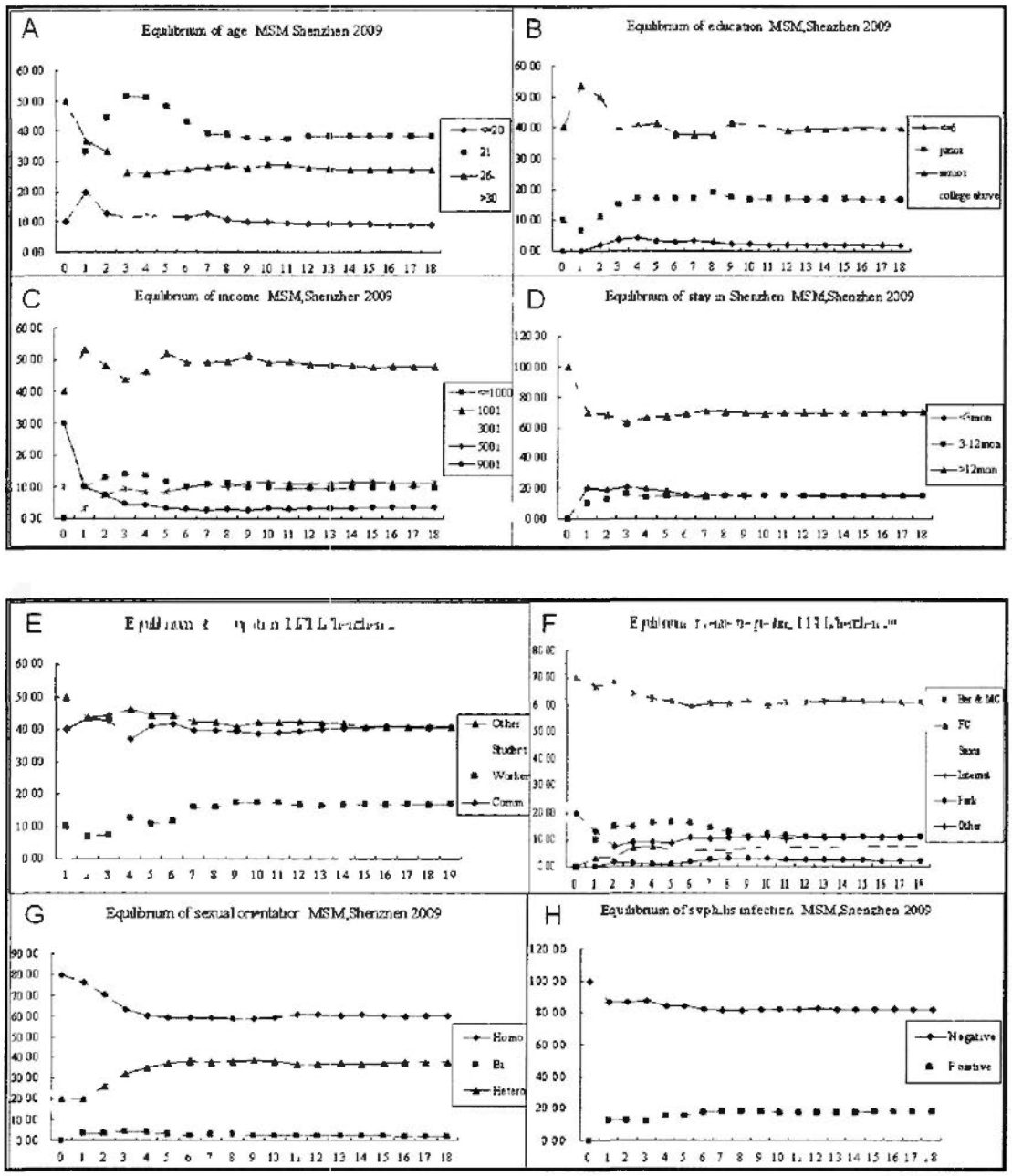

Figure 3-4 Equilibrium for demographic variables in RDS survey of MSM, Shenzhen, 2009
A: age; B: education level; C: income level; D: length of stay in Shenzhen;
E: occupation; F: venue for finding sex partners; G: sexual orientation; H: syphilis infection

Data for the adjusted analyses were sufficient when the sample reaches "equilibrium", that was, when additional waves of recruitment do not substantially change the composition of the sample with respect to key variables In this survey, the composition of socio-economic status (SES) was tracked in the sample In practice, equilibrium was usually achieved in four to five waves for most variables [111]

Equilibrium was achieved by wave 7 with regard to age, hukou (official residence status), educational level, income level, occupation, venue where they typically meet sexual partners, sexual orientation, and current syphilis infection rate Recruitment continued to the 18th wave (Figure 3-4)

3.6.2.2. Demographic characteristics of MSMs by RDS

Thirty MSMs were tested HIV-seropositive, with a crude Prevalence of HIV infection of 6 1% and adjusted Prevalence of HIV infection (adjusted for network size and linkage between recruiters and recruits) of 4 6% (95% CI 2 59-6 7%) (Table 3-19) The crude and adjusted prevalence of current syphilis was 18 3% and 16 4% (95% CI 11 9-21 0%) The average age of the subjects was 27 2 ±6 3 years Nearly all of the MSMs (98 4%) had received at least a junior high school education (over 9 years) 8 5% of the participants held official residence cards (hukou) in Shenzhen Most of the MSMs came from other provinces of China, while 15 6% came from other parts of Guangdong province Only 7% came from high HIV prevalence areas More than 70% of the participants had been living in Shenzhen for more than 1 year, most of whom lived in Luohu district, the most economically vibrant district in Shenzhen Over 90% of the MSMs earned over 1,000 RMB (approximately 147 US Dollars) per month, and more than half earned over 3,000 RMB (approximately 441 US Dollars, the average income level in Shenzhen) 67 9% of the participants self-identified as homosexual or gay, and 25 2% self identified as bisexual In univariate analysis, being less educated, staying shorter in Shenzhen and ever being married were positively associated with HIV infection (Table 3-19)

3.6.2.3. HIV-related sexual behaviors of the MSMs recruited from RDS

Table 3-20 showed sexual practice and behavior patterns of the participants 45 MSMs self-reported involving in paid sex with men in the past 6 months (adjusted percentage of 8 7%) According to the information from the individuals that attended both TLS and RDS survey, the behaviors of selling sex to men were underreported in RDS There were 42 4% (14/33) of the MSMs who participated in both TLS and

RDS survey admitted their paid sex behaviors in TLS while denied in RDS Took these 14 MBs into consideration, the proportion of MBs in Shenzhen MSMs would be at least 12 0% More than 60% of the participants typically meet their sex partners through the Internet, while 8 9% in saunas or parks and 9 6% in bars or massage centers The average number of male and female sex partners within the previous 6 months was 2 37 and 0 17, respectively While as much as 69 5% of the MSMs reported only having one male anal sex partners and less than 20% had sexual contact with female in the previous 6 months More than 80% of the MSMs had no sex partner from Hong Kong 32 2% reported that they had engaged in unprotected male-to-male anal sex within the past 6 months, including 31 5% who reported unprotected non-commercial male anal sex

3.6.2.4. Self-reported STIs, drug use, accessible services and knowledge from RDS

Table 3-21 showed that 54 2% of the participants never received HIV test before, and 17 8% received a HIV test in the previous 6 months The level of HIV-related knowledge was low, with only 61 1% responded correctly to at least six out of the nine items Their level of condom use knowledge was also poor, indicated by that more than 44 3% gave incorrect answer to at least 2 out of 5 items

Table 3-19 Demographic characteristics for MSMs recruited from RDS in Shenzhen, 2009

Variable	*Crude %(n)*	*Adjusted %(95%CI)**	*HIV+ %(95%CI)**	*P*
Overall	100 (492)	/	4.6 (2.5-6.7)	
Age（Years）				0.577
<=20	9.0 (44)	7.6 (4.6-11.1)	4.3 (0.0-10.4)	
21-25	38.5 (189)	37.2 (30.9-42.9)	4.0 (0.9-8.3)	
26-30	27.5 (135)	24.6 (20.0-30.4)	3.0 (0.7-5.5)	
>30	25.1 (123)	30.6 (24.1-36.6)	6.8 (2.4-11.6)	
Education level				0.006
Primary school or lower	1.8 (9)	1.4 (0.3-3.0)	15.7 (0.0-59.8)	
Junior high school	16.7 (82)	14.3 (10.2-18.4)	11.1 (2.5-21.9)	
Senior high school	39.8 (196)	34.4 (28.8-39.8)	6.0 (2.2-10.6)	
College or above	41.7 (205)	50.0 (44.0-56.2)	1.7 (0.5-3.0)	
Residence card held				0.252
Shenzhen	5.5 (27)	8.5 (5.2-12.3)	1.1 (0.0-2.8)	
Guangdong province	15.0 (74)	15.6 (11.4-20.4)	7.7 (0.8-17.5)	
others	79.5 (391)	75.9 (70.3-81.0)	4.5 (2.3-6.6)	
Hometown				0.053
Low prevalent	88.8 (437)	93.0 (90.6-95.4)	4.1 (2.0-6.4)	
High prevalent	11.2 (55)	7.0 (4.6-9.4)	11.2 (3.1-21.4)	
Length of stay in Shenzhen				0.044
<=3 months	15.2 (75)	13.6 (9.7-17.0)	10.7 (2.3-22.3)	
4-12 months	14.4 (71)	12.2 (8.1-16.3)	5.6 (1.1-11.8)	
>1 year	70.3 (346)	74.2 (69.3-80.0)	3.4 (1.4-5.4)	
Occupation				0.439
Clerk or others	40.7 (200)	26.0 (21.2-31.3)	6.3 (3.1-9.6)	
Student	1.8 (9)	2.5 (0.9-4.3)	1.2 (-)[#]	
Migrant worker	16.9 (83)	14.9 (10.7-19.0)	5.4 (1.6-10.2)	
Commercial services	40.7 (200)	56.6 (50.5-63.0)	3.8 (0.7-7.7)	
Monthly income				0.623
<=1000 RMB	9.8 (48)	6.9 (4.6-9.7)	13.4 (3.3-21.4)	
1001-3000 RMB	47.8 (235)	37.4 (31.0-42.8)	4.9 (2.7-7.3)	
3001-5000 RMB	27.6 (136)	33.1 (27.2-39.0)	4.3 (0.4-9.0)	
>5000 RMB	14.9 (73)	22.6 (16.3-30.7)	4.1 (0.0-8.0)	
Marital status				<0.001
Unmarried	82.7 (407)	82.1 (77.3-86.8)	3.3 (1.5-5.2)	
Married	13.8 (68)	16.7 (11.9-21.3)	8.6 (1.5-17.1)	
Divorced or other	3.5 (17)	1.2 (0.7-2.1)	41.5 (6.6-65.1)	
Sexual orientation				0.151
Homosexual/gay	60.2 (296)	67.9 (62.0-73.3)	3.8 (1.7-6.1)	
Bisexual	32.1 (158)	25.2 (20.6-30.3)	7.9 (2.6-13.9)	
Heterosexual/unsure	7.7 (38)	6.9 (4.1-10.2)	0.7 (-)[#]	

*: Adjusted for network size and linkage between recruiters and recruits.

#: RDSAT failed to generate 95% CI since the number of some subgroups was too small

Table 3-20 Sexual behavior patterns for MSMs recruited from RDS in Shenzhen, 2009

Variable	*Crude %(n)*	*Adjusted %(95%CI)**	*HIV+ %(95%CI)**	*P*
Venue frequenting				0.072
Bar & MC	11.0 (54)	9.6 (6.0-13.4)	9.3 (0.0-23.2)	
FC	7.7 (38)	5.5 (3.3-7.6)	6.2 (0.6-14.0)	
Sauna	6.9 (34)	5.5 (3.2-8.2)	14.3 (2.9-27.6)	
Internet	61.0 (300)	62.3 (56.7-68.5)	3.8 (1.7-6.4)	
Park	2.2 (11)	3.4 (1.1-5.9)	2.3 (-)[#]	
Other	11.2 (55)	13.7 (9.6-18.5)	0.9 (0.0-2.2)	
Number of male sex partners				0.026
0	4.3 (21)	5.4 (2.8-8.4)	0.8 (-)[#]	
1	52.6 (259)	69.5 (63.7-74.6)	3.1 (1.2-5.6)	
>1	43.1 (212)	25.1 (20.5-30.4)	9.7 (4.2-15.6)	
Gender of first sex partner				0.029
Male	66.5 (327)	71.7 (65.9-76.7)	3.2 (1.4-5.3)	
Female	33.5 (165)	28.3 (23.3-34.1)	8.0 (2.9-13.5)	
Age of 1st sex				0.689
<=18	26.8 (132)	20.4 (15.8-25.2)	3.2 (0.9-5.1)	
19-20	28.5 (140)	23.7 (18.9-29.0)	7.3 (1.7-14.4)	
21-22	20.3 (100)	27.8 (21.8-33.5)	4.5 (0.2-9.4)	
>22	24.4 (120)	28.0 (22.5-34.2)	5.0 (2.0-8.3)	
No. of female sex partner				0.274
0	81.3 (400)	82.6 (78.2-87.1)	4.2 (2.2-6.1)	
1	11.2 (55)	11.6 (7.8-15.2)	9.4 (0.4-21.4)	
>1	7.5 (37)	5.8 (3.3-8.8)	4.0 (0.0-9.4)	
Sold sex to men				0.244
Yes	9.1 (45)	8.7 (5.0-12.8)	0.2 (0.0-0.3)	
No	90.9 (447)	91.3 (87.2-95.0)	5.1 (2.7-7.3)	
UAI in male anal sex				0.002
Yes	43.7 (215)	32.2 (26.7-38.1)	8.8 (4.4-14.3)	
No	56.3 (277)	67.8 (61.9-73.3)	2.5 (0.7-4.8)	
UAVI in female sex				0.257
Yes	10.2 (50)	9.6 (6.4-13.6)	10.3 (0.0-24.1)	
No	89.8 (442)	90.4 (86.4-93.6)	4.1 (2.3-6.0)	
Consistent condom use				0.009
Not always use	48.4 (238)	35.7 (30.2-41.8)	7.9 (3.9-12.7)	
Always use	51.6 (254)	64.3 (58.2-69.8)	2.7 (0.8-5.0)	
Sexrole in male anal sex				0.759
Insertive only	33.2 (159)	32.3 (26.7-38.3)	4.8 (0.9-9.6)	
Both	50.5 (242)	49.8 (43.7-55.9)	5.7 (2.3-8.8)	
Receptive only	16.3 (78)	17.9 (13.2-22.5)	2.0 (0.0-4.2)	

*: Adjusted for network size and linkage between recruiters and recruits.

[#]: RDSAT failed to generate 95% CI since the number of some subgroups was too small

Table 3-21 STIs, drug use, HIV knowledge and services in MSMs recruited from RDS

Variable	*Crude %(n)*	*Adjusted %(95%CI)**	*HIV+ %(95%CI)**	*P*
Ever diagnosed with STIs				0 330
Yes	8 5 (42)	4 8 (3 3-7 1)	11 1 (1 7-16 7)	
No	91 5 (450)	95 2 (92 9-96 7)	4 6 (2 1-6 3)	
Most recent test for HIV				0 495
<=6m	21 5 (106)	17 8 (13 5-22 8)	3 1 (0 3-5 0)	
>6m	26 4 (130)	28 0 (23 0-34 1)	5 7 (0 8-9 9)	
Never	52 0 (256)	54 2 (47 3-59 0)	5 2 (2 5-8 6)	
Male sex partner from Hong Kong				0 265
Yes	14 2 (70)	9 7 (6 6-13 2)	4 2 (0 0-8 0)	
No	77 8 (383)	83 1 (78 5-87 0)	5 2 (2 7-7 7)	
Unsure	7 9 (39)	7 2 (4 6-10 6)	2 2 (-)[#]	
Drug use ever				1 000
No	90 2 (444)	92 5 (89 3-95 1)	4 7 (2 5-6 9)	
Yes	9 8 (48)	7 5 (4 9-10 7)	5 9 (0 0-11 2)	
Alcohol use before sex				0 164
No	56 8 (197)	66 7 (61 5-75 4)	3 6 (1 4-5 6)	
Yes	43 2 (150)	33 3 (24 6-38 5)	5 1 (0 1-3 2)	
HIV-related knowledge				0 096
Low	16 1 (79)	38 9 (31 3-46 4)	3 6 (0 0-6 8)	
High	83 9 (413)	61 1 (53 6-68 7)	6 0 (3 2-8 5)	
Condom use knowledge				0 033
Low	22 0 (108)	44 3 (37 4-51 2)	1 4 (0 0-2 7)	
High	78 0 (384)	55 7 (48 8-62 6)	6 7 (3 5-9 3)	
Any kind of HIV-related services				0 001
No	28 5 (140)	26 3 (20 7-30 8)	8 9 (3 8-15 4)	
Yes	71 5 (352)	73 7 (69 2-79 3)	3 0 (1 2-5 0)	
Syphilis infection				0 388
Yes	81 5 (401)	83 5 (79 0-88 0)	4 2 (2 0-6 9)	
No	18 3 (90)	16 4 (11 9-20 9)	6 2 (2 6-10 5)	

* Adjusted for network size and linkage between recruiters and recruits

[#] RDSAT failed to generate 95% CI since the number of some subgroups was too small

3.6.2.5. Multivariate analysis for examining factors associated with HIV infection

Table 3-22 showed the results of multivariate logistic regression analysis of data from MSM who were recruited from RDS survey Variables associated with HIV infection including staying in Shenzhen for less than 3 months, being married, coming from high HIV prevalence areas, having more than one non-commercial

male sex partners and receiving HIV-related services in the previous 6 months The risk of HIV infection was 4 3 times higher among MSM who had multiple male sex partners than those who did not, after adjusting for age, education, sexual orientation, venue for seeking partners, UAI in male sex and UAVI in female sex, HIV test and drug use history, HIV-related knowledge and condom use knowledge, and syphilis infection The risk of HIV infection was 5 2 times higher among MSM who came from high HIV prevalence area in China, 2 9 and 2 7 times higher among recent arrived MSM and married MSM, respectively Receiving HIV-related services was a protective factor for HIV infection in multivariate analysis

Table 3-22 Risk factors for HIV infection among MSM in Shenzhen, 2009 RDS survey

	AOR* (95%CI)	P
No. of male anal sex partners >1, past 6 months	4.30 (1.69-10.94)	<0 001
Hometown in high HIV prevalence area	5.22 (1.49-18.33)	0.011
Live in Shenzhen for less than 3 months	2.94 (1.16-7.40)	0.021
Ever married	2.74 (1 07-7 02)	0 041
Ever received HIV-related services, past 6 months	0.26 (0.10-0.66)	<0.001

*: AOR was adjusted for other confounder variables including. age, education level, sexual orientation, venue for meeting partners, UAI in male sex and UAVI in female sex, HIV test and drug use history, HIV-related knowledge and condom use knowledge, and syphilis infection

3.6.3. Comparison of characteristics of OMSMs from TLS and RDS survey

3.6.3.1. Demographic Characteristics and HIV/syphilis infections

Since MBs accounted for a very small proportion of the total MSMs that recruited from the RDS survey, but MBs and OMSMs were equally recruited in TLS survey, only characteristics of OMSMs were compared There were 465 OMSMs recruited from TLS and 447 OMSMs from RDS, from May to November 2009

Table 3-24 showed that the HIV positive rate was 5 8% in TLS and 5 1% in RDS, while the syphilis positive rate was 18 3% in TLS and 16 7% in RDS The average age of the OMSMs recruited from TLS and RDS was 30 2±8 0 and 28 2±6 4, respectively, with significant difference OMSMs recruited from RDS were younger, higher educated and had a higher income level More single and homosexual

orientated persons were found in RDS participants In addition, a greater proportion of RDS participants reported a long stay in Shenzhen than those in TLS

3.6.3.2. Sexual Practices

As displayed in Table 3-25, RDS could recruit more MSMs who were more likely to use Internet for partner seeking, have homosexual experience before having heterosexual contact, have no female partner, sustain only 1 male partner in the past 6 months, have no male partner from Hong Kong and practice receptive anal intercourse in male sex For the condom use, RDS tended to recruited OMSMs with lower unprotected anal or vaginal intercourse with both male and female

3.6.3.3. Self-reported STIs, drug use, accessible services and knowledge

There was a significantly greater proportion of OMSMs in RDS survey reporting ever diagnosed with sexually transmitted infections, while a smaller proportion with illegal drug use or drinking alcohol before having sex The HIV-related knowledge and condom use knowledge level were significantly lower in RDS survey Accordingly, a significantly smaller proportion of participants in RDS survey received HIV-related education and services in the past 1 year They were also less likely to receive a HIV test within 6 months (Table 3-25)

Table 3-23 Demographic data of OMSMs recruited by TLS and RDS in Shenzhen, 2009

	TLS n (%) (n = 465)	RDS n (%)* (n = 447)	Total n (%)* (n = 912)	χ^2	P
Test result					
HIV positive	27 (5.8)	23 (5.1)	50 (5.5)	0.21	0.644
Syphilis positive	85 (18.3)	75 (16.7)	160 (17.5)	0.41	0.521
Age（Years）				7.28	0.026
<=25	156 (33.5)	189 (42.0)	345 (37.7)		
26-30	130 (28.0)	116 (25.8)	246 (26.9)		
>30	179 (38.5)	145 (32.2)	324 (35.4)		
Education level				29.82	<0.001
Junior high school or lower	106 (22.8)	65 (14.4)	171 (18.7)		
Senior high school	199 (42.8)	152 (33.7)	351 (38.3)		
College or above	160 (34.4)	234 (51.9)	394 (43.0)		
Residence card holding				0.41	0.814
Shenzhen	38 (8.2)	42 (9.3)	80 (8.7)		
Guangdong	73 (15.7)	72 (16.0)	145 (15.8)		
Others	354 (76.1)	337 (74.7)	691 (75.4)		
Length of stay in Shenzhen				13.49	0.001
<=3 months	100 (21.5)	56 (12.4)	156 (17.0)		
4-12 months	47 (10.1)	54 (12.0)	101 (11.0)		
>1 year	318 (68.4)	341 (75.6)	659 (71.9)		
Occupational status				7.25	0.064
Commercial services	226 (48.6)	241 (53.6)	467 (51.0)		
Student	3 (0.6)	10 (2.2)	13 (1.4)		
Migrant worker	82 (17.6)	72 (16.0)	154 (16.8)		
Clerk or other	154 (33.1)	127 (28.2)	281 (30.7)		
Monthly income				20.53	<0.001
<=1000	33 (7.1)	32 (7.1)	65 (7.1)		
1001-3000	228 (49.0)	173 (38.3)	401 (43.7)		
3001-5000	131 (28.2)	143 (31.6)	274 (29.9)		
5000-9000	50 (10.8)	89 (19.7)	139 (15.2)		
>9000	23 (4.9)	15 (3.3)	38 (4.1)		
Marital status				24.63	<0.001
Unmarried	317 (68.2)	364 (80.7)	681 (74.3)		
Married	118 (25.4)	80 (17.7)	198 (21.6)		
Divorced or other	30 (6.5)	7 (1.6)	37 (4.0)		
Self-identified sexual orientation				12.81	0.002
Homosexual/gay	271 (58.3)	312 (69.2)	583 (63.6)		
Bisexual	150 (32.3)	114 (25.3)	264 (28.8)		
Heterosexual or unsure	44 (9.5)	25 (5.5)	69 (7.5)		

*: Adjusted for network size and linkage between recruiters and recruits.

Table 3-24 Sexual behavior of OMSM by TLS and RDS, Shenzhen, 2009

	TLS n (%) (n = 465)	RDS n (%)* (n = 447)	Total n (%)* (n = 912)	χ^2	P
Venue for finding partners				171.06	<0.001
Bar	62 (13.3)	36 (8.0)	98 (10.7)		
FC	78 (16.8)	18 (4.0)	96 (10.5)		
Sauna	116 (24.9)	27 (6.0)	143 (15.6)		
Internet	144 (31.0)	298 (66.2)	442 (48.3)		
Park	33 (7.1)	12 (2.7)	45 (4.9)		
Other	32 (6.9)	59 (13.1)	91 (9.9)		
Gender of first sex partner				29.70	<0.001
Male	262 (56.3)	331 (73.6)	593 (64.8)		
Female	203 (43.7)	119 (26.4)	322 (35.2)		
Female sex partner				316.00	<0.001
0	364 (77.9)	383 (85.1)	747 (81.5)		
1	84 (18.0)	52 (11.6)	136 (14.8)		
>1	19 (4.1)	15 (3.3)	34 (3.7)		
Number of anal sex partners				90.51	<0.001
0	37 (8.0)	26 (5.8)	63 (6.9)		
1	192 (41.3)	324 (72.0)	516 (56.4)		
>1	236 (50.8)	100 (22.2)	336 (36.7)		
Age of first sex				8.55	0.036
<=18	109 (23.4)	75 (16.6)	184 (20.1)		
19-20	120 (25.8)	111 (24.6)	231 (25.2)		
21-22	106 (22.8)	127 (28.2)	233 (25.4)		
>22	130 (28.0)	138 (30.6)	268 (29.3)		
Hong Kong male partners				6.92	0.031
Yes	186 (41.8)	150 (33.9)	336 (37.8)		
No or Unsure	259 (58.2)	293 (66.1)	552 (62.2)		
Sex role in male anal intercourse				6.92	0.031
Insertive only	186 (41.8)	150 (33.9)	336 (37.8)		
both	202 (45.4)	218 (49.2)	420 (47.3)		
Receptive only	57 (12.8)	75 (16.9)	132 (14.9)		
UAI in male sex				9.25	0.002
Yes	200 (42.8)	149 (33.0)	349 (38.0)		
No	267 (57.2)	302 (67.0)	569 (62.0)		
UAVI with female partners				4.13	0.042
Yes	62 (13.3)	41 (9.1)	103 (11.2)		
No	403 (86.7)	410 (90.9)	813 (88.8)		
Unprotected sex in all sex				12.00	0.001
Yes	221 (47.5)	163 (36.2)	384 (42.0)		
No	244 (52.5)	287 (63.8)	531 (58.0)		

*: Adjusted for network size and linkage between recruiters and recruits.

#: UAI: unprotected anal intercourse; UAVI: unprotected anal or vaginal intercourse.

Table 3-25 STIs, drug use, HIV knowledge and service in OMSM from TLS and RDS

	TLS n (%) (n =465)	RDS n (%)* (n =447)	Total n (%)* (n =912)	χ^2	P
Ever diagnosed with STIs				4.66	0.031
Yes	38 (8.2)	21 (4.7)	59 (6.4)		
No	427 (91.8)	429 (95.3)	856 (93.6)		
Ever took illegal drugs				5.36	0.021
No	434 (93.3)	436 (96.7)	870 (95.0)		
Yes	31 (6.7)	15 (3.3)	46 (5.0)		
Alcohol use before sex				17.50	<0.001
Never	334 (71.8)	376 (83.4)	710 (77.5)		
Ever	131 (28.2)	75 (16.6)	206 (22.5)		
History of HIV test				9.72	0.008
No	211 (45.4)	247 (54.8)	458 (50.0)		
Before 6 months	143 (30.8)	127 (28.2)	270 (29.5)		
Within 6 months	111 (23.9)	77 (17.1)	188 (20.5)		
Any kind of HIV education and service				24.42	<0.001
No	63 (13.5)	120 (26.6)	183 (20.0)		
Yes	402 (86.5)	331 (73.4)	733 (80.0)		
HIV-related knowledge				9.01	0.003
Low	130 (28.0)	168 (37.3)	298 (32.5)		
High	335 (72.0)	283 (62.7)	618 (67.5)		
Condom use knowledge				27.94	<0.001
Low	120 (25.8)	191 (42.4)	311 (34.0)		
High	345 (74.2)	260 (57.6)	605 (66.0)		
Self-perception of HIV infection				4.32	0.229
No risk	415 (89.2)	386 (85.6)	801 (87.4)		
Little risk	45 (9.7)	54 (12.0)	99 (10.8)		
High risk	5 (1.1)	10 (2.2)	15 (1.6)		

*: Adjusted for network size and linkage between recruiters and recruits.

STIs: sexually transmitted infections.

3.6.3.4. Comparison of OMSM recruited through RDS and TLS

Multivariate analysis was conducted to further evaluate the the difference in social-behavioral characteristics between MSMs recruited from TLS and RDS. The following candidate variables were considered in the models: age, education level, length of stay in Shenzhen, occupation, hometown, income level, marital status, sexual orientation, venue for finding partners, gender of first sex partner, age of first sex, sex role in male anal intercourse, number of male anal sex partners, male sex

partner from Hong Kong, condom use in male sex, condom use in female sex, drug use history, having drunk alcohol before sex, STIs diagnosed history, HIV test history, HIV-related services, HIV-related knowledge, condom use knowledge, and syphilis infection

Table 3-26 showed that a greater proportion of OMSMs recruited from RDS survey were single, came from low HIV prevalence area, used Internet for finding partners, had less anal sex partner, self-identified as homosexual or bisexual, had lower condom use knowledge, did not drink alcohol before sex and received no HIV services

Table 3-26 Multivariate analysis for the characteristics in OMSM by RDS and TLS

	RDS vs TLS	P
Hometown for high HIV prevalence area	0 30 (0 17-0 50)	<0 001
Ever been married	0 81 (0 66-0 98)	0 031
Venue type		<0 001
Bar and massage center	1	
Family club	0 31 (0 15-0 63)	0 001
Sauna and Park	0 44 (0 24-0 80)	0 007
Internet and other	2 65 (1 62-4 32)	<0 001
Sexual orientation		0 003
Homosexual	2 67 (1 45-4 92)	0 002
Bisexual	3 18 (1 62-6 25)	0 001
Heterosexual or uncertain	1	
Number of male anal sex partners >1	0 70 (0 59-0 83)	<0 001
Condom use knowledge	0 65 (0 46-0 91)	0 013
Drank alcohol before sex, past 6 months	0 53 (0 36-0 77)	0 001
Received HIV services, past 1 year	0 41 (0 27-0 62)	<0 001

3.6.4. Summary

The overall Prevalence of HIV infection in OMSMs by RDS (5 1%) and TLS (5 8%) was not significantly different, indicating that the recruitments of both RDS and TLS survey were acceptable and no significant difference in HIV infection was found between MSMs frequenting physical existed venues and MSMs not visiting these venues

A greater proportion of OMSMs in RDS self-identified as gay/homosexual, while

fewer of them reported multiple male sex partners Keeping only one sexual partner might be a protective factor from infection if the partner was also HIV negative

Since the HIV-related education and intervention for MSMs mainly based on outreach efforts in MSM venues, lower coverage of HIV services and lower condom use knowledge were reported in RDS than TLS

The two methods were similarly good to use based on the finding of this study, while TLS had significant advantage in reaching MSMs with more risky behaviors including multiple partners and UAI in male sex In addition, TLS could provide information on HIV infection rate and HIV-related risk behaviors in MSMs being recruited from different venues, which was essential for the decision makers to choose suitable venue-based HIV intervention and services for MSMs However, it was still important to conduct RDS survey to identify the HIV infection rate among Internet-based MSMs

3.7. Discussion

3.7.1. Prevalence and risk factors for HIV infection among MSMs in Shenzhen

In this study, a total of 2143 MSMs were recruited in Shenzhen through two sampling methods including 1651 persons from time-location sampling (TLS) recruited in either 2008 or 2009, and 492 from respondent-driven sampling (RDS) recruited in 2009 A separate recruitment of money boys and other MSMs was done for the TLS surveys and a mixed sample of MSMs was conducted for RDS In total, the Prevalence of HIV infection among these MSMs was 5 5% (5 7% in TLS and 4 6% in RDS), which was similar to the national rate of 4 9% in MSMs [88], and equal to other study findings in Shenzhen [24, 58] Prevalence of HIV infection in Shenzhen MSMs continuously increased from the beginning of MSM surveillance in 2002 when it was 0 91% to 1 53% in 2004, 1 67% in 2005, 2 48% in 2006 [57], 3 90%-4 13% in 2007[58], and 5 87% in 2008, according to surveillence data in Shenzhen CDC combining the routine snowball sampling survey and our TLS survey

However, the prevalence dropped slightly to 5 3% in 2009 (a combination of routine surveillance data with our data) (Figure 3-5) According to the prevalence of HIV infection found in our own study, a more obvious decrease in prevalence of HIV infection was observed in 2009 TLS (4 9%) compared with 2008 (6 7%) Although it's still hard to say that HIV was effectively controlled in this high risk population in Shenzhen, a possible stabilization in prevalence of HIV infection could be expected Other cities or areas in China also reported a significant increase in prevalence of HIV infection among MSMs no matter how high it was at the baseline, including Beijing (from 0 4% in 2004, 4 6% in 2005, and 5 8% in 2006) [30], Jinnan (from 0 05% in 2007 to 3 1% in 2008) [23, 27], Chongqing (from 10 4% in 2005 to 12 5% in 2007) [60] and Yunnan (from 4 0% in 2005 to 13 2% in 2007) [219] The trend of a stabilized increase in prevalence of HIV infection after a strong increase that was observed in Shenzhen is similar to the trend found in Bangkok, Thailand [131] and San Francisco, USA [220] But prevalence of HIV infection in both of these two cities has stabilised at a hyperendemic level of over 25%, which was more than 5 times higher than that in Shenzhen

Among the MSMs recruited from TLS surveys in the two years, syphilis infection (AOR=5 5, 95%CI 3 5-8 5), occupation as clerk or others (AOR=1 8, 95%CI 1 1-2 9), being recruited from small family clubs (AOR=4 1, 95%CI 1.9-9 1), parks (AOR=3 3, 95%CI 1 5-7 3) and saunas (AOR=2 8, 95%CI 1 2-6 5), coming from high HIV prevalence areas (AOR=1 8, 95%CI 1 0-3 1), and self-identifying as homosexual/gay (AOR=2 5, 95%CI 1 1-5 8) were significantly associated with HIV infection after adjusting for covariates and confounding factors

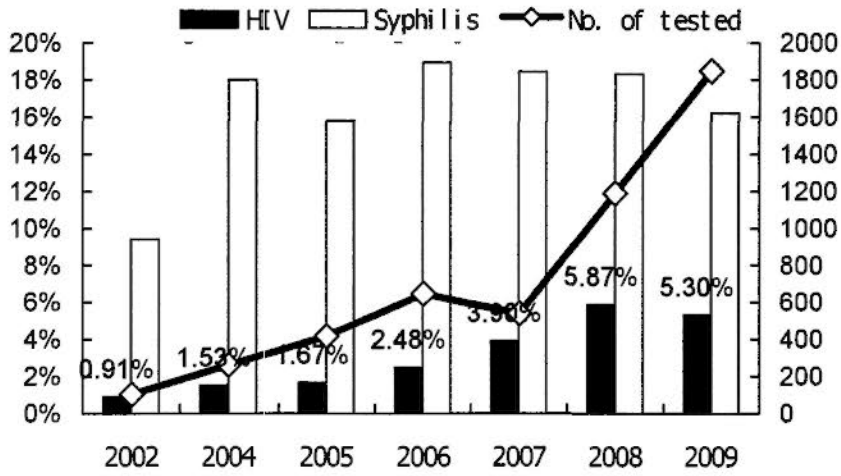

Figure 3-5 HIV/syphilis prevalence among MSM in Shenzhen by year

Syphilis infection and sexual orientation

Syphilis infection was a strong risk factor for HIV infection, with numerous studies reporting a correlation between HIV and syphilis infection [212, 221, 222] Consistent with the literature targeting MSMs [23, 223-225], syphilis infection and sexual orientation were found to be associated with HIV infection However, it was good to find that not all MSMs self-identified as gay or homosexual (42 6%) More than half of MSMs were either bisexual or heterosexual men engaging in sex with both men and women concurrently and around one quarter of the MSMs by TLS self-reported heterosexual orientation This was quite different from the proportion in Western countries and other cities in China showing that the MSM population consisted mostly of gay-identified men [22, 204, 223, 226] The large proportion of heterosexual MSMs could be explained by considering the large proportion of MBs in the TLS study Being heterosexual might protect them from risky sex behaviors in pursuit of their own pleasure in male-male sex Although the overall Prevalence of HIV infection among MSMs in Shenzhen was not higher than MSMs in other cities in China, the positive rate of the homosexual-identified MSMs (8 8%) was much higher than the documented prevalence among MSMs in Shenzhen This finding implied that the HIV-related health education and intervention targeting MSMs especially MBs should focus on this subgroup of people On the other hand, although a lower HIV infection rate was found among non-gay identified MSMs than among

gay identified MSMs (3 3% vs 8 8%), the high frequency of bisexual behaviors in non-gay identified MSMs implied that they might have higher possibility in spreading HIV from the risky MSM population into heterosexual networks

Hometown was a possible predictor for the HIV infection rate among MSMs

The HIV infection rate was associated with the hometown of the participants MSMs from high HIV prevalence areas were more likely to be infected with HIV than those coming from low HIV prevalence areas This finding probably could be explained by the fact that some study participants might have contracted HIV prior to arrival in Shenzhen However, among the 26 HIV-positive participants that came from high HIV prevalence areas, 10 (38 5%) had previously tested negative in the past 1 year, including 2 (7 7%) tested negative within 6 months after their arrival in Shenzhen Since no significant difference was found in the socio-economic status of this subgroup of MSMs compared with the others, the higher HIV infection rate could probably be explained by the higher proportion of risky behaviors that the MSMs perhaps carried over from their hometowns Lower rates of condom use with non-commercial partners were found among this group compared with the other groups (52 6% vs 45 3%) In addition, in Chinese societies, close relationships (networks) tend to form among people from the same hometown (*Laoxiang*) when they migrate to other locations The close networks, which likely include sexual networks, could further spread the virus within this population

Phylogenetic analysis of HIV strains from positive subjects in this study was also consistent with the hypothesis of internal transmission of virus between men from the same hometown who frequented the same venues (data showed in Chapter 4) Seven venue-*Laoxiang* groups (2-3 members per group, totally 15) of HIV positive MSMs from TLS survey were found to be located in the same genetic clusters in phylogenetic analysis with genetic distances of 0 1-0 3%, indicating a possibility of direct transmission In addition, the correlation of hometown HIV prevalence and HIV risk found in this study was consistent with the high percentage and wide

variety of MSM population in Shenzhen Therefore, Shenzhen could serve as a window in China for identifying the geographic distribution of HIV infection rates among MSMs based on their hometown information This finding implied that we should pay more attention to the subgroups of MSMs coming from high HIV prevalence areas and provide more intensive and suitable intervention and services to them

3.7.2. A comparison of HIV infection and its risk factors between MBs and OMSMs

The prevalence and risk factors for HIV infection were compared between MBs and OMSMs using a combined data from 2008 and 2009 from time-location surveys The HIV and syphilis prevalence among MBs was 4 5% and 12 9%, respectively, which was significantly lower than that among OMSMs (7 0% and 20 2%, respectively) The result was inconsistent with some studies conducted in Western countries and one city in China that indicated a higher HIV infection rate and higher likelihood of sexual risk behaviors among MBs [22, 24, 39, 215], while it was consistent with a study showing that prevalence of HIV infection among agency-based MBs were not higher than among other MSMs [227]

The difference in prevalence of HIV infection between MBs and OMSMs could be explained by the different distribution of age and the difference in their behaviors A greater proportion of MBs were in young age compared to OMSMs Increasing age has been found to be a risk factor to HIV infection[25], but age was not significantly related to HIV infection in our data after multivariate analysis for both MBs and OMSMs On the other hand, although MBs were more likely to have multiple male partners including both paying and non-paying partners, they were also more likely to use condoms in male anal sex It was found in Western countries that agency-based MBs (i e , escorts, with comparatively higher income) exhibit fewer HIV-related risk behaviors with clients than men working on the street (with lower income) [50, 54, 228, 229] Survival sex was found to be a risk factor for practicing

risk behaviors and consequently contracting HIV [45, 100] MBs in Shenzhen had similar monthly income levels compared with OMSMs, which was much higher than common migrant workers (600-800 RMB per month earned by most migrant workers in Shenzhen, approximately US$88-118, Shenzhen Longgang government online, http //www lg gov cn) The higher income empowered the MBs in negotiating condom use or rejecting unprotected anal intercourse with clients Engaging in sex work as an occupational choice instead of merely survival sex could partly explain why they exhibit lower levels of risk behavior and sexually transmitted infections However, MBs had significantly lower likelihood of using condoms in female sex, indicating that they might serve as bridge population to transmit HIV to the low risk heterosexual communities

In addition, a smaller proportion of OMSMs reported encountering male sex partners from Hong Kong, compared with MBs Combined with the finding that a greater proportion of OMSMs were infected by HIV, this implies that cross-border Hong Kong MSMs in Shenzhen mainly contacted with MBs, who had comparatively lower HIV infection rates This finding could partially explain the previous finding that different clusters of circulating HIV strains were observed in Shenzhen and Hong Kong [67, 68] Our own molecular epidemiological study on HIV positive MSMs collected from TLS and RDS surveys also showed that no interrelationship between clustered HIV strains in Hong Kong and Shenzhen (Chapter 4) This finding also implies a low risk of HIV transmission from Hong Kong MSMs to Shenzhen people Since Shenzhen MBs reported a higher likelihood of having Hong Kong partners than OMSMs, MBs might have lower risk in contracting HIV from male sex partners

MBs and OMSMs should be separately recruited in surveillance

According to the above findings that indicated a significant difference between MBs and OMSMs in prevalence and risk factors for HIV infection, a separate analysis of these two subgroups could be more adequate in interpreting the key risk factors for

HIV infection among them [39] Since MBs liked to strategically maintain divisions between their professional and personal lives [40], the commercial and non-commercial sex behaviors among MBs could be significantly different Combining the commercial and non-commercial sex behaviors of MBs and including them in a mixed MSMs study might introduce bias into the analysis However, most previous studies on MSMs mixed MBs with OMSMs The proportion of MBs in a mixed sample was unknown [30, 230, 231], or could be underreported or overreported [24, 28, 58] [39, 232] In our RDS survey that recruited a mixed sample of MSMs, although questions related to commercial sex were included, a significant size of MBs concealed their sex trade behaviors Nearly half (14/33) of the MBs participating in both the TLS and RDS surveys denied having had paid sex behaviors in RDS locations After private conversation with some of these MBs after the survey, most of them admitted that they concealed a sex trade experience in RDS In general, it was more acceptable in the communities to disclose a homosexual identity rather than disclose a sex trade behavior MBs recruited in their working venues had significantly higher willingness to admit their paid sex behaviors, while it would be difficult for them to admit such experiences when in a clinic if it was not absolutely necessary Therefore, the effect of self-reporting bias on paid sex could not be excluded in a survey recruiting a mixed sample of MSMs including both MBs and OMSMs

3.7.3. HIV infection and HIV-risk related characteristics of MSMs in different venues

Only a few previous studies have described the difference in HIV infection and related risk factors among MSMs in saunas and entertainment venues in China by convenience sampling [58, 60] However, MSMs in parks and family clubs had never been touched The current study was the first survey targeting MSMs by using time-location sampling in China that might have better representativeness than other non-probability methods In addition, the TLS survey identified the different

infection rates among MSMs in almost all different types of venues, and provided information on the difference of HIV-related risk factors among MSMs frequenting different venues These background surveillance data would therefore help health care providers to modify the strategy of venue-based intervention

The key findings related to venue were 1) Both MBs and OMSMs recruited from entertainment venues including bars and massage centers had significantly lower HIV risk compared with MSMs frequenting other venues including family clubs, parks and saunas 2) Higher likelihood of risky sexual behaviors was observed among MSMs from venues with higher HIV infection rates

HIV-risk related characteristics among MBs in different venues

Compared with MBs in entertainment venues, MBs in parks and family clubs were more likely to self-identify as gay or homosexual and earned less in each sexual encounter with clients More intensive intervention and education should be provided to MBs in parks and family clubs

PMBs had more commercial and non-commercial partners and were more likely to practice unprotected anal intercourse These two factors were the widely accepted predictors for HIV infection among MSMs [83, 233-236] Therefore, it was easy to understand the higher HIV infection rate in PMBs compared with EMBs This finding was also consistent with the reports in Western countries showing that street male prostitutes had higher HIV infection and related risk behaviors than agency-based MBs [40, 229, 237]

However, FMBs had a similar number of commercial or non-commercial male partners and similar level of UAI in male sex compared with EMBs The possible factors for FMBs in acquiring HIV might be the longer experience in selling sex, older in age, higher proportion of gay/homosexual and shorter stay in Shenzhen Among the 23 HIV positive FMBs, 14 (60 9%) sold sex for more than 1 year, 8 (34 8%) were older than 25 years in age and 16 (69 6%) self-identified as gay These

factors were well-known factors for the acquisition of HIV infection [64, 75, 238, 239] In addition, 17 (73 9%) had come to Shenzhen within the previous 3 months and 1 (4 3%) in the previous 6 months These FMBs might have contracted HIV prior to arrival in Shenzhen 39 1% (9 out of 23) of the positive FMBs came from high HIV prevalence areas, twice the percentage of HIV negative FMBs (16 8%) Besides these factors that might result in the higher HIV infection rate among FMBs compared with EMBs, another potential reason could be the different distribution of clients of these MBs (defined as MSMs that reported paying sex in the previous 6 months) OMSMs reporting paying sex in the previous 6 months were defined as clients of MBs Clients in entertainment venues (n=116) had significantly lower HIV infection rates (0 8%) than clients in family clubs (8 9%, n=56) (P=0 014, Fisher's exact test) Clients in entertainment venues also reported higher income levels than those in family clubs However, since the total number of clients was small and risk factors related to paying sex besides condom use was not collected, a study focused on MSMs involved in paying sex was needed in order to better interpret the HIV transmission relationships between MBs and clients

HIV-risk related characteristics among OMSMs in different venues

Similar to MBs, OMSMs frequenting entertainment venues also had lower likelihood of being infected with HIV and syphilis compared with others The characteristics associated with OMSMs frequenting parks were similar to the predictors for MBs working in parks, including greater proportion of unprotected anal intercourse with males and lower income However, SOMSMs had some unique characteristics SOMSMs were more likely to be older, married, less educated, and have multiple male partners compared with EOMSMs Since only a slightly greater proportion of SOMSMs practiced UAI in male sex compared with EOMSMs, multiple partners should be the key factor for SOMSMs contracting HIV These findings suggested that intervention efforts in saunas should not only focus on condom promotion, and behavior change in reducing sexual partners should be strengthened in saunas

In addition, contradictory characteristics of being married and self-identifying as homosexual or gay were found to be predictors for OMSMs frequenting parks and saunas other than entertainment venues We found that 28% (141/504) of the MSMs in parks and saunas had been married, 43% (60/141) of these married MSMs self-indentified as gay, and half (30/60) of these married gay men reported UAI in male sex Furthermore, 15% (6 in parks and 3 in saunas) of the married gay men were found to be HIV positive and none of them consistently use condoms in male-male sex The high HIV infection rates and large proportion of married gay men in parks and saunas further indicated that MSMs in parks and saunas might perhaps have higher risks than other MSMs for acquiring HIV and transmitting it not only to other MSMs but also to the general population

3.7.4. A comparison of RDS and TLS in recruiting high risk MSMs in Shenzhen

Both TLS and RDS have their strengths and weaknesses Since there is no standard sampling frame for groups at behavioral risk for HIV infection, the generalizability and representativeness of TLS and RDS are unknown If a very high percentage of venues are identified and a very high percentage of target populations visit such venues, TLS is probably suitable for producing probability samples of the population that attend venue-day-time segments, although they could not represent the whole population Given the situation that frequent gay venue visitors were more likely to engage in high-risk sexual behaviors [117], HIV prevention research among MSMs could better use TLS for the purpose of analyzing more risky subgroups [118]

RDS, on the other hand, is a long-chain referral process that is implemented in a manner allowing for the calculation of selection probabilities [112, 135] RDS can be used if a population is not immediately available or if persons at risk are currently very hidden and hard to reach Although RDS may not provide reliable eligibility and response rates, which make it hard to estimate the representativeness, it is still

acceptable in recruiting hidden populations after adjusting for the selection probabilities based on network sizes

In this study, the overall prevalence of HIV infection among OMSMs by TLS (5 8%) was slightly higher than by RDS (5 1%), and a significantly greater proportion of MSMs recruited from RDS reported that they liked to seek male sex partners through the Internet (a subgroup of MSMs that were more hidden than venue-based MSMs) compared with MSMs in TLS (66 2% vs 31 0%) Furthermore, MSMs who reported seeking partners in venues were more likely to be HIV positive than MSMs seeking partners through the Internet (9 1% vs 3 4%) in RDS survey These findings might imply that HIV infection rates among Internet-based MSMs are lower than that among venue-based MSMs In addition, MSMs recruited through RDS were less likely to visit the high HIV risk venues including family clubs, parks and saunas, compared with MSMs in TLS They also reported fewer high risk behaviors including multiple male partners and UAI in male sex These characteristics might be associated with a lower HIV infection rate in RDS Although it was widely accepted that RDS had the advantage in reaching more hidden populations than TLS, these hidden MSMs in Shenzhen showed even lower risks than the venue-based MSMs There were several possible reasons why RDS achieved a lower risk sample than TLS First, the MSMs in TLS were more active in seeking multiple partners and practicing sexual risk behaviors than MSMs in RDS Second, a significant proportion of MSMs in RDS might have the willingness and knowledge to get an HIV test, since the clinic where we conducted the RDS survey was a community-based VCT (Voluntary counseling and testing) clinic The VCT normally had lower HIV positive rates than MSM surveillance Third, although RDS had a prior known sampling bias from the fact that the incentives might have a much stronger appeal to MSM of lower socio-economic status (SES) than higher SES MSM [240], such a bias was not observed in this study MSMs recruited in our RDS had even higher socio-economic status than MSMs in TLS This finding indicated that RDS in Shenzhen might not

have an advantage in reaching MSMs of low SES Further investigations targeting MSMs in low SES should be considered for future surveillance

The comparison of the results from TLS and RDS showed that the two sampling methods had a mutually compensable role in reaching high risk MSMs in Shenzhen TLS could reach more sexually active MSMs in venues based on a random selection strategy, while there was a possibility that a sub-group of MSMs who did not go to any physical existing venues would not be recruited in TLS In contrast, RDS had the advantage to reach more hidden subgroups, such as Internet-based MSMs, while it might not provide reliable eligibility and response rates In addition, TLS was a simple, but labor intensive method, RDS was convenient to use, but its analytic methods have not been well established and are comparatively sophisticated Using the two methods together could compensate for the disadvantages of each, and cover more MSMs with different characteristics as well as identify the proportion of different subgroups of MSMs

Venue of recruitment was better than self-reported venue for seeking partners in interpreting the characteristics of MSMs in different venues

Although a correlation of HIV infection and venue of MSMs recruitment was observed in this TLS study, no similar correlation was observed between HIV infection and self-reported venue for seeking sex partners in this study This finding indicated that venue comparison data from other sampling methods including RDS using the self-reported variable of venue for seeking sex partners might be unsuitable to fully understand the venue correlation on HIV transmission Using venue for recruitment in TLS survey instead of the self-reported venue where the participants would like to visit and seek partners could exclude the possibility of self-reporting bias TLS might be an irreplaceable method that provides reliable venue based information for finding suitable HIV intervention and service activities in different venues

TLS could provide more information for designing intervention strategies

TLS surveys had additional strengths over RDS or other sampling methods besides the advantage of reaching more risky subgroups of MSMs in Shenzhen TLS needs the researchers go in-depth to the communities and forces public health workers to identify, describe and map venues, and to interact sensitively with gatekeepers and members of the target population, which consequently makes it easier to integrate in sentinel surveillance systems and has a high degree of replicability and reliability [241] Better understanding of the situation in communities and venues helped researchers in deciding which venue-specific intervention strategies to use according to the characteristics of each venue In this TLS study, we found that MSMs in some venues (family clubs, parks and saunas) had higher HIV infection rates, and higher likelihood of having sex with women, which might push them into a more risky position in transmitting HIV to the general population After obtaining the information from TLS survey in 2008, a venue specific intervention strategy based on TLS data was applied to the community

Most of the health education and interventions targeting high-risk populations relied on outreach efforts that were fully based in venues Before the time of TLS 2008, all HIV-related health education and intervention strategies mainly focused on big entertainment venues in Shenzhen for the purpose of covering more target populations in one outreach effort Health education and condom distribution were occasionally provided in saunas The variance in coverage of HIV services in different venues might influence the HIV risks among MSMs visiting the venues Intensive venue-targeting interventions were provided in Shenzhen according to the result from 2008 TLS during the one-year interval of the two surveys Outreach interventions were designed to ensure that efforts covered all different types of MSM including those frequenting small venues The HIV infection rate in each venue was shared with the manager or boss of the venue to achieve their support in outreach efforts and on-venue surveillance Further, intensive peer education and intervention

efforts were provided to venues with significantly higher HIV and syphilis infection rates including family clubs, parks and saunas For example, peer education and condom distribution was provided in each sauna every week for the purpose of increasing condom use rates and reducing casual sex partners, condoms were made available in each small dark room in saunas instead of only being available at the counter Peer training was provided each month for MBs in family clubs on HIV knowledge, sexual behaviors and negotiation skills, and condom availability in each clubs was insured, weekly condom distribution was provided in parks The comparison of TLS data between 2008 and 2009 could partly interpret the effect of TLS-monitored intervention efforts HIV infection rate was significantly decreased in family clubs (11 1% to 6 0%) and saunas (10 1% to 6 7%) in 2009 TLS The infection rate in parks was not decreased because no intervention strategy was provided in parks besides condom distribution

However, no intervention evaluation study was performed to evaluate the effects of the TLS-monitored venue-based intervention strategies, it was hard to make any conclusion at this time In addition, HIV prevalence and sexual risk behaviors of MSM in 2009 TLS might be influenced by other factors First, it might be due to the selection bias and reporting bias Data were collected using a hand-held computer-assisted self-interview system in 2009, which has been shown to increase more candid and reliable self-reports of sensitive behaviors [242] and reduce self-reporting bias However, the use of computers might conversely affect the willingness of participation of some MSMs of low SES and became a source of selection bias Second, a national anti-pornography campaign was conducted in China for the celebration of the 60th anniversary of the founding of the People's Republic of China Since MSM could be convicted on charges of assembled pruriency and MBs for selling sex, some venues restricted their clients' behaviors and even closed (such as family clubs that involved in sex trade) The restriction of behaviors and reduction of on-business venues might result in fewer sexual trade activities as well as sexual behaviors Therefore, a trend study with a serial

cross-sectional survey should be conducted in the future to further investigate the prevalence of HIV infection and HIV-related risk behaviors of MSMs by TLS in accordance with TLS-based intervention efforts

3.7.5. Strengths and Limitations

Strengths of the study

The strengths of this study lied in the setting and design First, this is the first study in China that investigated the prevalence and risk factors for HIV infection in men who have sex with men using a powerful sampling approach in a hard-to-reach population, time-location sampling method It is also one of the largest regional studies in China, with the number of study subjects exceeded 2100

Second, it is also the first study comparing the HIV risks among money boys and other MSMs based on an equal sample size and parallel recruitment by TLS The results showed that prevalence of HIV infection among MBs was lower than OMSMs, which was different from our prior estimation HIV-related risk factors for MBs and OMSMs were also different from each other, indicating that a separate analysis is necessary

Third, detailed information of relationship between venue and HIV infection rates /risk related characteristics among MBs and OMSMs was also obtained from the TLS survey A significant difference in HIV infection and related risk factors was observed in different venues visited by MSMs from the TLS survey MSMs frequenting family clubs, parks and saunas had significantly higher HIV infection rates than in bars and massage centers This finding was the first report in China showing a correlation of HIV risk with venue, based on the venues where MSMs were recruited in TLS

Fourth, a parallel comparison of TLS with respondent-driven sampling, another approximate probability sampling method, was conducted for the first time in China The results showed that TLS and RDS had a mutually compensable role in reaching

high risk MSMs in Shenzhen, while TLS could provide more information on venue comparison and observation The information was essential for developing suitable venue-specific HIV intervention and services approaches for the affected communities

Finally, according to our knowledge, this study was the first MSM survey that introduced a computer-assisted self-interview system in China Replacing the interviewer with a computer can provide conditions, including privacy and the perception of anonymity, that facilitate reliable and frank reporting, thereby increasing reports of sensitive behavior in surveys in sensitive populations [213] In addition, the CASI system was hugely beneficial in helping to enforce the strict quality control measures and allowed the researchers to concentrate on counseling and recording contact information of each participant on field

Limitations of the study

Although we conducted a rigorous TLS and RDS study among the Chinese MSM, limitations should be noted There are some limitations to the way in which the survey was conducted

1) Because of the cross-sectional design of the study and the nature of the items used in the logistic regression analysis, the analysis can only provide evidence of statistical association between those items and HIV infection and can not show cause-effect relationships Since a very high proportion of Shenzhen MSMs were internal migrants (> 90%), a cohort study is nearly impossible to conduct in this city A serial cross-sectional design for a trend study might be considered for future research

2) Because the population was recruited and sampled in MSM venues by TLS, there was a possibility that a sub-group of MSMs who did not go to any physical existing venues would not be recruited in TLS In addition, although we tried to cover all MSM venues in Shenzhen, it was still quite possible that some venues were not

identified and some venues were inaccessible A RDS survey was conducted in order to cover these hidden populations The result of RDS was also compared with TLS in order to evaluate the representativeness of the study

3) The non-response bias (14 3% in MBs and 32 4% in OMSMs) in TLS rose according to the nature of recruitment sites People in venues might not like to participate in the survey at those times and locations Since the refusal rate in each venue was recorded, adjustment could be conducted based on the number of enumerates and participants in each venue if necessary It is possible that HIV-related service-users were more likely than others to join our study If such is true, non-participants might be in more adverse situations and their condom use behaviors and associated factors might be different from participants of this study

4) Past sexual behaviors, and illicit drug use, might be subject to reporting bias Thus, responses might have been biased by recall errors or intentional misreporting of behavior MSMs were difficult to identify especially for MBs It was possible that some of the survey participants falsely represented themselves as MSM or took part in the survey more than once in order to receive more incentives However, many efforts had been made to minimize this bias, including the anonymous nature of the study, using of self-administered questionnaires, using experienced outreach fieldworkers and MSM volunteers, and ensuring the respondents that data will not be released to a third party We also checked the mobile phone numbers of each participant as well as key variables in an effort to exclude doubles In addition, different sample strategies including a parallel recruitment of MBs and OMSMs in TLS and a mixed recruitment of all MSMs in RDS were applied to further evaluate the reporting bias

5) The sample sizes for MBs or OMSMs in some venues were small, especially for PMBs, which might introduce type II error into the study Attention should be paid in interpreting the result Further studies with larger sample sizes are needed to improve the precision of these estimates

Chapter 4 Molecular epidemiological analysis of HIV positive MSMs

4.1. Background and objectives

A couple of researches on sero-prevalence of the MSM population in China had been reported [22-31], while not much information was available on viral subtyping Molecular epidemiology was rarely performed in MSMs especially in China A molecular epidemiological study conducted in Hong Kong and Shenzhen found that no genetic cluster was formed in HIV positive individuals infected through transmission routes other than homosexual contact and most HIV positive MSM in both cities were clustered into 3 genetic clusters However, no overlap or crossover was observed between the Shenzhen HIV genetic clusters and Hong Kong clusters [67, 68] Information from virus sequences could help us to figure out the origin of HIV-1 pandemic and to track the transmission pattern of risk groups Among the rare researches that performed on specific risk groups, few of them collected samples with a scientific sampling method [66] Most studies on HIV molecular epidemiology were based on a relatively small sample size for economic consideration, while the compensation would also be promising if a probability sampling strategy was applied

This chapter aimed at describing a molecular epidemiological study on newly-diagnosed HIV-1 positive MSMs in Shenzhen from two surveys using approximate probability sampling methods (TLS and RDS) from 2008 to 2009

Nucleotide sequencing analysis was performed on the pol (RT and PR gene) region of HIV-1 viral genome for subtype determination and drug resistance identification In addition, env (C2-V4) and gag (p17/p24 junction) regions on HIV strains were also analyzed in a majority of HIV positive MSM samples to confirm the findings in pol analysis Moreover, phylogenetic analysis was performed to understand the genetic interrelationships among these samples by aligning the sequences together

with reference sequences This study may provide supplementary information for the understanding of the HIV transmission pattern among MSMs in Shenzhen and its correlation with Hong Kong MSMs

4.2. Subjects and Methods

4.2.1. Framework of molecular epidemiological study

Flaw diagram of molecular epidemiological study was showed in Figure 4-1

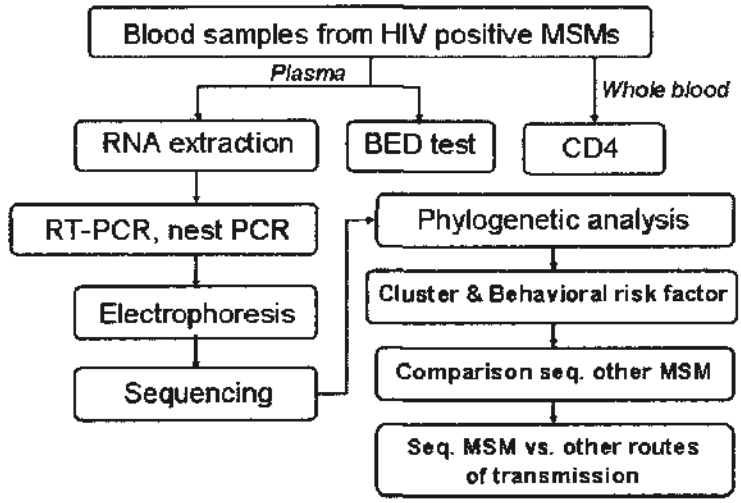

Figure 4-1 Flaw diagram of molecular epidemiological study

4.2.2. Subjects

Samples used in this study including sera from HIV positive MSMs recruited from the TLS and RDS survey in 2008 and 2009, and cumulated HIV positive samples in Shenzhen from year 2006 to 2009 The cumulated samples included HIV patients who have been infected through homosexual contact and other modes of transmission HIV sequences of these samples were compared and analyzed in the study

4.2.3. Laboratory test

Besides the HIV and syphilis serological testing that was described in the previous chapter, further laboratory detection and analysis were conducted targeting on HIV

strains including subtyping, phylogenetic tree construction, recent infection identification and drug resistance analysis.

4.2.3.1. CD4 counting

Three-color fluorescence kit for CD4/CD8/CD3 (Cat No. 340298, Becton-Dickinson, BD) counting was used according to kit manual.

Fresh whole blood was analyzed on a FACS Calibur cytometer (BD) within 8 hours. Multiset software was used for CD4/CD8/CD3 analysis TruCount tube (BD) was used to count the absolute number of lymphocyte in each subset. The countes of CD3, CD4 T cells and CD8 T cells were analyzed by Cellquest software. For all flow cytometric analyses, quadrant markers were set by using negative isotype controls. A live gate was set around CD4 or CD8 T cells and a minimum of 10000 gated events were collected for each sample.

4.2.3.2. Test for recent infection

The recent infection was tested by using HIV-1 BED Incidence Assay, which was to test HIV positive individuals who had been infected within the past 153 days (the window period that is recommended by the manufacturer of the commercially available BED assay) [203, 243].

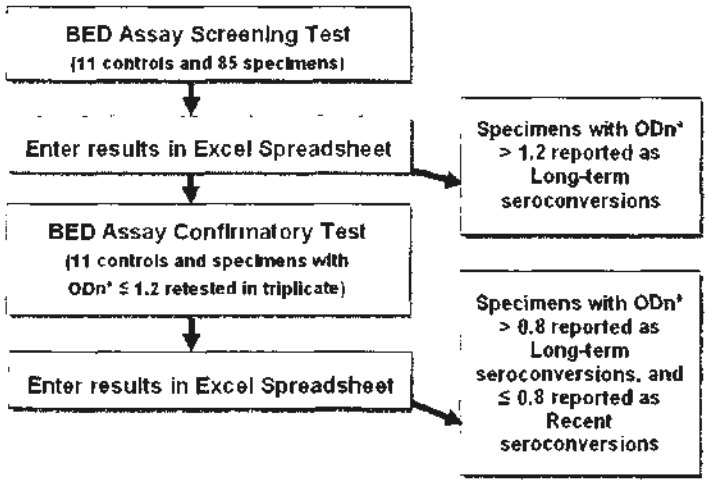

Figure 4-2 BED assay procedure in a glance

ODn: calculated by dividing the OD for each specimen (or midian OD when testing in riplicate) by the median OD of the calibrator.

- Prepare 1X Wash Buffer from 10X Wash Buffer Concentrate, prepare Diluent Buffer which is 3% bovine serum albumin (BSA) in 1 X Wash Buffer, and prepare 1:101 dilutions of controls and specimens.
- Prepare 2 dilutions of the Negative Control, 3 dilutions each of the Calibrator, Low Positive Control, and High Positive Control. Prepare a single dilution of each specimen.
- Transfer 100 µL of diluted specimen to the Goat Anti-human IgG coated microwell test plate; incubate the plate for 1 hour at 37°C.
- Wash the plate 4 times with 300 µL of 1X Wash Buffer /well, with a 10 second soak between each wash.
- Prepare 1:1001 dilution of the HIV-1 BED Peptide. Add 100 µL to every well. Incubate the plate for 1 hour at 37°C. Wash as described previously
- Prepare 1:1001 dilution of the Streptavidin-HRP Conjugate. Add 100 µL of diluted Conjugate to every well.Incubate for 90 minutes at 37 °C and wash.
- Add 100 µL of TMB Substrate to every well, incubate the plate at 25°C for exactly 15 minutes for color development.
- Place the plate in a spectrophotometer set at 450 nanometer wavelength with reference wavelength at 630 to 650 nanometers
- The confirmation procedure is the same as the screening but with different plate well arrangement. Normalized OD (ODn) is calculated by dividing the OD for each specimen (or median OD when testing in triplicate) by the median OD of the Calibrator.

4.2.4. Sequence-based analysis of HIV strains

4.2.4.1. HIV viral RNA extraction

The HIV viral RNA extraction was performed by using QIAmp viral RNA mini kit (QIAGEN, Germany).

(1). Dissolve lyophilized carrier RNA to 1 µg/µL in Buffer AE. Add 25mL ethanol (96–100%) to buffer AW1 and 30uL ethanol to buffer AW2.

(2) Pipet 560 µL of prepared Buffer AVL containing carrier RNA into a 1 5 ml microcentrifuge tube

(3) Add 140 µL plasma, serum, urine, cell-culture supernatant, or cell-free body fluid to the Buffer AVL–carrier RNA in the microcentrifuge tube Mix by pulse-vortexing for 15 s Incubate at room temperature (15–25°C) for 10 min

(4) Briefly centrifuge the tube, add 560 µL of ethanol (96–100%) to the sample, and mix by pulse-vortexing for 15 s

(5) Apply 630 µL of the solution from step 5 to the QIAamp Mini column (in a 2 ml collection tube) Centrifuge at 6000g for 1 min Place the column into a clean 2 ml collection tube, and repeat this step

(6) Add 500 µL of Buffer AW1 and centrifuge at 6000g for 1 min Place the column in a clean 2 ml collection tube

(7) Add 500 µL of Buffer AW2 Centrifuge at full speed (20,000g) for 3 min

(8) Place the QIAamp Mini column in a clean 1 5 ml tube Add 60 µL of Buffer AVE Incubate at room temperature for 1 min Centrifuge at 6000g for 1 min

4.2.4.2. Primer sets and Pol region amplification

Pol region [covering protease (PR) and reverse transcriptase (RT), 1 3 kb] was amplified from extracted viral RNA The primers used in PCR and sequencing were listed in Table 4-1

Table 4-1 Primer set for pol amplification and sequencing

Primers	Position (HXB2*)	Sequence (5'→3')
AMPLIFICATION PRIMERS		
For subtype B amplification		
Outer sense (MAW26)	2251-2277	5'-TTGGAAATGTGGAAAGGAAGGAC-3'
Outer antisense (RT21	2588-2605	5'-CTGTATTTCTGCTATTAAGTCTTTTGATGGG-3'
Inner sense (RT20)	3441-3462	5'-CTGCCAGTTCTAGCTCTGCTTC-3'
Inner antisense(PRO1)	2147-2166	5'-CAGAGCCAACAGCCCCACCA-3'
For CRF 01AE amplification		

MAW26-01AE	2251-2277	5'-CTGCCAATTCTAATTCTGCTTC-3'
RT21-01AE	2588-2605	5'-CRGARCCAWCAGCYCCACCA-3'
RT20-01AE	3441-3462	5'-TTGGAAATGTGGRAAGGARGGMC-3'
PRO1-01AE	2147-2166	5'-CTGTATTTCTGCTAYTAARTCTTTTGATGGG-3'
SEQUENCING PRIMERS		
RT-A	2519-2539	5'-GTTGACTCAGATTGGTTGCAC-3'
RT-B	2946-2967	5'-CCTAGTATAAACAATGAGACAC-3'
RT20-A1	3441-3462	5'-CTGCCAATTCTAATTCTGCTTC-3'
PRO1-A1	2147-2166	5'-CRGARCCAWCAGCYCCACCA-3'
RTAS-qian	2702-2719	5'-GGACCTACACCTGTCAAC-3'

* Remarks reference isolate HXB2 (Genebank accession # K03455)

Protocol for RT-PCR (PrimeScript™ one-step RT-PCR Kit, TAKARA, Japan)

(1) 25μL One Step RT PCR Reaction Mix contains 2×1 Step Buffer 12 5μL, PrimeScript 1 Step Enzyme Mix 1μL, Outer primer set (5umol each/μL) 2μL, Template RNA 7 5μL

(2) First-round RT-PCR: 50 °C 30 min, 94 °C 2 min, 94 °C 30 sec, 55 °C 30 sec, 72 °C 2 5min for 30 cycles, 72 °C 10 min

(3) Second round PCR of the nest PCR by using ExTaq ^{TM} from TAKARA, Japan 50μL PCR Reaction Mix contains 10×Ex Taq Buffer 5μL, 10mM 4dNTP Mix 4μL, Inner primer set (5umol each/μL) 4μL, Ex Taq (5u/mL) 0 3μL, First-round PCR product 5μL

(4) Second-round PCR 50 °C 30 min, 94 °C 2 min, 94 °C 30 sec, 63 °C 30 sec, 72 °C 1 5min for 35 cycles, 72 °C 10 min

(5) All amplicans were identified by gel electrophoresis and sequencing The PCR products were identified by 1 2% agarose gel electrophoresis, and then sequenced with sequencing primers

4.2.4.3. env and gag amplification

Protocol for env and gag amplification was similar to pol amplification (primers were listed in Table 4-2)

Table 4-2 Primer set for env and gag amplification and sequencing

Primers	Position (HXB2*)	Sequence (5'→3')
AMPLIFICATION PRIMERS		
For *env* gene		
Outer sense (44F)	6954 - 6973	5'-ACA GTR CAR TGY ACA CAT GG-3'
Outer antisense (35R)	7668 - 7648	5'-CAC TTC TCC AAT TGT CCI TCA-3'
Inner sense (33F)	7002 - 7021	5'-CTG TTI AAT GGC AGI CTA GC-3'
Inner antisense (48R)	7541 - 7523	5'-RAT GGG AGG RGY ATA CAT-3'
For *gag* gene		
Outer sense (332)	796 - 818	5'-GCG AGA GCG TCA RTA TTA AGI GG-3'
Outer antisense (308)	1319 -1297	5'-TCT GAT AAT GCT GWR AAC ATG GG-3'
Inner sense (306)	836 - 857	5'-GGG AAA AAA TTC GGT TAA GGC C -3'
Inner antisense (307)	1271 - 1249	5'-CTT CTA YTA CTT TYA CCC ATG C-3
SEQUENCING PRIMERS		
For *env* gene		
Sense (207)	826 - 848	5'- CTG TTA AAT GGC AGT CTA GC -3'
For *gag* gene		
Sense (306)	836 - 857	5'-GGG AAA AAA TTC GGT TAA GGC C -3'

* Remarks reference isolate HXB2 (Genebank accession # K03455)

First-round RT-PCR: 50°C 30 min, 94°C 2 min, 94°C 30 sec, 55°C 30 sec, 72°C 1 5min for 30 cycles, 72°C 10 min

Second-round PCR 50°C 30 min, 94°C 2 min, 94°C 30 sec, 56°C 30 sec, 72°C 1 min for 35 cycles, 72°C 10 min

4.2.4.4. Sequencing and sequence analysis

PCR products were purified and sequenced by commercial services Brief sequencing procedure was purified PCR products were sequenced directionally with fluorescent dye terminators (Prism BigDye terminator cycle sequencing ready reaction kit, Applied Biosystems) by an automated DNA sequencer (Applied Biosystems model 3110xl) Sequence fragments were then assembled by ContigExpress (InforMax Inc , USA) Pol sequences were uploaded to an HIV drug resistance database at Stanford University (http //hivdb stanford edu) for the identification of mutation sites and assessment of its impact on first line therapeutic responses

4.2.5. Phylogenetic analysis

Neighbor joining phylogeny construction

The nucleotide sequences of HIV pol, env and gag regions were compared with reference sequences from HIV genotypes and circulating recombinant forms (http //www hiv lanl gov/content/sequence/HIV/SUBTYPE_REF/Table1 html)

Genotype of HIV was determined after aligning with reference sequences and followed by phylogenetic analysis The pol sequences of some strains from a CRF01_AE cluster (EF122532, EF122534, and EF122541) which was reported in Hong Kong this year [66] was also included besides the reference strains for each subtype (Table 4-3) NJ phylogeny serves the purpose of constructing large phylogenetic trees that are virtually impossible to be constructed with more sophisticated methods (e g Maximum Likelihood method) In the study, phylogenetic trees were constructed using NJ method from the samples collected from our own 2008 and 2009 surveys together with reference sequences

Table 4-3 Standard reference sequences used in the study

COMMON NAME	LOCUS	ACC #
A1 UG92UG037	H92UG037	U51190
A1 UG85 U455	U455	U455
A1 SE SE7253	ASOSE7253	AF069670
B FR HXB2-LAI-IIIB-BRU	HIVHXB2R	K03455
C BR BR025-d	H92BR025	U52953
C ET ETH2220	HIVETH2220	U46016
C BW 96 96BW0502	BW0502	BW0502
D CD ELI	HIVELICG	K03454
D CD 83NDK	83NDK	83NDK
F1 BR 93BR020_1	93BR020	AF005494
F2 CM MP255	95CMMP255C	AJ249236
G SE SE6165	AF061642	AF061642
H CF 056	90CF056	AF005496
J SE SE7887	SE92809	AF082394
K CD EQTB11C	97EQTB11C	AJ249235
01_AE CF90 90CF11697	90CF11697	90CF11697
01_AE TH 99TH MU2079	MU2079	MU2079
01_AE CF 90 90CF402	90CF402	90CF402
01_AE TH CM240	HIV1CM240	U54771
02_AG NG IBNG	HIVIBNG	L39106
03_AB RU KAL153_2	KAL1532	AF193276
04_cpx CY CY032	CY0323	AF049337
05_DF BE VI1310	VI1310	AF193253

06_cpx AU BFP90	BFP90	AF064699
07_BC CN CN001	CN001	AY043176
07_BC CN CN54	CN54b	AX149771
08_BC CN 97CNGX_6F	97CNGX6F	AY008715
10_CD TZ 96TZ_BF061	96TZBF061	AF289548
11_cpx GR GR17	GR17	AF179368
12_BF AR ARMA159	ARMA159	AF385936
13_cpx CM 1849	1849	AF460972
14_BG ES X397	X397	AF423756
15_01B TH 99TH_MU2079	01AEB	AF516184
N CM YBF106	YBF106	AJ271370
O BE ANT70	HIVANT70C	L20587

Sequences were aligned using CLUSTAL_W (http //www ebi ac uk/Tools/clustalw/ index html) and then edited by BioEdit software The aligned nucleotide sequences were translated into amino acid sequences with standard genetic codes The genetic distances (as measured by substitutions per 100 sites with an estimated 95% standard of error) were estimated by Kimura's 2- parameter's nucleotide substitution model for the calculation of genetic distance matrix, with both synonymous and non-synonymous site were taken into account Phylogenetic and molecular evolutionary analysis was performed with MEGA 4 0 (The Biodesign Institute, Tempe, AZ) The reliability of the phylogenetic tree analysis was assessed and assured by bootstrap re-sampling with 1000 replicates Lineages or clusters with bootstrap value greater than 95% or with bootstrap value greater than 80% and branch length <0 015% were defined as bootstrap-well-supported The scale bars at the bottom of the NJ phylogenies are in K2P genetic distance unit

Maximum Likelihood phylogeny

Maximum likelihood (ML) methods are regarded as the most accurate method of phylogeny reconstruction In this study, the individual phytogenies for HIV subtype B, CRF01_AE and CRF07_BC were reconstructed using this method The ML methods in this thesis used MEGA 5 0 (BETA) The general-time-reversible model with invariable sites and 4 categories of gamma distribution of site heterogeneity was used for ML search Neighbor joining trees were built as the starting tree Heuristic

search (hs) started from the previous NJ tree was used to obtain the best ML tree Robustness of the ML phylogenies was assessed using 100 replicates of ML bootstrap in MEGA

4.2.6. Statistic analysis

A descriptive study in percentage terms of all recorded variables was performed using SPSS v16 0 (SPSS Inc , USA) Frequencies were compared using the Pearson's χ^2 test or Fisher's exact test Trent test was used for year of diagnosing $P < 0\ 05$ and $P < 0\ 01$ were used as the criterion to determine statistical significance The correlations between genetic clustering and HIV-related socio-behavioral characteristics were also examined by logistic regression analysis When doing logistic regression analysis, univariate analysis was first conducted to select variables for inclusion in models based on these results and the hypothesized relationships to outcomes Since the sample size was comparatively small, variables with P values less than 0 15 were included in the logistic regression model for multivariate analysis Other potential factors that may be associated with outcomes are also included in the model

4.3. Results

4.3.1. Subtype distribution among HIV-1 positive MSM cases

A total of 124 leftover sera were collected from HIV-1 individuals who were recruited through MSM survey by TLS and RDS sampling methods 106 (85 5%) HIV-1 positive samples were successfully identified for HIV-1 subtypes based on the sequences of pol region There was no statistically significant difference in the proportion of MSMs between sequenced and non-sequenced samples CRF01_AE was the predominant (57 5%) genetic form in Shenzhen followed by CRF07_BC (34 0%) and subtype B (8 5%) No other subtype was found among these MSMs (Table 4-4)

Table 4-4 HIV positive samples collected from the MSM survey, 2008-2009

	2008TL	2009TLS	2009RDS	Total

	S			
Tested HIV positive	49	45	30	124
Repeat cases		2	1	
Sequenced	43	38	25	106
Subtype				
CRF01_AE	23	20	18	61
B	4	4	1	9
CRF07_BC	16	14	6	36

TLS: time-location sampling, RDS: respondent driven sampling

4.3.2. Phylogenetic analysis and detection of transmission clusters

The phylogenetic analysis was performed on the 106 pol nucleotide sequences from HIV positive MSMs in the TLS and RDS survey (Figure 4-3 and Figure 4-4).

Figure 4-3 Phylogenetic tree of CRF01_AE strains from TLS and RDS (n=61) in Shenzhen Open circle: OMSM 2008TLS; solid circle: OMSM 2009TLS; open square: MSW 2008TLS; solid square: MSW 2009TLS; solid triangle: MSM by 2009RDS; red: sauna user; blue: park user; yellow: family club user; green: bar and massage center user; black: Internet user.

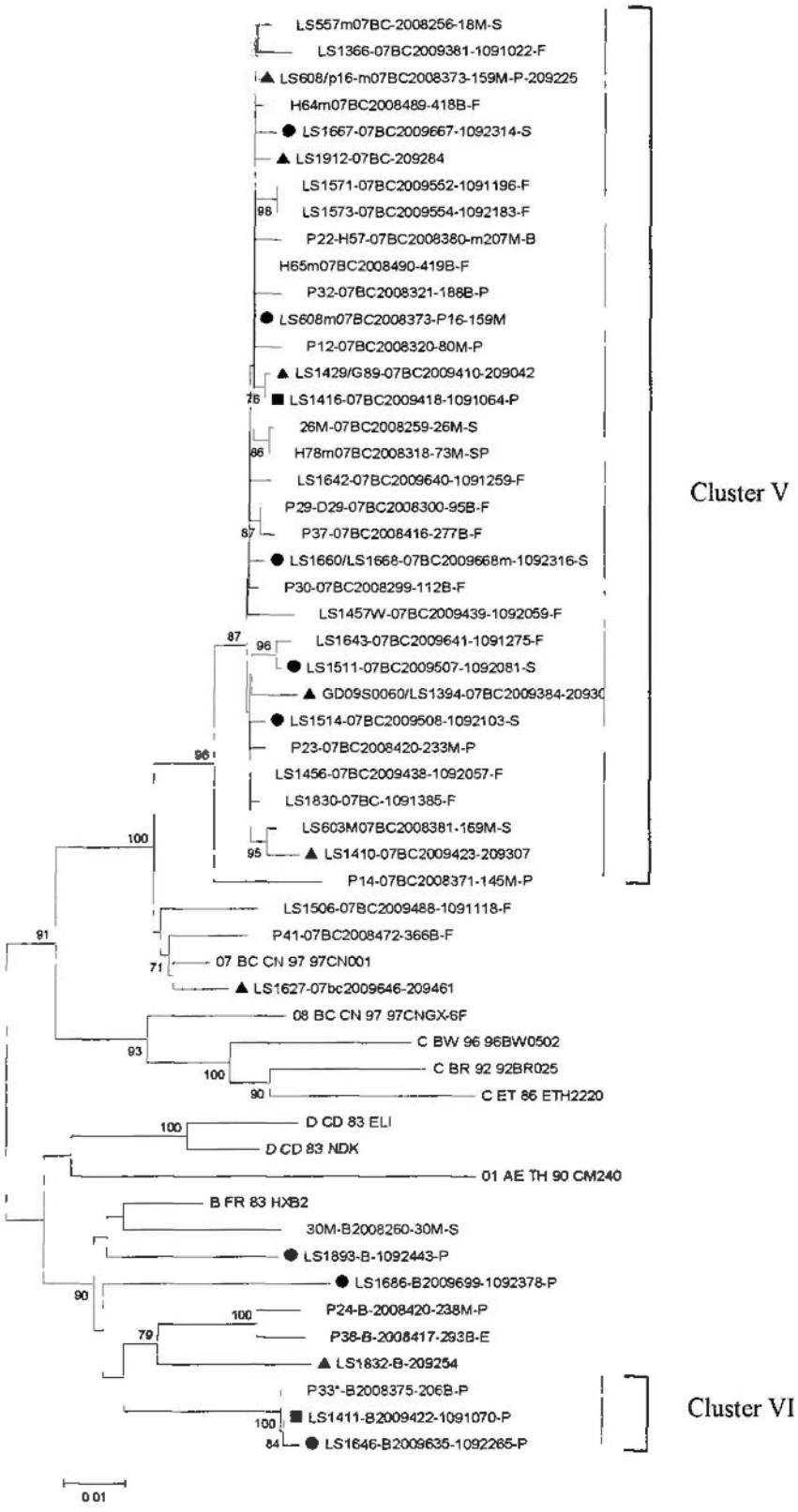

Figure 4-4 Phylogenetic tree of CRF07_BC and B strains from TLS and RDS (n=45) in Shenzhen

Open circle: OMSM 2008TLS; solid circle: OMSM 2009TLS; open square: MSW 2008TLS; solid square: MSW 2009TLS; solid triangle: MSM by 2009RDS; red: sauna user; blue: park user; yellow: family club user; green: bar and massage center user; black: Internet user.

Four CRF01_AE genetic clusters [cluster I (23 members), II (8), III (4) and IV (10)], one CRF07_BC cluster [cluster V (33)] and one B cluster [cluster VI (3)] were observed with a determination of bootstrap value exceeding 95 in NJ tree ML trees were similar to NJ tree There were 81 members within the genetic clusters, which accounted for 76 4% of the total samples Genetic clusters were not equally distributed within different subtypes More than 90% of CRF07_BC strains were clustered together, and around three fourth of the CRF01_AE strains were located in 4 separate clusters, while the majority of subtype B members were non-clustered unique transmission events (Table 4-5) The CRF07_BC and B clusters were defined, whereas the CRF01_AE genetic cluster had longer branch lengths, especially cluster I, which indicated a loose interrelationships

Table 4-5 Relationship of subtypes and genetic clusters among MSM

Subtype	*Clustered (n=81) n (%)*	*Non-clustered (n=25) n (%)*	χ^2	*P*
CRF01_AE	45(73 8)	16(26.2)	14.153	0.001
B	3(33.3)	6(66 7)		
CRF07_BC	33(91.7)	3(8.3)		

*: percentage of each category of the variable

For HIV positive MSMs, the venue of recruitment in TLS survey were recorded and compared with the distribution of genetic clusters From the phylogenetic tree, all MSMs in subtype B cluster were recruited from park, while individuals in other clusters showed no venue-type specific characteristics The majority of individuals in cluster II and III (CRF01_AE) were OMSMs with no sex trade experience From RDS survey, venue-based and Internet-based MSMs were compared for clustering Since only 5 internet-based MSMs were found to be HIV positive in RDS survey, no significant difference was observed (40% vs 81%, fisher's exact test P =0 10)

Epidemiologic characteristics from the behavioral survey showed that HIV genetic clustering could be attributed to the differences in behavioral characteristics MSMs engaging in receptive anal intercourse in male sex in the previous 6 months, living in Shenzhen for more than 1 year, having first sex experience before the age of 18,

coming from high HIV prevalence areas were significantly more likely to be included in clusters. After multivariate logistic regression analysis, sex role and length of stay in Shenzhen were found to be associated with genetic clustering (Table 4-6).

Table 4-6 Correlation between behavioral risk factors and genetic clusters among MSM

Variable	*Clustered (n=81)n (%)*	*Non-clustered (n=25) n (%)*	*P*	Univariate	Multivariate
Venue for recruitment[$]				/	/
Bar & MC	6(9.5)	3(16.7)	0.839		
FC	21(33.3)	7(36.8)			
Park	20(31.7)	5(27.8)			
Sauna	16(25.4)	3(15.8)			
Sexual practices in male anal intercourse					
Insertive only	17(21.8)	11(44.0)	0.017*	4.3(1.4-13.0)	5.2(1.6-16.3)*
Both	47(60.3)	7(28.0)		1.3(0.4-4.2)	1.3(0.4-4.4)
Receptive only	14(17.9)	7(28.0)		1	1
Hometown prevalence of HIV infection					
Low	57(73.1)	23(92.0)	0.048*	4.0(1.0-18.6)	NS
High	21(26.9)	2(8.0)			
Length of stay in Shenzhen					
<=1 year	30(37.0)	15(60.0)	0.042*	0.3(0.1-0.8)	NS
>1 year	51(63.0)	10(40.0)			
Age of first sex					
<=18	38(46.9)	5(20.0)	0.017*	2.6(1.0-6.4)	1.8(1.1-2.9)*
>18	43(53.1)	20(80.0)		1	1
Number of anal sex partners					
<=1	24(29.6)	9(36.0)	0.548	2.0(0.8-5.1)	/
>1	57(70.4)	16(64.0)			
UAI in non-commercial male sex					
No	38(46.9)	16(64.0)	0.135	1.3(0.5-3.4)	/
Yes	43(53.1)	9(36.0)			
Ever received HIV test					
No	40(49.4)	8(32.0)	0.127	0.5(0.2-1.2)	/
Yes	41(50.6)	17(68.0)			
Condom use knowledge					
Low	17(21.0)	9(36.0)	0.127	2.1(0.8-5.6)	/
High	64(79.0)	16(64.0)			

*: P<0.05; [$]: n= 63 (cluster)+18 (non-cluster); NS:not significant

Since the venue-type differentiation could hardly explain the distribution of HIV strains, the relationship between possible genetic transmission chains and specific

venues of recruitment was further investigated based on the 6 genetic clusters observed in the phylogenetic tree In total, 79 pol sequences obtained from positive samples were collected from 26 different venues through TLS survey, and 61 of them were located in one of the 6 genetic clusters Among them, the subgroups of MSM from the exact same venues were identified within each genetic cluster Overall, 47 members (59 5% of the total) were found to be clustered into 17 MSM venue-groups These groups were defined as venue-specific transmission chains The number of members in these 17 chains was ranged from two to seven (two, 70 6%, three, 11 8%, more than four, 17 6%) The two largest chains (contain 6 and 7 members) of CRF07_BC were observed within two big venues including one park and one sauna, and most of the 12 pairs of MSMs in venue-specific transmission chains were collected from family clubs (8 from family clubs, 2 from park, 1 from a small bar and 1 from a sauna) MSMs included in these transmission chains had significantly greater proportion of being recruited from sauna or park (69 8% vs 30 2%, χ^2 = 8 635, P = 0 035) The high percentage of members within the venue-specific transmission chains (74 6%) showed a close relationship between HIV genetic characteristics and the specific MSM venue where MSM visited and found sex partners, which further indicated a high possibility of HIV transmission within venues

4.3.3. CD4 counts, recent infection and primary drug resistance

Among HIV positive cases recruited from TLS and RDS surveys, 84 were randomly selected and tested for recent infection (Figure 4-5) 31 (36 9%) of them were identified as being infected within the past 5 months, with an incidence estimated as 4 63% (95% CI 3 07-6 19) Individuals from cluster I and II (15% of recent infection) were mainly past infection, while MSMs from cluster III (100%) and other clusters (50%) had much higher proportion of recent infection Only a slightly higher percentage of recent infected individuals was observed in clustered MSMs compared with non-clustered samples (83 9% vs 71 7%, χ^2 = 1 598, P = 0 206), while the

variation in distribution of recent infect among different clusters was significant (χ^2 = 13.462, P = 0.019). The average CD4 count of the MSMs in this study was 391.41 cells/mm^3. 47.8% of them had a CD4 count less than 350 cells/mm^3, which is the WHO recommended threshold for initiating antiretroviral therapy in developing countries, and is also the threshold for providing free treatment in China. Non-clustered MSMs had significantly higher likelihood of having lower CD4 counts and were in need of initiating HAART immediately after the diagnose (83.3% vs. 39.4%, χ^2 = 3.944, P = 0.045). There was only one sample among the 106 HIV positive MSMs recruited in our study with drug resistant mutation on PR region of the virus (M46L), which indicated that the primary drug resistant rate among HIV positive MSMs in Shenzhen was low.

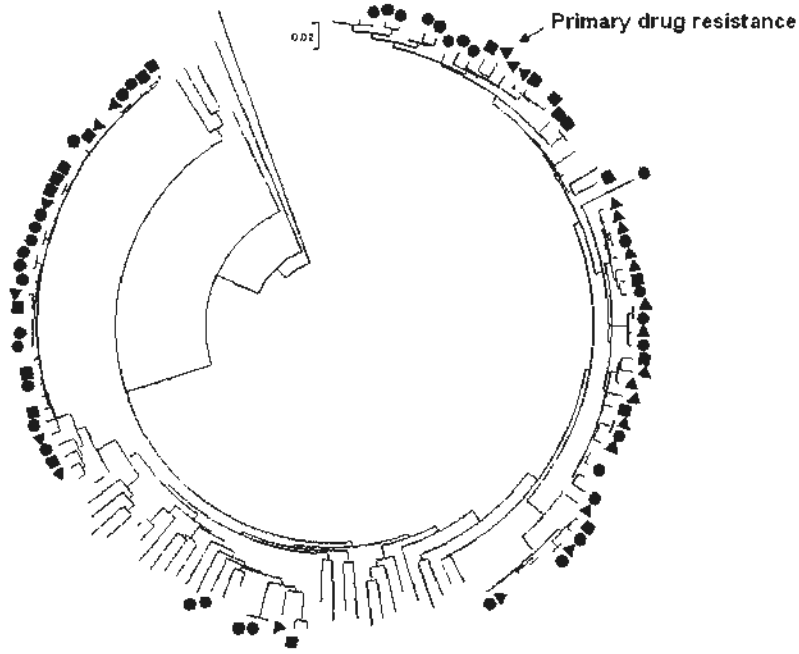

Figure 4-5 Strains of recent infection in the phylogenetic tree of MSM in the study
Blue: long term infection; red: recent infection
Circle: TLS 2009; square: TLS 2008; triangle: RDS

4.3.4. Comparison of HIV sequences collected from positive MSMs by TLS and RDS with MSMs from other sources

The 106 pol sequences collected in this study were compared with other 219 MSM samples collected during 2006 and 2009 by other sampling methods from other sources including STI clinics, VCT (voluntary counseling and testing), and integrated surveillance system (Figure 4-6) Since a CRF01_AE cluster was recently reported in Hong Kong MSMs [66], clustered CRF01-AE sequences in this study were compared with the reference sequences from Hong Kong CRF01-AE cluster members None of the Hong Kong clustered strains was found to be located in any clusters in our study, indicating no cross-border HIV transmission Phylogenetic analysis on env and gag regions of HIV genome were also conducted, similar genetic cluster distribution was observed in both env and gag trees (Figure 4-7)

The phylogenetic tree with a total of 325 sequences from HIV positive MSMs were compared for the year of diagnosis A significant greater proportion of recent diagnosed cases was found in CRF07_BC group compared with subtype B group

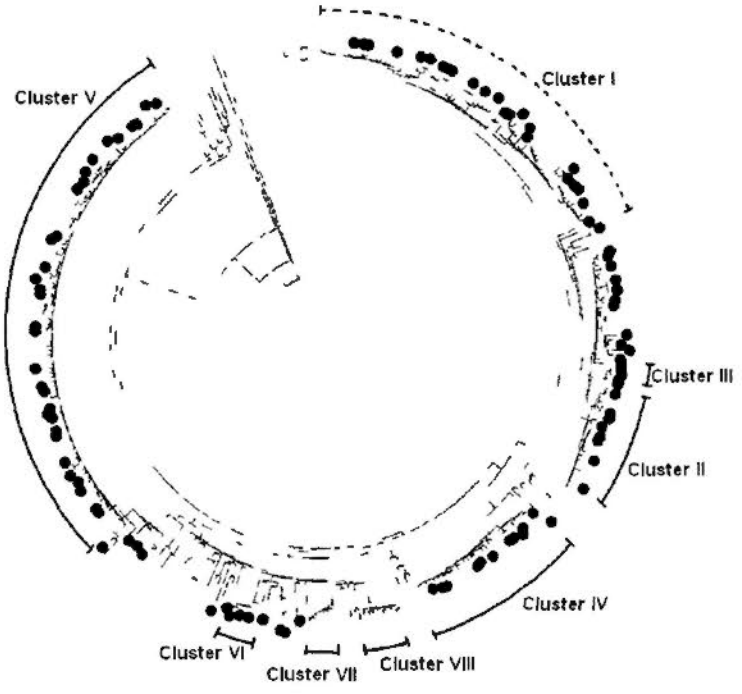

Figure 4-6 Phylogenetic tree of HIV strains from MSM in Shenzhen by program based on pol sequences (n=325)
Black solid circle MSM recruited from TLS and RDS survey

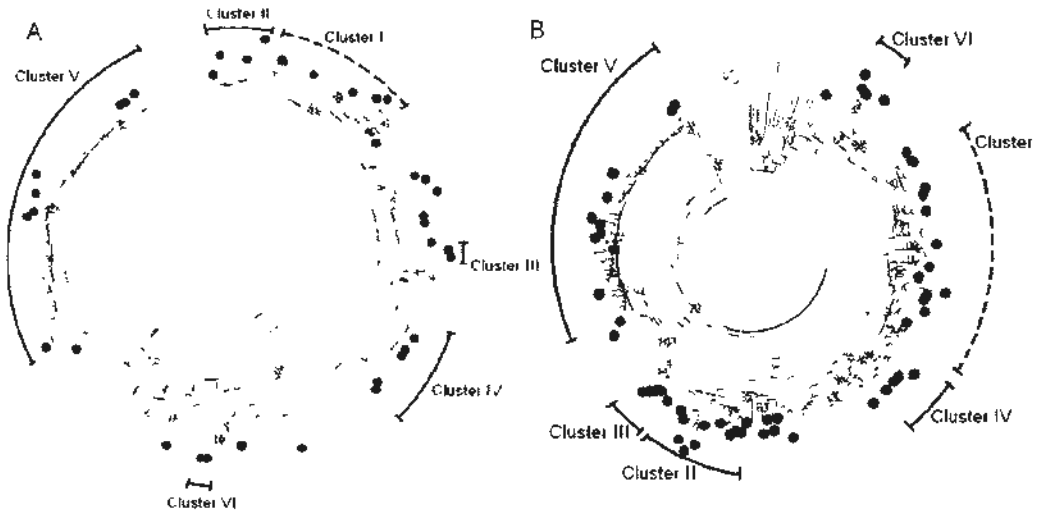

Figure 4-7 Phylogenetic tree of HIV strains from MSM in Shenzhen by program based on gag (n=151) and env (n=165) sequences

A gag tree; B: env tree; Black solid circle: MSM recruited from TLS and RDS survey.

Strains isolated from HIV positive MSMs in early epidemics stage (2006 and before) were classified into three genetic forms CRF01_AE (63 9%), B (27 8%) and CRF07_BC (8 2%) In year 2007, the proportion of CRF07_BC was increased among MSMs (CRF01_AE 47 1%, B 29 4%, CRF07_BC 20 6%) From 2008, less and less subtype B strains were observed among this population (Table 4-7 and Figure 4-7) In 2009, subtype B only accounted for only 9 1% of the total infected MSMs Although CRF01_AE was continuously the predominant genetic form in Shenzhen, the second predominant HIV subtype circulating among MSMs was shifted from B to CRF07_BC The distribution of HIV strains collected in this study was different from other HIV strains collected from MSMs who were diagnosed in 2008 and 2009 A slightly greater proportion of CRF01_AE and lower proportion of CRF07_BC were observed among MSMs from this study than other MSMs in both year 2008 and 2009 (Table 4-7) In addition, HIV strains from positive MSMs in our study were more likely to be clustered than strains from MSMs by other sampling methods (Table 4-8) These findings indicated that the distribution of HIV positive MSMs recruited from the approximate probability sampling (TLS and RDS) were different from MSMs by other methods, and therefore implied that the result from

previous used convenience sampling might not correctly represent the HIV distribution among MSMs.

Among the 6 transmission clusters identified in previous section with the study samples, only cluster I was no longer identified as a cluster due to the decreased bootstrap value and longer branch lengths. However, two new subtype B clusters (cluster Ⅶ and Ⅷ) were observed. Both of them contained a majority of early isolated strains with more than 50% were diagnosed before 2007.

Table 4-7 Subtype distribution among MSM in Shenzhen

Variable	CRF01_AE n (%)	B n (%)	CRF07_BC n (%)	X^2	*P*
Year of diagnosis					
2009	82(53.2)	14(9.1)	58(37.7)	22.69	**0.007**
2008	64(49.2)	20(15.4)	45(34.6)		
2007	16(47.1)	10(29.4)	7(20.6)		
2006 and before	62(63.9)	10(27.8)	4(8.2)		
2009 Samples				3.425	0.180
MSMs in project	38(62.3)	5(8.2)	18(29.5)		
Other MSMs	44(47.3)	9(9.7)	40(43.0)		
2008 Samples				1.792[$]	0.689
MSMs in project	23(51.1)	5(11.1)	17(37.8)		
Other MSMs	41(47.7)	16(18.6)	28(32.6)		

[$]: Fisher's exact value.

Table 4-8 Distribution of genetic clusters among MSM in Shenzhen

Variable	Clustered MSM n (%)	Non-clustered MSM n (%)	X^2	*P*
Year of diagnosis				
2009	98 (63.6)	56 (36.4)	4.707	0.753
2008	82 (63.1)	48 (36.9)		
2007	11 (61.1)	7 (38.9)		
2006 and before	14 (73.7)	5 (26.3)		
2009 Samples				
MSMs in project	46 (75.4)	15 (24.6)	6.051	**0.014**
Other MSMs	52 (55.9)	41 (44.1)		
2008 Samples				
MSMs in project	35 (77.8)	10 (22.2)	6.748	**0.009**
Other MSMs	82 (62.6)	49 (37.4)		

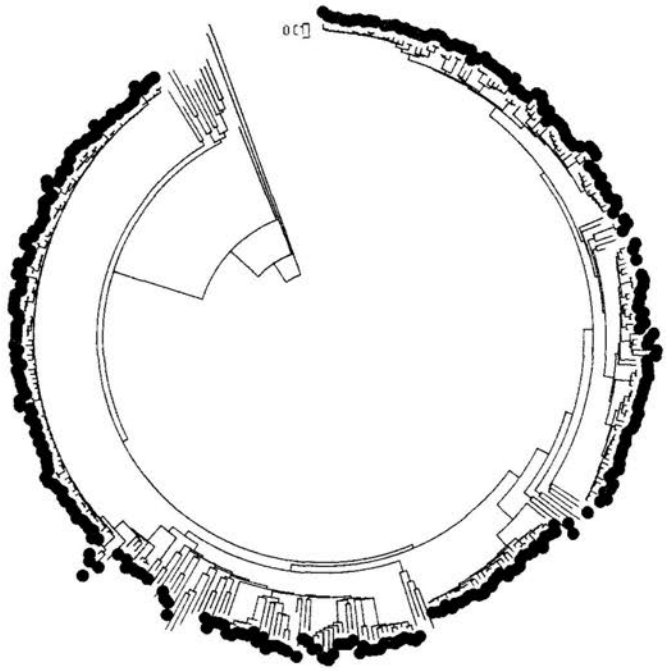

Figure 4-8 Pol tree of HIV strains from MSM in Shenzhen by year of diagnosis (n=325)
Red: before 2006; Green: 2007, blue: 2008; purple: 2009

4.3.5. Comparison of HIV sequences from positive MSM with individuals infected through other routes

The 325 MSM sequences were also compared with other 347 samples that were collected during year 2006 and 2009 from HIV positive individuals who were infected through other transmission routes including heterosexual contact, IDU and blood transmission (Figure 4-9) From the phylogenetic tree, HIV strains circulated among MSM were clustered together and were separated from other HIV individuals infected through blood-borne transmission or heterosexual transmission No cluster was found among the specimens from persons infected through blood-borne and heterosexual contact transmission

We then analyzed the associations of genetic clustering with mode of infection as well as diagnosing year Pearson's chi square test was used for the comparison of genetic cluster with transmission route, and trend test was used for year of diagnosis Individuals infected through homosexual contact had significantly higher likelihood to be clustered together compared with individuals through other routes of

transmission (Table 4-9) A trend of increasing proportion of clustered HIV strains isolated from newly diagnosed patients was observed by year After multivariate analysis, only transmission route was found to be the key determinant for genetic clusters, which indicated a closer transmission relationship among MSM population than other high risk populations. A transmission network among MSMs was probably formed from year 2007

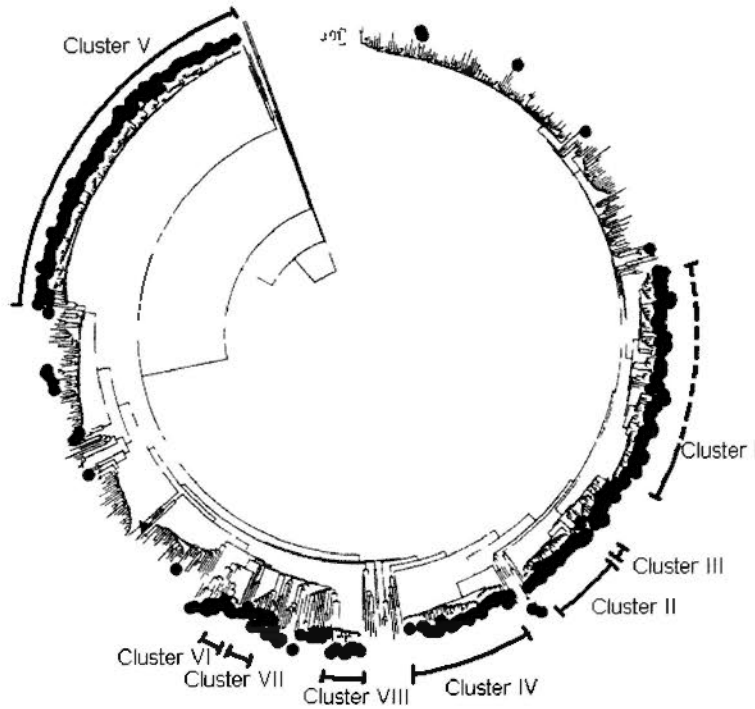

Figure 4-9 Phylogenetic tree of HIV pol sequences collected in Shenzhen (n=672)
Black solid circle: HIV strains from samples of MSM.

Table 4-9 Relationship of genetic clusters and epidemic data among MSM

Variable	*Cluster (n=252) n (%)*	*Non-cluster (n=419) n*	X^2	*P*	*AOR*[$]
Transmission route					
Homosexual	206(63.6)	118(36.4)	182.63	<0.001	1
Heterosexual	37(15.5)	201(84.5)			0.11(0.07-0.16)*
Other	9(8.3)	100(91.7)			0.07(0.02-0.25)*
Year of diagnosis					
2009	118(44.5)	147(55.5)	21.49[#]	<0.001	1
2008	97(38.5)	155(61.5)			0.89(0.59-1.34)
2007	17(39.5)	26(60.5)			1.29(0.59-2.83)
2006 or before	20(17.9)	92(82.1)			0.66(0.29-1.49)

*: P<0.05. [#]: trend test value; [$] : adjusted odds ratio in multivariate analysis.

4.4. Discussion

4.4.1. Genetic clusters and epidemiological characteristics of HIV positive MSMs

This chapter mainly investigated the molecular interrelationships among HIV positive MSMs and the association between genetic clustering and social behavioral characteristics of MSMs based on probability sampling methods Similar to result of Hong Kong showing that MSMs tended to form their own genetic clusters in molecular epidemiological analysis [67], several genetic clusters were also observed among Shenzhen MSMs Contrast to some studies reporting that no correlation was found between the distribution of MSMs in phylogenetic tree and social behavioral characteristics of MSMs [244], the findings in this study revealed that HIV positive MSM engaging in receptive anal intercourse in male sex in the previous 6 months, living in Shenzhen for more than 1 year, having first sex experience before the age of 18, coming from high HIV prevalence areas were more likely to be included in genetic clusters

Although no association was found between venue-type and transmission clusters, 17 venue-specific transmission chains which covered a majority of samples found in phylogenetic analysis might indicate an underlying HIV transmission in venue Since more than 50% of the MSMs in TLS surveys self-reported seeking partners in multiple types of venue, they might act as mediums in transmitting HIV to different venues This finding might be the reason that no association between genetic clusters and venue-types was observed in our study However, the large proportion of MSMs in venue-specific transmission chains still indicated the possibility of direct HIV transmission in venue Further study with a larger sample size and more detailed information on sexual contact between the MSMs in each chain should be considered to further evaluate the relationship between venue and HIV transmission

4.4.2. Genetic clusters and recent infection

Although all HIV cases used in this study were recently diagnosed, they did not indicate that the person was recently infected with HIV BED test could be an evidence for evaluating population based incidence Testing for recent infection is an alternative for follow-up and record-based studies when direct seroconversion time could hardly be identified for positive individuals A significant greater proportion of patients infected through homosexual contact were identified as recent infection (around 40%), compared with patients infected via injecting drug use and blood transmission (2-3%) [203] This finding urged the need of effective intervention and education among men who have sex men compared with other high risk populations Cluster III and V were probably newly emerged strains with a majority of members within clusters were diagnosed in 2008 and 2009

4.4.3. Sampling methods is crutial for molecular epidemiological study

Almost all phylogenetic analysis studies on genetic clusters were based on all reachable samples (or newly infected individuals) in a specific area [66, 67, 165, 186, 244-247], while sampling methods might influence the pattern of transmitted strains as viruses from some individuals could been sequenced retrospectively Traditional epidemiological method of contact tracing was widely used in identifying more unknown infected cases if the recent partner of a person was identified Contact tracing on individual basis thereby is easier to identify genetic clusters in accompany with the transmission chains, while it might bring bias to the population based analysis of transmission clustering The cluster of sequences obtained from contact tracing could have an impact on the percentage of clustered transmission events found in the respective year of infection, which was larger than expected when sampling was random It was found that samples collected from community-based sampling and snowball sampling methods were more likely to be clustered MSMs than samples in TLS and RDS (85% vs 76%) In addition, two additional subtype B clusters were observed among MSMs samples collected from other sources

In contrast, HIV positive MSMs found in VCT or STI clinics might be difficult to trace the place and time of infection, and therefore could hardly represent the MSMs in Shenzhen Identification of transmission route in patients from VCT or STI clinics was based on their self-judgements The lack of detailed epidemiological information especially recent behavioral data in male-male sex in these samples might lead to a false identification of transmission route Therefore, individuals recruited from TLS and RDS could be a representative sample based on random selection for molecular epidemiological analysis

The comparison between samples recruited in this study with other cumulated samples indicated that the most widely observed genetic clusters in CRF07_BC and CRF_01 AE among Shenzhen MSMs were still formed when a sampling strategy was applied However, the proportion of CRF01_AE was larger and subtype B was smaller among our samples compared with MSM samples being collected by other methods, though the differences were not significant due to the small sample size In addition, a majority of clusters in subtype B were not observed among MSMs in TLS and RDS studies Project samples were found to be distributed in all genetic clusters in the two recombinant forms (CRF07_BC and CRF01_AE), while they only located in 1 of the 3 genetic clusters in subtype B Most of MSMs infected by subtype B virus were recruited through other sources This situation might result from the trend of decreased proportion of subtype B virus in recent years among Shenzhen HIV positive population

4.4.4. Relationships between Shenzhen MSMs and Hong Kong MSMs

Genetic clusters in neighbor city Hong Kong were found exclusively among the subtype B specimens, and no sequence clustering was identified among CRF01_AE specimens, though CRF01_AE was the predominant genetic form in Hong Kong [67] Another recent study indicated a CRF01_AE MSM cluster was observed based on a part of Hong Kong MSM samples [66] The sequences were compared in our study, but they were allocated in a non-clustered form, which indicated that no molecular

evidence was observed for cross-border HIV transmission among MSMs However, near half of the MSMs in our epidemiological survey reported having sexual contact with Hong Kong partners

Based on a recent study on social network and genotypic clustering, it was found that the cluster was related to sauna and Internet [248] MSMs visiting sauna in Hong Kong might not be similar to MSMs visiting Shenzhen venues In addition, Internet-based MSMs might have different transmission network compared with venue-based MSMs Although Internet-based participants were recruited from RDS survey, with 50 2% of MSMs in RDS reported Internet only as the way of partner sourcing, the overall number of Internet-based HIV positive individuals (19) was comparatively small and could hardly provide adequate information for analysis Further Internet-based MSMs surveillance should be conducted to better interpret the HIV transmission pattern among MSM in Shenzhen and the relationship between Shenzhen and Hong Kong MSMs Finally, the conflict on phylogenetic analysis and population study for HIV transmission could probably due to the finding that the majority of MSMs having sex with Hong Kong MSMs were MBs (81%), a subgroup of MSMs that had significantly lower prevalence of HIV infection and higher condom use rate than other MSMs

However, we could hardly make any conclusion at this time since no parallel study was conducted among Hong Kong MSMs focusing on their cross-border sexual behaviors A cooperative program between the Chinese University of Hong Kong and Shenzhen CDC on cross-border Hong Kong MSMs was conducted in Shenzhen to investigate the social-behavioral characteristics of this unique subpopulation, and the data would be published soon We hope that data could help to explain the HIV transmission pattern among Shenzhen and Hong Kong MSMs

Chapter 5 Conclusions

1 This study is the first study conducted in China that investigated the prevalence of HIV infection and its risk factors in men who have sex with men (MSM) using two powerful sampling approaches in hard-to-reach population, time-location sampling method and respondent-driven sampling method The study is also the largest regional study on MSMs, to date, in the country, with the number of study subjects exceeding 2100 Another unique feature of this study is that money boys and other MSMs was separated for analysis and the HIV risks between them was compared based on parallel recruitments by TLS Furthermore, significant differences in HIV infection and related risk factors were observed among MSMs frequenting different MSM venues, which was the first report in China showing a correlation of HIV risk with venue The study determined molecular interrelationships among HIV positive MSMs sampled from TLS and RDS surveys based on the sequences of HIV strains

2 The prevalence of HIV infection among the MSMs was 5 5%, similar to the national level of 4 9% in MSMs However, there was a difference between MBs and other MSMs, in which a significantly lower infection rate was observed in MBs Factors found to be associated with HIV infection in MSMs included syphilis infection, occupation, sexual orientation, venue for recruitment and hometown prevalence of HIV infection Again, the risk factors were different between MBs and other MSMs The findings suggest that a separate analysis on MBs and other MSMs is necessary for a better interpretation of the HIV-related risk factors In addition, a separate recruitment of MBs and OMSMs might be helpful after comparing the result of mixed and separate sampling of these two subgroups Based on the results, HIV prevention and intervention should focus on developing a suitable strategy for different subgroups

3 The results from this study also showed that MSMs frequenting family clubs, parks and saunas, had significantly higher HIV infection than in entertainment

venues, such as bars and massage centers The socio-behavioral characteristics of MSMs in various venues were also different, which indicated a possible venue effects on MSMs' behaviors and preferences, and therefore urged the need to develop venue-specific interventions

4 The comparison of the results from TLS and RDS showed that the two sampling methods had a mutually compensable role in reaching high risk MSMs TLS could reach more sexually active MSMs in venue based on a random selection strategy, while it could only represent the population attending venues In contrast, RDS had the advantage to reach more hidden subgroup, such as Internet-based MSMs, while it might be hard to estimate the representativeness due to the lack of information on reliable eligibility and response rates In addition, TLS was a simple, but labor intensive method, RDS was convenient to use, but its analytic methods have not been well established and are comparatively sophisticated Using both of the methods together could compensate for the disadvantages of each, and cover more MSMs with different characteristics and different subgroups

5 From the molecular phylogenetical study on HIV strains of positive MSMs, certain molecular interrelationships were found There were genetic clusters, which indicated a high HIV transmission possibility among the members in clusters The close relationships among cluster members could be linked to the social behavioral characteristics of the subjects Furthermore, the high proportion (60%) of subjects in venue-specific transmission chains might indicate an underlying transmission of HIV in venue This finding was consistent with our epidemiologic study showing that MSMs frequenting different venues had different HIV infection rate and HIV-risk related characteristics The information may be helpful to formulate an intervention strategy to break the transmission chain in each high risk venue On the other hand, further studies with larger sample sizes and combination of both epidemiological and molecular data are needed to confirm the possible venue relationship with HIV transmission among MSMs

References

1 Zhou L, Guo J, Fan L, Tian J, Zhou B Survey of motivation for use of voluntary counseling and testing services for HIV in a high risk area of Shenyang, China BMC Health Serv Res2009 Feb 5,9(1) 23

2 Nguyen TA, Nguyen HT, Le GT, Detels R Prevalence and risk factors associated with HIV infection among men having sex with men in Ho Chi Minh City, Vietnam AIDS Behav2008 May.12(3) 476-82

3 Hidaka Y, Ichikawa S, Koyano J, Urao M, Yasuo T, Kimura H, et al Substance use and sexual behaviours of Japanese men who have sex with men a nationwide internet survey conducted in Japan BMC Public Health2006,6 239

4 Gupta A, Mehta S, Godbole SV, Sahay S, Walshe L, Reynolds SJ, et al Same-sex behavior and high rates of HIV among men attending sexually transmitted infection clinics in Pune, India (1993-2002) J Acquir Immune Defic Syndr2006 Dec 1,43(4) 483-90

5 Choi KH, Hudes ES, Steward WT Social discrimination, concurrent sexual partnerships, and HIV risk among men who have sex with men in Shanghai, China AIDS Behav2008 Jul.12(4 Suppl) S71-7

6 Chen KT, Chang HL, Chen CT, Chen YA The changing face of the HIV epidemic in Taiwan a new challenge for public health policy strategies AIDS Patient Care STDS2009 Mar.23(3) 195-201

7 UNAIDS/WHO AIDS epidemic update 2009 Genova UNAIDS2009 Jan 15, 2010

8 World Health Organization World health statistics 2008 Geneva World Health Organization2008

9 Smith AD, Tapsoba P, Peshu N, Sanders EJ, Jaffe HW Men who have sex with men and HIV/AIDS in sub-Saharan Africa Lancet2009 Aug 1,374(9687) 416-22

10 Mills S, Saidel T, Magnani R, Brown T Surveillance and modelling of HIV, STI, and risk behaviours in concentrated HIV epidemics Sexually transmitted infections2004 Dec,80 Suppl 2 ii57-62

11 Sharma M, Oppenheimer E, Saidel T, Loo V, Garg R A situation update on HIV epidemics among people who inject drugs and national responses in South-East Asia Region AIDS2009 Jul 17,23(11) 1405-13

12 Crago AL, Rakhmetova A, Karadafov M, Islamova S, Maslova I Central & Eastern Europe and Central Asia police raids and violence put sex workers at risk of HIV HIV AIDS Policy Law Rev2008 Dec.13(2-3) 71-2

13 Asia rapid increase in cases of HIV among MSM HIV AIDS Policy Law Rev2009 May.14(1) 35

14 Cheng MH Asian countries urged to address HIV/AIDS in MSM Lancet2009 Feb 28,373(9665) 707

15 Rodrigo C, Rajapakse S Current Status of HIV/AIDS in South Asia J Glob Infect Dis2009 Jul.1(2) 93-101

16 Lau KA, Wang B, Saksena NK Emerging trends of HIV epidemiology in Asia AIDS Rev2007 Oct-Dec,9(4) 218-29

17 Normile D AIDS Asia grapples with unexpected wave of HIV infections Science2009

Nov 27.326(5957) 1174
18 China State Council AIDS Working Committee Office & UN Theme Group on AIDS in China A joint assessment of HIVAIDS prevention. treatment and care in China(2007) Beijing2007
19 State Council AIDS Working Committee Office. UN Theme Group on AIDS in China A joint assessment of HIV/AIDS prevention. treatment and care in China 20072007 March 1. 2008
20 Ministry of Health of China AIDS epidemic update of China. December 2009 2009 [December 20. 2009]. Available from http //www gov cn/jrzg/2009-12/01/content_1477101 htm
21 Wu Z HIV epidemics in China national annual meeting on HIV/AIDS. Beijing. China2010
22 Feng Y. Wu Z. Detels R. Qin G. Liu L. Wang X. et al HIV/STD prevalence among men who have sex with men in Chengdu. China and associated risk factors for HIV infection J Acquir Immune Defic Syndr2010 Feb.53 Suppl 1 S74-80
23 Ruan S. Yang H. Zhu Y. Wang M. Ma Y. Zhao J. et al Rising HIV prevalence among married and unmarried among men who have sex with men Jinan. China AIDS Behav2009 Aug.13(4) 671-6
24 Liu H. Cai Y. Rhodes AG. Hong F Money boys. HIV risks. and the associations between norms and safer sex a respondent-driven sampling study in Shenzhen. China AIDS Behav2009 Aug.13(4) 652-62
25 Lau JT. Wang M. Tse YK. Gu J. Tsui HY. Zhang Y. et al HIV-related behaviors among men who have sex with men in China 2005-2006 AIDS Educ Prev2009 Aug.21(4) 325-39
26 Wong FY. Huang ZJ. He N. Smith BD. Ding Y. Fu C. et al HIV risks among gay- and non-gay-identified migrant money boys in Shanghai. China AIDS Care2008 Feb.20(2) 170-80
27 Ruan S. Yang H. Zhu Y. Ma Y. Li J. Zhao J. et al HIV prevalence and correlates of unprotected anal intercourse among men who have sex with men. Jinan. China AIDS Behav2008 May.12(3) 469-75
28 He Q. Wang Y. Li Y. Zhang Y. Lin P. Yang F. et al Accessing men who have sex with men through long-chain referral recruitment. Guangzhou. China AIDS Behav2008 Jul.12(4 Suppl) S93-6
29 Cai YM. Liu H. Pan P. Hong FC. Lai YH. Zhou H Investigation of STD/AIDS high risk behavior among male sex workers in Shenzhen Chinese Journal of AIDS & STD2008.14(02) 142-4
30 Ma X. Zhang Q. He X. Sun W. Yue H. Chen S. 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 J Acquir Immune Defic Syndr2007 Aug 15.45(5) 581-7
31 Li QH. Wu H. Sun LJ. Huang XJ. Liu Y. Sun X. et al Infections of STDs/HIV and Assessment of Risk Factors in Male Sex Workers Journal of Capital University of Medical Sciences2006(06)
32 Zha ZX Shenzhen population's present situation. problem and countermeasure research special zone economy2006.10 12-5

33 Chen L, Feng TJ, Tan JG, Cai WD, Shi XD, Wang XH, et al Estimate the Male Homosexual Population in Shenzhen by Capture-Mark-Recapture Method in 2006 Journal of Tropical Medicine2008;8(2) 175-6
34 Zhang BC, Chu QS MSM and HIV/AIDS in China Cell Res2005 Nov-Dec;15(11-12) 858-64
35 Lipovsek V, Mukherjee A, Navin D, Marjara P, Sharma A, Roy KP Increases in self-reported consistent condom use among male clients of female sex workers following exposure to an integrated behaviour change programme in four states in southern India Sex Transm Infect2010 Feb;86 Suppl 1 i25-32
36 Baral S, Kizub D, Masenior NF, Peryskina A, Stachowiak J, Stibich M, et al Male sex workers in Moscow, Russia a pilot study of demographics, substance use patterns, and prevalence of HIV-1 and sexually transmitted infections AIDS Care2010 Jan;22(1) 112-8
37 Mimiaga MJ, Reisner SL, Tinsley JP, Mayer KH, Safren SA Street Workers and Internet Escorts Contextual and Psychosocial Factors Surrounding HIV Risk Behavior among Men Who Engage in Sex Work with Other Men J Urban Health2009 Jan;86(1) 54-66
38 Infante C, Sosa-Rubi SG, Cuadra SM Sex work in Mexico vulnerability of male, travesti, transgender and transsexual sex workers Cult Health Sex2009 Feb;11(2) 125-37
39 Tun W, de Mello M, Pinho A, Chinaglia M, Diaz J Sexual risk behaviours and HIV seroprevalence among male sex workers who have sex with men and non-sex workers in Campinas, Brazil Sex Transm Infect2008 Nov;84(6) 455-7
40 Smith MD, Seal DW Sexual Behavior, Mental Health, Substance Use, and HIV Risk Among Agency-Based Male Escorts in a Small U S City Int J Sex Health2008 Mar 1;19(4) 27-39
41 Smith MD, Grov C, Seal DW Agency-Based Male Sex Work A Descriptive Focus on Physical, Personal, and Social Space J Mens Stud2008 May 1;16(2) 193-210
42 Timpson SC, Ross MW, Williams ML, Atkinson J Characteristics, drug use, and sex partners of a sample of male sex workers Am J Drug Alcohol Abuse2007;33(1) 63-9
43 Parsons JT, Koken JA, Bimbi DS Looking beyond HIV eliciting individual and community needs of male internet escorts J Homosex2007;53(1-2) 219-40
44 Kaye K Sex and the unspoken in male street prostitution J Homosex2007;53(1-2) 37-73
45 Hwahng SJ, Nuttbrock L Sex Workers, Fem Queens, and Cross-Dressers Differential Marginalizations and HIV Vulnerabilities Among Three Ethnocultural Male-to-Female Transgender Communities in New York City Sex Res Social Policy2007 Dec;4(4) 36-59
46 Bimbi DS Male prostitution pathology, paradigms and progress in research J Homosex2007;53(1-2) 7-35
47 Williams ML, Bowen AM, Timpson SC, Ross MW, Atkinson JS HIV prevention and street-based male sex workers an evaluation of brief interventions AIDS Educ Prev2006 Jun;18(3) 204-15
48 Sethi G, Holden BM, Gaffney J, Greene L, Ghani AC, Ward H HIV, sexually transmitted infections, and risk behaviours in male sex workers in London over a 10 year period Sex Transm Infect2006 Oct;82(5) 359-63
49 Parker M Core groups and the transmission of HIV learning from male sex workers J

Biosoc Sci2006 Jan.38(1) 117-31
50 Bacon O, Lum P, Hahn J, Evans J, Davidson P, Moss A, et al Commercial sex work and risk of HIV infection among young drug-injecting men who have sex with men in San Francisco Sex Transm Dis2006 Apr.33(4) 228-34
51 Aynalem G, Smith L, Bemis C, Taylor M, Hawkins K, Kerndt P Commercial sex venues a closer look at their impact on the syphilis and HIV epidemics among men who have sex with men Sex Transm Infect2006 Dec.82(6) 439-43
52 Pisani E, Girault P, Gultom M, Sukartini N, Kumalawati J, Jazan S, et al HIV, syphilis infection, and sexual practices among transgenders, male sex workers, and other men who have sex with men in Jakarta, Indonesia Sex Transm Infect2004 Dec.80(6) 536-40
53 Williams ML, Bowen AM, Timpson S, Blair Keel K Drug injection and sexual mixing patterns of drug-using male sex workers Sex Transm Dis2003 Jul.30(7) 571-4
54 Minichiello V, Marino R, Browne J, Jamieson M, Peterson K, Reuter B, et al Male sex workers in three Australian cities socio-demographic and sex work characteristics J Homosex2001.42(1) 29-51
55 Kelly JA, Amirkhanian YA, McAuliffe TL, Dyatlov RV, Granskaya J, Borodkina OI, et al HIV risk behavior and risk-related characteristics of young Russian men who exchange sex for money or valuables from other men AIDS Educ Prev2001 Apr.13(2) 175-88
56 Cai YM, Liu H, Pan P, Hong FC, Zhou H Survey of KABP on STD/AIDS among male sexual workers in Shenzhen City China Tropical Medicine2007.7(11) 2131-3
57 Zeng HF, Qin YM, Ye BY, Lin AH, Cai WD Survey of infectious status of HIV/AIDS in male homosexuals in Shenzhen City China Tropical Medicine2006.6(9) 1686-8
58 Hong FC, Zhou H, Cai YM, Pan P, Feng TJ, Liu XL, 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 Sex Transm Infect2009 Feb.85(1) 42-4
59 Choi KH, Gibson DR, Han L, Guo Y High levels of unprotected sex with men and women among men who have sex with men a potential bridge of HIV transmission in Beijing, China AIDS Educ Prev2004 Feb.16(1) 19-30
60 Feng L, Ding X, Lu R, Liu J, Sy A, Ouyang L, et al High HIV Prevalence Detected in 2006 and 2007 Among Men Who Have Sex With Men in China's Largest Municipality An Alarming Epidemic in Chongqing, China J Acquir Immune Defic Syndr2009 Sep.52(1) 79-85
61 Feng Y, Wu Z, Detels R Evolution of men who have sex with men community and experienced stigma among men who have sex with men in Chengdu, China J Acquir Immune Defic Syndr2010 Feb.53 Suppl 1 S98-103
62 Reisner SL, Mimiaga MJ, Mayer KH, Tinsley JP, Safren SA Tricks of the trade sexual health behaviors, the context of HIV risk, and potential prevention intervention strategies for male sex workers J LGBT Health Res2008.4(4) 195-209
63 Padilla M, Castellanos D, Guilamo-Ramos V, Reyes AM, Sanchez Marte LE, Soriano MA Stigma, social inequality, and HIV risk disclosure among Dominican male sex workers Soc Sci Med2008 Aug.67(3) 380-8
64 Kong TS Risk factors affecting condom use among male sex workers who serve men in China a qualitative study Sex Transm Infect2008 Nov.84(6) 444-8
65 Lau JT, Kim JH, Lau M, Tsui HY Prevalence and risk behaviors of Hong Kong males

who seek cross-border same-sex partners in mainland China Sex Transm Dis2004 Sep.31(9) 568-74
66 Chen JH, Wong KH, Li P, Chan KC, Lee MP, Lam HY, et al Molecular epidemiological study of HIV-1 CRF01_AE transmission in Hong Kong J Acquir Immune Defic Syndr2009 Aug 15.51(5) 530-5
67 Leung TW, Mak D, Wong KH, Wang Y, Song YH, Tsang DN, et al Molecular epidemiology demonstrated three emerging clusters of human immunodeficiency virus type 1 subtype B infection in Hong Kong AIDS Res Hum Retroviruses2008 Jul.24(7) 903-10
68 Zhao GL, Feng TJ, Zhao J, Wang XH, Shi XD, Zhang Y, et al Study on molecular epidemiology of human immunodeficiency virus type 1 infection in men who have sex with men(MSM)in Shenzhen CHINESE JOURNAL OF AIDS & STD2008.14(2) 137-41
69 UNAIDS/APCOM Men who have Sex with Men (MSM) – Update for ICAAP, Bali, 2009 report on the commission of AIDS in Asia ICAAP, Bali2009
70 van Griensven F, de Lind van Wijngaarden JW, Baral S, Grulich A The global epidemic of HIV infection among men who have sex with men Curr Opin HIV AIDS2009 Jul.4(4) 300-7
71 Fenton K Possibilities for Moving Forward the 2010 National STD Prevention Conference, March 8-11, 2010, Atlanta2010
72 Stephane LV, Strat YL, Cazein F, Pillonel J, Brunet S, Bousquet V, et al Population-based HIV Incidence in France, 2003 to 2008 the 17th Conference on Retroviruses and Opportunistic Infections (CROI) Feburary 18,2010, San Francisco2010
73 Staff Writer HIV infections in gay men 'increasing in homophobic countries' 2010 [updated March 16, 2010March 18, 2010]. Available from http //www pinknews co uk/2010/03/16/hiv-infections-in-gay-men-increasing-in-homophobic-countries/
74 Choi KH, Liu H, Guo Y, Han L, Mandel JS, Rutherford GW Emerging HIV-1 epidemic in China in men who have sex with men Lancet2003 Jun 21.361(9375) 2125-6
75 Mi G, Wu Z, Zhang B, Zhang H Survey on HIV/AIDS-related high risk behaviors among male sex workers in two cities in China AIDS2007 Dec.21 Suppl 8 S67-72
76 Geibel S, van der Elst EM, King'ola N, Luchters S, Davies A, Getambu EM, et al 'Are you on the market?' a capture-recapture enumeration of men who sell sex to men in and around Mombasa, Kenya AIDS2007 Jun 19.21(10) 1349-54
77 Lan YJ, Gu Y, Wang B, Zhou DL, Zhang JX [Behavioral features of men who have sex with men] Sichuan Da Xue Xue Bao Yi Xue Ban2004 May.35(3) 372-5
78 McFarland W, Busch MP, Kellogg TA, Rawal BD, Satten GA, Katz MH, et al Detection of early HIV infection and estimation of incidence using a sensitive/less-sensitive enzyme immunoassay testing strategy at anonymous counseling and testing sites in San Francisco J Acquir Immune Defic Syndr1999 Dec 15.22(5) 484-9
79 do Espirito Santo ME, Etheredge GD HIV prevalence and sexual behaviour of male clients of brothels' prostitutes in Dakar, Senegal AIDS Care2003 Feb.15(1) 53-62
80 Zhu WM, Lin HJ, Zhang YF, Qiu DH, Feng JF, Gao MY, et al [Human immunodeficiency virus/sexually transmitted infection, risk behavior and sexual networks among men who have sex with men in Taizhou city, Zhejiang province] Zhonghua Liu Xing Bing Xue Za Zhi2008 Oct.29(10) 994-8

81 Li A, Varangrat A, Wimonsate W, Chemnasiri T, Sinthuwattanawibool C, Phanuphak P, et al Sexual behavior and risk factors for HIV infection among homosexual and bisexual men in Thailand AIDS Behav2009 Apr;13(2) 318-27
82 Fuller CM, Absalon J, Ompad DC, Nash D, Koblin B, Blaney S, et al A comparison of HIV seropositive and seronegative young adult heroin- and cocaine-using men who have sex with men in New York City, 2000-2003 J Urban Health2005 Mar;82(1 Suppl 1) i51-61
83 Hirshfield S, Remien RH, Walavalkar I, Chiasson MA Crystal methamphetamine use predicts incident STD infection among men who have sex with men recruited online a nested case-control study J Med Internet Res2004 Nov 29;6(4) e41
84 Smereck GA, Hockman EM Prevalence of HIV infection and HIV risk behaviors associated with living place on-the-street homeless drug users as a special target population for public health intervention Am J Drug Alcohol Abuse1998 May;24(2) 299-319
85 Ruan Y, Luo F, Jia Y, Li X, Li Q, Liang H, et al Risk factors for syphilis and prevalence of HIV, hepatitis B and C among men who have sex with men in Beijing, China implications for HIV prevention AIDS Behav2009 Aug;13(4) 663-70
86 Li CM, Xiao Y, Liu JB, Ding XB, Jia Y [HIV and syphilis infections among men who have sex with men in Chongqing municipality, China] Zhonghua Liu Xing Bing Xue Za Zhi2009 Feb;30(2) 126-30
87 Guo H, Wei JF, Yang H, Huan X, Tsui SK, Zhang C Rapidly increasing prevalence of HIV and syphilis and HIV-1 subtype characterization among men who have sex with men in Jiangsu, China Sex Transm Dis2009 Feb;36(2) 120-5
88 Hao Y Update on the AIDS epidemic in China the ninth international conference on HIV/AIDS in the Asia-Pacific (ICAAP) Aug , Bali, Indonesia2009
89 Choi KH, Ning Z, Gregorich SE, Pan QC The influence of social and sexual networks in the spread of HIV and syphilis among men who have sex with men in Shanghai, China J Acquir Immune Defic Syndr2007 May 1;45(1) 77-84
90 Zhang X, Wang C, Hengwei W, Li X, Li D, Ruan Y, et al Risk factors of HIV infection and prevalence of co-infections among men who have sex with men in Beijing, China AIDS2007 Dec;21 Suppl 8 S53-7
91 Jiang J, Cao N, Zhang J, Xia Q, Gong X, Xue H, et al High prevalence of sexually transmitted diseases among men who have sex with men in Jiangsu Province, China Sex Transm Dis2006 Feb;33(2) 118-23
92 Ma XY, Zhang QY, He X, Zhao JK, Li Y, Sun WD, et al [Epidemiological study on the status of HIV/STDs and relative behaviors among MSM in Beijing] Zhonghua Liu Xing Bing Xue Za Zhi2007 Sep;28(9) 851-5
93 Lau JT, Wang M, Wong HN, Tsui HY, Jia M, Cheng F, et al Prevalence of bisexual behaviors among men who have sex with men (MSM) in China and associations between condom use in MSM and heterosexual behaviors Sex Transm Dis2008 Apr;35(4) 406-13
94 Ruan Y, Li D, Li X, Qian HZ, Shi W, Zhang X, et al Relationship between syphilis and HIV infections among men who have sex with men in Beijing, China Sex Transm Dis2007 Aug;34(8) 592-7
95 Cai WD, Zhao J, Zhao JK HIV prevalence and related risk factors among male sex workers in Shenzhen, China - Results from a time-location-sampling survey Sexually transmitted infections [J] 2009

96 Special Preventive Programme. Centre for Health Protection. Department of Health HIV/AIDS Situation in Hong Kong [2009] Hong Kong2010
97 Special Preventive Programme Centre for Health Protection. Department of Health CRiSP - Community Based Risk Behavioural and Seroprevalence Survey for Female Sex Workers in Hong Kong 2006 Hong Kong2007
98 Special Preventive Programme Centre for Health Protection, Department of Health PRiSM-HIV Prevalence and Risk behavioural Survey of Men who have sex with men in Hong Kong 2008 Hong Kong2009
99 Chemnasiri T. Netwong T. Visarutratana S, Varangrat A. Li A, Phanuphak P. et al Inconsistent condom use among young men who have sex with men, male sex workers. and transgenders in Thailand AIDS Educ Prev2010 Apr,22(2) 100-9
100 Shinde S. Setia MS. Row-Kavi A. Anand V. Jerajani H Male sex workers are we ignoring a risk group in Mumbai, India? Indian J Dermatol Venereol Leprol2009 Jan-Feb.75(1) 41-6
101 Setia MS. Lindan C. Jerajani HR. Kumta S. Ekstrand M. Mathur M. et al Men who have sex with men and transgenders in Mumbai, India an emerging risk group for STIs and HIV Indian J Dermatol Venereol Leprol2006 Nov-Dec.72(6) 425-31
102 Cai WD. Zhao J. Zhao JK. Raymond HF. Feng YJ. Liu J. et al HIV prevalence and related risk factors among male sex workers in Shenzhen. China results from a time-location sampling survey Sex Transm Infect2010 Feb.86(1) 15-20
103 Lau JT. Cai WD. Tsui HY. Chen L. Cheng JQ Psychosocial factors in association with condom use during commercial sex among migrant male sex workers living in Shenzhen, mainland China who serve cross-border Hong Kong male clients AIDS Behav2009 Oct.13(5) 939-48
104 Geibel S, Luchters S. King'Ola N. Esu-Williams E. Rinyiru A, Tun W Factors associated with self-reported unprotected anal sex among male sex workers in Mombasa. Kenya Sex Transm Dis2008 Aug.35(8) 746-52
105 He N. Wong FY. Huang ZJ. Ding Y. Fu C. Smith BD, et al HIV risks among two types of male migrants in Shanghai, China money boys vs general male migrants Aids2007 Dec.21 Suppl 8 S73-9
106 Liu H. Liu H. Cai Y, Rhodes AG Hong F Money boys. HIV risks. and the associations between norms and safer sex a respondent-driven sampling study in Shenzhen. China AIDS Behav2009 Aug.13(4) 652-62
107 He N, Wong FY. Huang ZJ. Thompson EE. Fu C Substance use and HIV risks among male heterosexual and 'money boy' migrants in Shanghai. China AIDS care2007 Jan.19(1) 109-15
108 Magnani R. Sabin K. Saidel T. D H Review of sampling hard-to-reach and hidden populations for HIV surveillance AIDS2005.19(S2) S67-S72
109 Watters JK. Biernacki P Targeted sampling Options for the study of hidden populations Social Problems 1989.36(4) 416-30
110 Coryn CL. Gugiu PC. Davidson EJ. Schroter DC Needs assessment in hidden populations using respondent-driven sampling Eval J Aust2007.7(2) 3-11
111 Heckathorn DD Respondent-driven sampling a new approach to the study of hidden populations Social Problems1997.44(2) 174-99

112 Heckathorn DD Respondent driven sampling II Deriving valid population estimates from chain-referral samples of hidden populations Social Problems2002,49(1) 11-34
113 Ferreira LO, de Oliveira ES, Raymond HF, Chen SY, McFarland W Use of time-location sampling for systematic behavioral surveillance of truck drivers in Brazil AIDS and behavior2008 Jul,12(4 Suppl) S32-8
114 Gjenero-Margan I, Kolaric B Epidemiology of HIV infection and AIDS in Croatia--an overview Coll Antropol2006 Dec,30 Suppl 2 11-6
115 Carlson RG, Wang J, Siegal HA, Falck RS, Guo J An ethnographic approach to targeted sampling Problems and solutions in AIDS prevention research among injection drug and crack-cocaine users Human Organization 1994,53(3) 279-86
116 Muhib FB, Lin LS, Stueve A, Miller RL, Ford WL, Johnson WD, et al A venue-based method for sampling hard-to-reach populations Public health reports (Washington, DC 1974)2001,116 Suppl 1 216-22
117 Xia Q, Tholandi M, Osmond DH, Pollack LM, Zhou W, Ruiz JD, et al The effect of venue sampling on estimates of HIV prevalence and sexual risk behaviors in men who have sex with men Sex Transm Dis2006 Sep,33(9) 545-50
118 Mansergh G, Naorat S, Jommaroeng R, Jenkins RA, Jeeyapant S, Kanggarnrua K, et al Adaptation of Venue-Day-Time Sampling in Southeast Asia to Access Men Who Have Sex with Men for HIV Assessment in Bangkok Field Methods2006 May,18(2) 135-52
119 MacKellar D, Valleroy L, Karon J, Lemp G, Janssen R The Young Men's Survey methods for estimating HIV seroprevalence and risk factors among young men who have sex with men Public health reports (Washington, DC 1974)1996,111 Suppl 1 138-44
120 van Griensven F, Thanprasertsuk S, Jommaroeng R, Mansergh G, Naorat S, Jenkins RA, et al Evidence of a previously undocumented epidemic of HIV infection among men who have sex with men in Bangkok, Thailand AIDS2005 Mar 25,19(5) 521-6
121 HIV/STD risks in young men who have sex with men who do not disclose their sexual orientation--six U S cities, 1994-2000 MMWR Morb Mortal Wkly Rep2003 Feb 7,52(5) 81-6
122 Lemp GF, Hirozawa A M , Givertz D , Nieri G N , Anderson L , Lindegren M L, et al Seroprevalence of HIV and risk behaviors among young homosexual and bisexual men The San Francisco/Berkeley Young Men's Survey JAMA1994,272 449-54
123 Berry M, Raymond HF, Kellogg T, McFarland W The Internet, HIV serosorting and transmission risk among men who have sex with men, San Francisco AIDS2008 Mar 30,22(6) 787-9
124 Bohl DD, Raymond HF, Arnold M, McFarland W Concurrent sexual partnerships and racial disparities in HIV infection among men who have sex with men Sex Transm Infect2009 Sep,85(5) 367-9
125 Heimer R, Grau LE, Curtin E, Khoshnood K, Singer M Assessment of HIV testing of urban injection drug users implications for expansion of HIV testing and prevention efforts American Journal of Public Health2007 Jan,97(1) 110-6
126 McFarland W, Caceres CF HIV surveillance among men who have sex with men AIDS2001 Apr,15 Suppl 3 S23-32
127 Ramirez-Valles J, Heckathorn DD, Vazquez R, Diaz RM, Campbell RT From networks to populations the development and application of respondent-driven sampling among IDUs

and Latino gay men AIDS and behavior2005 Dec,9(4) 387-402
128 Raymond HF, McFarland W Racial mixing and HIV risk among men who have sex with men AIDS Behav2009 Aug,13(4) 630-7
129 Snowden JM, Raymond HF, McFarland W Prevalence of seroadaptive behaviours of men who have sex with men, San Francisco, 2004 Sex Transm Infect2009 Oct,85(6) 469-76
130 Yeka W, Maibani-Michie G, Prybylski D, Colby D Application of respondent driven sampling to collect baseline data on FSWs and MSM for HIV risk reduction interventions in two urban centres in Papua New Guinea J Urban Health2006 Nov,83(6 Suppl) i60-72
131 van Griensven F, Varangrat A, Wimonsate W, Tanpradech S, Kladsawad K, Chemnasiri T, et al Trends in HIV Prevalence, Estimated HIV Incidence, and Risk Behavior Among Men Who Have Sex With Men in Bangkok, Thailand, 2003-2007 J Acquir Immune Defic Syndr2009 Nov 5
132 MacKellar DA, Gallagher KM, Finlayson T, Sanchez T, Lansky A, Sullivan PS Surveillance of HIV risk and prevention behaviors of men who have sex with men--a national application of venue-based, time-space sampling Public Health Rep2007,122 Suppl 1 39-47
133 Watts DJ Small Worlds The Dynamics of Networks Between Order and Randomness Princeton NJ Princeton University Press, 2003
134 Heckathorn DD Extensions of Respondent-Driven Sampling Analyzing Continuous Variables and Controlling for Differential Recruitment Sociological Methodology2007,37(1) 151-207
135 Salganik MJ, Heckathorn DD Sampling and Estimation in Hidden Populations Using Respondent-Driven Sampling Sociological Methodology2004,34(1) 193-239
136 Colby D, Minh TT, Toan TT Down on the farm homosexual behaviour, HIV risk and HIV prevalence in rural communities in Khanh Hoa province, Vietnam Sex Transm Infect2008 Nov,84(6) 439-43
137 Deiss RG, Brouwer KC, Loza O, Lozada RM, Ramos R, Cruz MA, et al High-risk sexual and drug using behaviors among male injection drug users who have sex with men in 2 Mexico-US border cities Sex Transm Dis2008 Mar,35(3) 243-9
138 Des Jarlais DC, Arasteh K, Perlis T, Hagan H, Abdul-Quader A, Heckathorn DD, et al Convergence of HIV seroprevalence among injecting and non-injecting drug users in New York City AIDS2007 Jan 11,21(2) 231-5
139 Malekinejad M, Johnston L, Kendall C, Kerr L, Rifkin M, G R Using respondent-driven sampling methodology for HIV biological and behavioral surveillance in international settings a systematic review *AIDS and Behavior* 2008,**12**(S1) 105-30
140 Millett GA, Ding H, Lauby J, Flores S, Stueve A, Bingham T, et al Circumcision status and HIV infection among Black and Latino men who have sex with men in 3 US cities J Acquir Immune Defic Syndr2007 Dec 15,46(5) 643-50
141 Bozicevic I, Rode OD, Lepej SZ, Johnston LG, Stulhofer A, Dominkovic Z, et al Prevalence of sexually transmitted infections among men who have sex with men in Zagreb, Croatia AIDS Behav2009 Apr,13(2) 303-9
142 Gondim RC, Kerr LR, Werneck GL, Macena RH, Pontes MK, Kendall C Risky sexual practices among men who have sex with men in Northeast Brazil results from four

sequential surveys Cad Saude Publica2009 Jun.25(6) 1390-8
143 Gorbach PM, Murphy R, Weiss RE, Hucks-Ortiz C, Shoptaw S Bridging sexual boundaries men who have sex with men and women in a street-based sample in los angeles J Urban Health2009 Jul.86 Suppl 1 63-76
144 Lane T, Raymond HF, Dladla S, Rasethe J, Struthers H, McFarland W, et al High HIV Prevalence Among Men Who have Sex with Men in Soweto, South Africa Results from the Soweto Men's Study AIDS Behav2009 Aug 7
145 Mimiaga MJ, Goldhammer H, Belanoff C, Tetu AM, Mayer KH Men who have sex with men perceptions about sexual risk, HIV and sexually transmitted disease testing, and provider communication Sex Transm Dis2007 Feb,34(2) 113-9
146 Simic M, Johnston LG, Platt L, Baros S, Andjelkovic V, Novotny T, et al Exploring barriers to 'respondent driven sampling' in sex worker and drug-injecting sex worker populations in Eastern Europe Journal of urban health bulletin of the New York Academy of Medicine2006 Nov.83(6 Suppl) i6-15
147 Robinson WT, Risser JM, McGoy S, Becker AB, Rehman H, Jefferson M, et al Recruiting injection drug users a three-site comparison of results and experiences with respondent-driven and targeted sampling procedures J Urban Health2006 Nov,83(6 Suppl) i29-38
148 Des Jarlais DC, Arasteh K, Perlis T, Hagan H, Heckathorn DD, McKnight C, et al The transition from injection to non-injection drug use long-term outcomes among heroin and cocaine users in New York City Addiction2007 May,102(5) 778-85
149 Lansky A, Abdul-Quader AS, Cribbin M, Hall T, Finlayson TJ, Garffin RS, et al Developing an HIV behavioral surveillance system for injecting drug users the National HIV Behavioral Surveillance System Public Health Reports2007,122(S1) 48-55
150 Holmes EC RNA virus genomics a world of possibilities J Clin Invest2009 Sep,119(9) 2488-95
151 Kim GJ, Nam JG, Shin BG, Kee MK, Kim EJ, Lee JS, et al National survey of prevalent HIV strains limited genetic variation of Korean HIV-1 clade B within the population of Korean men who have sex with men J Acquir Immune Defic Syndr2008 Jun 1.48(2) 127-32
152 Kolader ME, Dukers NH, van der Bij AK, Dierdorp M, Fennema JS, Coutinho RA, et al Molecular epidemiology of Neisseria gonorrhoeae in Amsterdam, The Netherlands, shows distinct heterosexual and homosexual networks J Clin Microbiol2006 Aug,44(8) 2689-97
153 Katzenstein TL, Jorgensen LB, Permin H, Hansen J, Nielsen C, Machuca R, et al Nosocomial HIV-transmission in an outpatient clinic detected by epidemiological and phylogenetic analyses AIDS1999 Sep 10,13(13) 1737-44
154 Pistello M, Del Santo B, Butto S, Bargagna M, Domenici R, Bendinelli M Genetic and phylogenetic analyses of HIV-1 corroborate the transmission link hypothesis J Clin Virol2004 May,30(1) 11-8
155 Paraskevis D, Magiorkinis E, Theodoridou M, Mostrou G, Papaevangelou V, Kiosses VG, et al Molecular epidemiology of vertical human immunodeficiency virus type 1 transmission in Greece evidence of non-B subtypes J Hum Virol1999 Nov-Dec.2(6) 339-43
156 Delwart EL, Busch MP, Kalish ML, Mosley JW, Mullins JI Rapid molecular

epidemiology of human immunodeficiency virus transmission AIDS Res Hum Retroviruses1995 Sep;11(9) 1081-93
157 Trask SA, Derdeyn CA, Fideli U, Chen Y, Meleth S, Kasolo F, et al Molecular epidemiology of human immunodeficiency virus type 1 transmission in a heterosexual cohort of discordant couples in Zambia J Virol2002 Jan;76(1) 397-405
158 Robbins KE, Weidle PJ, Brown TM, Saekhou AM, Coles B, Holmberg SD, et al Molecular analysis in support of an investigation of a cluster of HIV-1-infected women AIDS Res Hum Retroviruses2002 Oct 10;18(15) 1157-61
159 Kim YB, Cho YK, Lee HJ, Kim CK, Kim YK, Yang JM Molecular phylogenetic analysis of human immunodeficiency virus type 1 strains obtained from Korean patients env gene sequences AIDS Res Hum Retroviruses1999 Feb 10;15(3) 303-7
160 Birch CJ, McCaw RF, Bulach DM, Revill PA, Carter JT, Tomnay J, et al Molecular analysis of human immunodeficiency virus strains associated with a case of criminal transmission of the virus J Infect Dis2000 Sep;182(3) 941-4
161 Albert J, Wahlberg J, Leitner T, Escanilla D, Uhlen M Analysis of a rape case by direct sequencing of the human immunodeficiency virus type 1 pol and gag genes J Virol1994 Sep;68(9) 5918-24
162 Resik S, Lemey P, Ping LH, Kouri V, Joanes J, Perez J, et al Limitations to contact tracing and phylogenetic analysis in establishing HIV type 1 transmission networks in Cuba AIDS Res Hum Retroviruses2007 Mar;23(3) 347-56
163 Lepej SZ, Vrakela IB, Poljak M, Bozicevic I, Begovac J Phylogenetic analysis of HIV sequences obtained in a respondent-driven sampling study of men who have sex with men AIDS Res Hum Retroviruses2009 Dec;25(12) 1335-8
164 Deng W, Fu P, Bao L, Vidal N, He Q, Qin C, et al Molecular epidemiological tracing of HIV-1 outbreaks in Hainan island of southern China AIDS2009 May 15;23(8) 977-85
165 Smith DM, May SJ, Tweeten S, Drumright L, Pacold ME, Kosakovsky Pond SL, et al A public health model for the molecular surveillance of HIV transmission in San Diego, California AIDS2009 Jan 14;23(2) 225-32
166 Buskin SE, Ellis GM, Pepper GG, Frenkel LM, Pergam SA, Gottlieb GS, et al Transmission cluster of multiclass highly drug-resistant HIV-1 among 9 men who have sex with men in Seattle/King County, WA, 2005-2007 J Acquir Immune Defic Syndr2008 Oct 1;49(2) 205-11
167 Sahbandar IN, Takahashi K, Djoerban Z, Firmansyah I, Naganawa S, Motomura K, et al Current HIV type 1 molecular epidemiology profile and identification of unique recombinant forms in Jakarta, Indonesia AIDS Res Hum Retroviruses2009 Jul;25(7) 637-46
168 Kaye M, Chibo D, Birch C Comparison of Bayesian and maximum-likelihood phylogenetic approaches in two legal cases involving accusations of transmission of HIV AIDS Res Hum Retroviruses2009 Aug;25(8) 741-8
169 Gurjar RS, Ravi V, Desai A Molecular epidemiology of HIV type 2 infections in South India AIDS Res Hum Retroviruses2009 Mar;25(3) 363-72
170 Gaur AH, Dominguez KL, Kalish ML, Rivera-Hernandez D, Donohoe M, Brooks JT, et al Practice of feeding premasticated food to infants a potential risk factor for HIV transmission Pediatrics2009 Aug;124(2) 658-66
171 Esteves A, Parreira R, Piedade J, Venenno T, Franco M, Germano de Sousa J, et al

Spreading of HIV-1 subtype G and envB/gagG recombinant strains among injecting drug users in Lisbon, Portugal AIDS Res Hum Retroviruses2003 Jun.19(6) 511-7
172 Roques P. Robertson DL. Souquiere S. Damond F. Ayouba A, Farfara I. et al Phylogenetic analysis of 49 newly derived HIV-1 group O strains high viral diversity but no group M-like subtype structure Virology2002 Oct 25.302(2) 259-73
173 Abebe A. Lukashov VV. Pollakis G. Kliphuis A. Fontanet AL. Goudsmit J. et al Timing of the HIV-1 subtype C epidemic in Ethiopia based on early virus strains and subsequent virus diversification AIDS2001 Aug 17.15(12) 1555-61
174 Montavon C. Toure-Kane C. Liegeois F. Mpoudi E, Bourgeois A. Vergne L. et al Most env and gag subtype A HIV-1 viruses circulating in West and West Central Africa are similar to the prototype AG recombinant virus IBNG J Acquir Immune Defic Syndr2000 Apr 15.23(5) 363-74
175 Triques K. Bourgeois A. Saragosti S. Vidal N, Mpoudi-Ngole E. Nzilambi N, et al High diversity of HIV-1 subtype F strains in Central Africa Virology1999 Jun 20.259(1) 99-109
176 Lukashov VV. Huismans R. Rakhmanova AG. Lisitsina ZN. Akhtyrskaya NA. Vlasov NN. et al Circulation of subtype A and gagA/envB recombinant HIV type 1 strains among injecting drug users in St Petersburg. Russia. correlates with geographical origin of infections AIDS Res Hum Retroviruses1999 Nov 20.15(17) 1577-83
177 Novitsky VA. Montano MA. Essex M Molecular epidemiology of an HIV-1 subtype A subcluster among injection drug users in the Southern Ukraine AIDS Res Hum Retroviruses1998 Aug 10.14(12) 1079-85
178 Liitsola K. Tashkinova I. Laukkanen T. Korovina G Smolskaja T. Momot O. et al HIV-1 genetic subtype A/B recombinant strain causing an explosive epidemic in injecting drug users in Kaliningrad AIDS1998 Oct 1.12(14) 1907-19
179 Bobkov A. Cheingsong-Popov R. Selimova L. Ladnaya N. Kazennova E. Kravchenko A. et al An HIV type 1 epidemic among injecting drug users in the former Soviet Union caused by a homogeneous subtype A strain AIDS Res Hum Retroviruses1997 Sep 20.13(14) 1195-201
180 Lihana RW. Khamadi SA. Lwembe RM. Kinyua JG Muriuki JK. Lagat NJ. et al HIV-1 subtype and viral tropism determination for evaluating antiretroviral therapy options an analysis of archived Kenyan blood samples BMC Infect Dis2009.9 215
181 Kousiappa I. van de Vijver DA. Demetriades I. Kostrikis LG Genetic analysis of HIV type 1 strains from newly infected untreated patients in cyprus high genetic diversity and low prevalence of drug resistance AIDS Res Hum Retroviruses2009 Jan.25(1) 23-35
182 Mintsa-Ndong A. Caron M. Plantier JC. Makuwa M. Le Hello S. Courgnaud V. et al High HIV Type 1 prevalence and wide genetic diversity with dominance of recombinant strains but low level of antiretroviral drug-resistance mutations in untreated patients in northeast Gabon. Central Africa AIDS Res Hum Retroviruses2009 Apr.25(4) 411-8
183 Holzmayer V. Aitken C. Skinner C. Ryall L. Devare SG Hackett J. Jr Characterization of genetically diverse HIV type 1 from a London cohort near full-length genomic analysis of a subtype H strain AIDS Res Hum Retroviruses2009 Jul.25(7) 721-6
184 Ho SK. Perez EE. Rose SL. Coman RM. Lowe AC. Hou W. et al Genetic determinants in HIV-1 Gag and Env V3 are related to viral response to combination antiretroviral therapy with a protease inhibitor AIDS2009 Aug 24.23(13) 1631-40

185 Han Z, Leung TW, Zhao J, Wang M, Fan L, Li K, et al A HIV-1 heterosexual transmission chain in Guangzhou, China a molecular epidemiological study Virol J2009.6 148
186 Brenner BG, Roger M, Routy JP, Moisi D, Ntemgwa M, Matte C, et al High rates of forward transmission events after acute/early HIV-1 infection J Infect Dis2007 Apr 1.195(7) 951-9
187 Hirsch MS, Conway B, D'Aquila RT, Johnson VA, Brun-Vezinet F, Clotet B, et al Antiretroviral drug resistance testing in adults with HIV infection implications for clinical management International AIDS Society--USA Panel JAMA1998 Jun 24.279(24) 1984-91
188 Grant RM, Hecht FM, Warmerdam M, Liu L, Liegler T, Petropoulos CJ, et al Time trends in primary HIV-1 drug resistance among recently infected persons JAMA2002 Jul 10.288(2) 181-8
189 Violin M, Velleca R, Cozzi-Lepri A, Riva C, Grossi PA, Carnevale G, et al Prevalence of HIV-1 primary drug resistance in seroconverters of the ICoNA cohort over the period 1996-2001 J Acquir Immune Defic Syndr2004 Jun 1.36(2) 761-4
190 Viani RM, Peralta L, Aldrovandi G, Kapogiannis BG, Mitchell R, Spector SA, et al Prevalence of primary HIV-1 drug resistance among recently infected adolescents a multicenter adolescent medicine trials network for HIV/AIDS interventions study J Infect Dis2006 Dec 1.194(11) 1505-9
191 Chaix ML, Descamps D, Harzic M, Schneider V, Deveau C, Tamalet C, et al Stable prevalence of genotypic drug resistance mutations but increase in non-B virus among patients with primary HIV-1 infection in France AIDS2003 Dec 5.17(18) 2635-43
192 Wong KH, Chan KC, Cheng KL, Chan WK, Kam KM, Lee SS Establishing CD4 thresholds for highly active antiretroviral therapy initiation in a cohort of HIV-infected adult Chinese in Hong Kong AIDS Patient Care STDS2007 Feb.21(2) 106-15
193 Han X, Zhang M, Dai D, Wang Y, Zhang Z, Liu J, et al Genotypic resistance mutations to antiretroviral drugs in treatment-naive HIV/AIDS patients living in Liaoning Province, China baseline prevalence and subtype-specific difference AIDS Res Hum Retroviruses2007 Mar.23(3) 357-64
194 Wang XQ, Tong X, Tang H, Liu PP, Zhang W, Yang RG [Study on genotypic resistance mutations to antiretroviral drugs on HIV strains of treated and treatment-naive HIV-1 infectious patients in Hubei province] Zhonghua Liu Xing Bing Xue Za Zhi2007 Nov.28(11) 1112-5
195 Zhang X, Li S, Li X, Xu J, Li D, Ruan Y, et al Characterization of HIV-1 subtypes and viral antiretroviral drug resistance in men who have sex with men in Beijing, China AIDS2007 Dec.21 Suppl 8 S59-65
196 Beltrami EM, Williams IT, Shapiro CN, Chamberland ME Risk and management of blood-borne infections in health care workers Clin Microbiol Rev2000 Jul.13(3) 385-407
197 Belyakov IM, Hel Z, Kelsall B, Kuznetsov VA, Ahlers JD, Nacsa J, et al Mucosal AIDS vaccine reduces disease and viral load in gut reservoir and blood after mucosal infection of macaques Nat Med2001 Dec.7(12) 1320-6
198 Yu G, Li Y, Li J, Diao L, Yan X, Lin P, et al Genetic diversity and drug resistance of HIV type 1 circulating recombinant Form_BC among drug users in Guangdong Province AIDS Res Hum Retroviruses2009 Sep.25(9) 869-75

199 Parekh BS, McDougal J New approaches for detecting recent HIV-1 infection AIDS Reviews2001,3 183-93
200 Brookmeyer R, Quinn TC Estimation of current human immunodeficiency virus incidence rates from a cross-sectional survey using early diagnostic tests Am J Epidemiol1995 Jan 15,141(2) 166-72
201 Vanichseni S, Kitayaporn D, Mastro TD, Mock PA, Raktham S, Des Jarlais DC, et al Continued high HIV-1 incidence in a vaccine trial preparatory cohort of injection drug users in Bangkok, Thailand AIDS2001 Feb 16,15(3) 397-405
202 Cleghorn FR, Jack N, Murphy JR, Edwards J, Mahabir B, Paul R, et al Direct and indirect estimates of HIV-1 incidence in a high-prevalence population Am J Epidemiol1998 May 1,147(9) 834-9
203 Xiao Y, Jiang Y, Feng J, Xu W, Wang M, Funkhouser E, et al Seroincidence of recent human immunodeficiency virus type 1 infections in China Clin Vaccine Immunol2007 Oct,14(10) 1384-6
204 Xiao Y, Sun J, Li C, Lu F, Allen KL, Vermund SH, et al Prevalence and correlates of HIV and syphilis infections among men who have sex with men in seven provinces in China with historically low HIV prevalence J Acquir Immune Defic Syndr Feb,53 Suppl 1 S66-73
205 Ammaranond P, Cunningham P, Oelrichs R, Suzuki K, Harris C, Leas L, et al Rates of transmission of antiretroviral drug resistant strains of HIV-1 J Clin Virol2003 Feb,26(2) 153-61
206 Pillay D, Cane PA, Shirley J, Porter K Detection of drug resistance associated mutations in HIV primary infection within the UK AIDS2000 May 5,14(7) 906-8
207 UK Collaborative Group on Monitoring the Transmission of HIV Drug Resistance Analysis of prevalence of HIV-1 drug resistance in primary infections in the United Kingdom BMJ2001 May 5,322(7294) 1087-8
208 Han X, Dai D, Zhao B, Liu J, Ding H, Zhang M, et al Genetic and epidemiologic characterization of HIV-1 infection In Liaoning Province, China J Acquir Immune Defic Syndr Feb,53 Suppl 1 S27-33
209 Hsieh FY, Bloch DA, Larsen MD A simple method of sample size calculation for linear and logistic regression Statistics in Medicine1998,17 1623-34
210 Everitt BS Multivariate analysis the need for data, and other problems British Journal of Psychiatry 1975,126 237-40
211 Zhao JK, Ma XY, Wang LY, Lu F A new approach of sampling hard-to-reach population, respondent-driven sampling (RDS) Chin J Dis Control Prev2005,9(6) 634-7
212 Zhong F, Lin P, Xu H, Wang Y, Wang M, He Q, et al Possible Increase in HIV and Syphilis Prevalence Among Men Who Have Sex with Men in Guangzhou, China Results from a Respondent-Driven Sampling Survey AIDS Behav2009 Oct 14
213 Williams ML, Freeman RC, Bowen AM, Zhao Z, Elwood WN, Gordon C, et al A comparison of the reliability of self-reported drug use and sexual behaviors using computer-assisted versus face-to-face interviewing AIDS Educ Prev2000 Jun,12(3) 199-213
214 China State Council AIDS Working Committee Office Monitoring and Evaluation Framework for China's HIV/AIDS Prevention,Treatment and Care Program Beijing People's Health Publishing House, 2007

215 Roy E, Haley N, Leclerc P, Lemire N, Boivin JF, Frappier JY, et al Prevalence of HIV infection and risk behaviours among Montreal street youth Int J STD AIDS2000 Apr,11(4) 241-7
216 Ruan FF, Tsai YM Male homosexuality in contemporary mainland China Arch Sex Behav1988 Apr,17(2) 189-99
217 Qi SZ, Zhang GC, Wang QQ MSM, MB and HIV/STD transmission Chinese Journal of AIDS & STD2006(01)
218 Iguchi MY, Ober AJ, Berry SH, Fain T, Heckathorn DD, Gorbach PM, et al Simultaneous recruitment of drug users and men who have sex with men in the United States and Russia using respondent-driven sampling sampling methods and implications J Urban Health2009 Jul,86 Suppl 1 5-31
219 Jia M, Luo H, Ma Y, Wang N, Smith K, Mei J, et al The HIV epidemic in Yunnan Province, China, 1989-2007 J Acquir Immune Defic Syndr Feb,53 Suppl 1 S34-40
220 Scheer S, Kellogg T, Klausner JD, Schwarcz S, Colfax G, Bernstein K, et al HIV is hyperendemic among men who have sex with men in San Francisco 10-year trends in HIV incidence, HIV prevalence, sexually transmitted infections and sexual risk behaviour Sex Transm Infect2008 Nov,84(6) 493-8
221 Fleming DT, Wasserheit JN From epidemiological synergy to public health policy and practice the contribution of other sexually transmitted diseases to sexual transmission of HIV infection Sex Transm Infect1999 Feb,75(1) 3-17
222 Zou H, Wu Z, Yu J, Li M, Ablimit M, Li F, et al Sexual risk behaviors and HIV infection among men who have sex with men who use the internet in Beijing and Urumqi, China J Acquir Immune Defic Syndr Feb,53 Suppl 1 S81-7
223 Huber JD, Kleinplatz PJ Sexual orientation identification of men who have sex with men in public settings in Canada J Homosex2002,42(3) 1-20
224 Klein H Sexual orientation, drug use preference during sex, and HIV risk practices and preferences among men who specifically seek unprotected sex partners via the internet Int J Environ Res Public Health2009 May,6(5) 1620-35
225 Myers T, Allman D, Jackson EA, Orr K Variation in sexual orientations among men who have sex with men, and their current sexual practices Can J Public Health1995 Nov-Dec,86(6) 384-8
226 Rietmeijer CA, Wolitski RJ, Fishbein M, Corby NH, Cohn DL Sex hustling, injection drug use, and non-gay identification by men who have sex with men Associations with high-risk sexual behaviors and condom use Sex Transm Dis1998 Aug,25(7) 353-60
227 Vanwesenbeeck I Another decade of social scientific work on sex work A review of the research 1990-2000 Annu Rev Sex Res2001,12 242-83
228 Minichiello V, Marino R, Browne J Knowledge, risk perceptions and condom usage in male sex workers from three Australian cities AIDS Care2001 Jun,13(3) 387-402
229 Simon PM, Morse EV, Osofsky HJ, Balson PM, Gaumer HR Psychological characteristics of a sample of male street prostitutes Arch Sex Behav1992 Feb,21(1) 33-44
230 Baral S, Trapence G, Motimedi F, Umar E, Iipinge S, Dausab F, et al HIV prevalence, risks for HIV infection, and human rights among men who have sex with men (MSM) in Malawi, Namibia, and Botswana PLoS ONE2009,4(3) e4997
231 Bingham TA, Harawa NT, Johnson DF, Secura GM, MacKellar DA, Valleroy LA The

effect of partner characteristics on HIV infection among African American men who have sex with men in the Young Men's Survey, Los Angeles, 1999-2000 AIDS Educ Prev2003 Feb,15(1 Suppl A) 39-52
232 Brahmam GN, Kodavalla V, Rajkumar H, Rachakulla HK, Kallam S, Myakala SP, et al Sexual practices, HIV and sexually transmitted infections among self-identified men who have sex with men in four high HIV prevalence states of India AIDS2008 Dec,22 Suppl 5 S45-57
233 Benn PD, Rooney G, Carder C, Brown M, Stevenson SR, Copas A, et al Chlamydia trachomatis and Neisseria gonorrhoeae infection and the sexual behaviour of men who have sex with men Sex Transm Infect2007 Apr,83(2) 106-12
234 Zule WA, Bobashev GV, Wechsberg WM, Costenbader EC, Coomes CM Behaviorally bisexual men and their risk behaviors with men and women J Urban Health2009 Jul,86 Suppl 1 48-62
235 Van der Bij AK, Kolader ME, de Vries HJ, Prins M, Coutinho RA, Dukers NH Condom use rather than serosorting explains differences in HIV incidence among men who have sex with men J Acquir Immune Defic Syndr2007 Aug 15,45(5) 574-80
236 He Q, Wang Y, Lin P, Raymond HF, Li Y, Yang F, et al High prevalence of risk behavior concurrent with links to other high risk populations a potentially explosive HIV epidemic among men who have sex with men in Guangzhou, China Sex Transm Infect2009 Apr 22
237 Minichiello V, Marino R, Browne J, Jamieson M, Reuter B, Robinson K Commercial sex between men A prospective diary-based study Journal of Sex Research2000,37 151-61
238 Guadamuz TE, Kunawararak P, Beyrer C, Pumpaisanchai J, Wei C, Celentano DD HIV prevalence, sexual and behavioral correlates among Shan, Hill tribe, and Thai male sex workers in Northern Thailand AIDS Care2010 Apr 16 1-9
239 Russi JC, Serra M, Vinoles J, Perez MT, Ruchansky D, Alonso G, et al Sexual transmission of hepatitis B virus, hepatitis C virus, and human immunodeficiency virus type 1 infections among male transvestite comercial sex workers in Montevideo, Uruguay Am J Trop Med Hyg2003 Jun,68(6) 716-20
240 Kendall C, Kerr LR, Gondim RC, Werneck GL, Macena RH, Pontes MK, et al An empirical comparison of respondent-driven sampling, time location sampling, and snowball sampling for behavioral surveillance in men who have sex with men, Fortaleza, Brazil AIDS Behav2008 Jul,12(4 Suppl) S97-104
241 Griensven Fv, editor Time location sampling still relevant after all those years? Global HIV/AIDS Surveillance Meeting, 2009 March 2 - 5, Bangkok
242 van Griensven F, Naorat S, Kilmarx PH, Supaporn Jeeyapant, Chomnad Manopaiboon, Supaporn Chaikummao, et al Palmtop-assisted selfinterviewing for the collection of sensitive behavioral data randomized trial with drug use urine testing Am J Epidemiol2006,163 271-8
243 Li S-W, Zhang X-Y, Li X-X, Wang M-J, Li D-L, Ruan Y-H, et al Detection of recent HIV-1 infections among men who have sex with men in Beijing during 2005-2006 chinese medical journal2008,121(12) 1105
244 Bezemer D, van Sighem A, Lukashov VV, van der Hoek L, Back N, Schuurman R, et al Transmission networks of HIV-1 among men having sex with men in the Netherlands AIDS

Jan 16.24(2) 271-82
245 Yerly S, Junier T, Gayet-Ageron A, Amari EB, von Wyl V, Gunthard HF, et al The impact of transmission clusters on primary drug resistance in newly diagnosed HIV-1 infection AIDS2009 Jul 17.23(11) 1415-23
246 Thomson MM, Vinogradova A, Delgado E, Rakhmanova A, Yakovlev A, Cuevas MT, et al Molecular epidemiology of HIV-1 in St Petersburg, Russia predominance of subtype A, former Soviet Union variant, and identification of intrasubtype subclusters J Acquir Immune Defic Syndr2009 Jul 1.51(3) 332-9
247 Ahumada-Ruiz S, Flores-Figueroa D, Toala-Gonzalez I, Thomson MM Analysis of HIV-1 pol sequences from Panama identification of phylogenetic clusters within subtype B and detection of antiretroviral drug resistance mutations Infect Genet Evol2009 Sep.9(5) 933-40
248 Lee SS, Tam DK, Tan Y, Mak WL, Wong KH, Chen JH, et al An exploratory study on the social and genotypic clustering of HIV infection in men having sex with men AIDS2009 Jul 15

Appendix I (Questionnaire of the study)

MSM 健康调查问卷

本栏由采样者填写

1 场所名称：_____ 场所代码：□□□□ 所在地：_____区
调查日期：200_ 年__月__日 被访者编号（招募卡号）

2 在调查前，我们想确定你是否适合做这个调查，在过去六个月，你曾否有过男男同性性行为(包括肛交或口交)? □1 没有→非合适受访者 □2 有→继续访问

3 样本来源：□1 会所 □2 酒吧 □3 浴室 □4 公园 □5 休闲/按摩中心 □6 258 工作室. □7 社区门诊 □8 其它请注明：_____

4 在本地，您大约认识多少个圈内的朋友（男男性行为者）？（认识是指您能够认识对方面孔，知道名字或化名、昵称，和联系方式，并能在一个月内联系上对方，主您仔细回想一下： □1_____人 □2 拒答

5 介绍参加这次活动的人和您是什么关系？ □1 过去的性伴（6 个月以前） □2 近 6 个月的性伴 □3 好朋友 □4 普通朋友 □5 熟人 □6 陌生人 □7 拒答

以下对调查对象进行调查

A、人口特征	
6	您的出生日期： 19_____ 年_____ 月
7	您的家乡：_____省
8	您的家乡属于：□1 城市 □2 乡镇、□3 农村
9	您目前是否居住在深圳? □1 是 → 1 您的户口所在地? □1 深圳市 □2 本省其它地区 □3 外省(请注明_____省) □4 香港 □5 其它国家地区（请注明_____国） 11 您住哪个区？□1 罗湖 □2 福田 □3 南山 □4 宝安 □5 龙岗 □6 盐田 12 您在深圳居住：□1 <1 个月 □2 1-3 个月 □3 3-6 个月 □4 7-12 个月 □5 1-2 年 □6 2 年以上 □2 否 → 请问您居住在哪里？□1 广东省 □2 中国大陆其它省份 □3 香港 □4 外国
14	您的民族：□1 汉族 □2 其他. 请注明：_____
15	您的最高学历：□1 文盲 □2 小学 □3 初中 □4 高中.中专 □5 大专.大学 □6 研究生以上
16	您目前工作状况： □1 全职 □2 兼职 □3 待业/失业 □4 退休 □5 个体 □6 学生 □7 其它，请注明：_____
17	您目前职业：□1 学生 □2 教师 □3 保育员/保姆 □4 餐饮食品业 □5 商业服务

	□₆ 医务人员 □₇ 工人 □₈ 民工 □₉ 农民 □₁₀ 牧民 □₁₁ 渔(船)民 □₁₂ 干部/职员 □₁₃ 离退 □₁₄ 家务/待业 □₁₅ 军人 □₁₆ MB □₁₇ 其它，请注明_____
18	目前您的月收入水平：□₁ 无收入 □₂ 1000 以下 □₃ 1001－2000 □₄ 2001－3000 □₅ 3001－4000 □₆ 4001－5000 □₇ 5001－7000 □₈ 7001－9000 □₉ 9000 以上
19	您目前的婚姻状况：□₁ 未婚 → ₂₀ 您将来会与女性结婚吗？□₁ 会 □₂ 不会 □₃ 不确定 □₂ 在婚 □₃ 同居 □₄ 分居 □₅ 离异或丧偶
21	您现在与谁住在一起？□₁ 独居 □₂ 室友(无性行为) □₃ 同性性伴 □₄ 异性性伴 □₅ 妻子及家人 □₇ 父母、子女或亲戚 □₉ 其他，请注明_____

B、性行为

23	您如何评价自己现阶段的性取向？□₁ 同性恋 □₂ 异性恋 □₃ 双性恋 □₄ 不确定
24	您第一次发生性行为（口交，阴道交，肛交）的年龄是？_____周岁
25	您第一个性伴是男性还是女性？□₁ 男 □₂ 女 → ₂₆ 您第一次与男性发生性行为时是多大年龄？_____周岁
27	您寻找性伴最常去的场所是？（可多选，请按频繁程度标明次序） □₁ 酒吧/歌舞厅 □₂ 会所/家庭会所 □₃ 桑拿/浴池/按摩/健身中心 □₄ 互联网 □₅ 公园/公厕/草地 □₆ 朋友介绍 □₇ 小型聚会活动 □₈ 其它，请注明_____₂₈
29	您过去六个月内与谁发生过性行为？□₁ 男性 □₂ 男性及女性 □₃ 女性 ₃₀

C、同性性行为

C1	₃₂ 过去的六个月里您是否与男性有过肛交性行为？□₁ 是 □₂ 否 ₃₃ 过去的六个月里您曾与多少个不同的男性发生过肛交性行为？□₁ _____个 ₃₄ 他们与你的关系是：（可多选） 1. 确立关系的男朋友 2. 约会的情人 3. 嫖客 4. 一夜情 5. MB（给同志提供性服务的人员） 6. 妈咪 7. 其他，_____ ₃₅ 有外国人么？□ 有 □ 没有 □ 不确定 ₃₆ 同性性伴有香港人么？□₁ 有 □₂ 没有 □ 不确定 ₃₈ 你跟男性发生肛交性行为时，你的性角色是： □₁ 只做“1” □₂ 只做“0” □ 两个都有 ₃₉ 过去 6 个月内，你与男性发生肛交行为时（包括做 0 或做 1），是否使用润滑剂？ □₁ 是 → ₄₂ 使用哪些？□₁ 性行为专用润滑剂 □₂ 非专用润滑剂 □₃ 口水 □₂ 否 ₄₃ 最近 6 个月跟男性发生口交时使用安全套的频率如何？ □₁ 从不 □₂ 有时（>50%） □₃ 经常（>50%） □₄ 每次都用 □₅ 没有口交
C2	下面是关于一些跟你有确立关系的男朋友（BF，固定性伴）方面的问题：

	44 你曾处过多少个确立关系的男朋友？_____个 （0 个跳至 C3） 47 最近 6 个月跟男朋友发生肛交时使用安全套的频率如何？ □1 从不 □2 有时(>50%) □3 经常(>50%) □4 每次都用 □5 无肛交 □6 无男朋友
C3	下面是关于一夜情（419）方面的问题： 48 最近 6 个月，您有过多少个一夜情的男性性伴？_____个 （如果为 0，跳至 C4） 50 总体来讲，最近 6 个月你与一夜情男性性伴发生肛交时使用安全套的频率如何？ □1 从不 □2 有时（>50%）□3 经常（>50%） □4 每次都用 □5 没有肛交
C4	下面是关于跟你有过肛交或口交行为的普通男性性伴（或约会的情人）方面的问题： 52 最近 6 个月，您与多少个普通男性朋友（性伴）发生过肛交或口交行为？_____个 （如果为 0，跳至 C5） 54 总体来讲，最近 6 个月你与这样的男性性伴发生肛交时使用安全套的频率如何？ □1 从不 □2 有时（>50%）□3 经常（>50%） □4 每次都用 □5 没有肛交
C5	55 在最近 6 个月内，你参与过多少次群交性行为（三人或三人以上）？_____次 （0 次跳至 C6） 56 最近 6 个月你参与群交性行为时，肛交中使用安全套的频率如何？ □1 从不 □2 有时（>50%）□3 经常（>50%） □4 每次都用 □5 没有肛交
C6	57 最近六个月，您通过付钱的方式得到过男性为您提供的性服务吗？ □1 是 → 58 近六个月，您通过付钱的方式得到过多少男性为您提供性服务？ □1 _____人 □2 记不清 59 最近六个月，您与这种男性发生肛交时使用安全套频率如何？ □2 否 □1 从不 □2 有时 □3 经常 □4 每次都用 60 最近一次与这类男性发生性行为时使用安全套了吗？□1 是 □2 否

D、异性性行为

61 您曾经与女性发生过阴道性交吗？ □1 有 □2 没有 *（如果没有，请跳到 E 部分）*

62 在过去的6个月里您与女性发生过性行为吗？
□1 有，_____位 □2 没有 *（如果没有，请跳到第E部分）*

D3 65 使用安全套的频率如何？ □1 从不 □2 有时 □3 经常 □4 每次都用

E、商业性行为	
72	您是否在最近六个月内为了得到钱，为男性提供过商业性性服务(口交或肛交)？ □1 是 □2 否 *(如果否，请跳至 94 题)*
73	您为男性提供商业性性服务多长时间了？____年（小于 1 年的请填____月）
74	您过去 6 个月的男性客人中大概几成是香港客人？_____ （0-10，请尽量估计）
77	过去 6 个月内，您为多少男性提供商业性性服务：□1 _____人 □2 记不清
78	过去 1 个月内，您为多少男性提供商业性性服务：_____人（请尽量估计人数）

79	过去 1 个月内，您为男性提供商业性性服务时，平均每人每次付您多少钱（含小费）？ __ __元。
81	81 过去 1 个月内，您大约与多少个商业性伴发生过肛交(包括做 0 或做 1)？_____ 82 几成是您完全做"1"__ 几成是您完全做"0"____ 几成是两个都做___ 83 与这类性伴肛交时使用安全套的频率如何？□1 从不 □2 有时 □3 经常 □4 每次
E9	90 你是否在深圳以外的地方向男性提供过商业性性服务？ □1 是 → 91 您过去 6 个月内在几个城市从事过商业性性服务？…… ____个。 □2 否 92 过去 6 个月内都在哪几个城市？_____ 93 如果过去 6 个月内去过外国做 MB，有哪些国家？_____
94	您是否在六个月内为女性客人提供了商业性性服务（肛交或阴道交）？ □1 是 □2 否 *(如果否，请跳到第 106 题)*
95	您第一次为女性客人提供商业性性服务的年龄是：____岁
96	您过去 6 个月的女性客人中大概几成是香港客人？_____（0-10，请尽量估计）
99	99 过去 6 个月内，您为多少女性提供商业性性服务：_____人（请尽量估计人数） 100 过去 1 个月内，您为多少女性提供商业性性服务：_____人（请尽量估计人数）
101	过去 1 个月内，您为女性提供商业性性服务时，平均每人每次付您多少钱（含小费）？ ____ __元
102	102 请您回想一下，在过去的 6 个月里您与女性客人性交时，您使用安全套的情况。 □1 从不使用 □2 有时使用 □3 经常使用 □4 每次都用 103 你最近一次与女性客人性交时使用安全套了吗？□1 是 □2 否

F、药物酒精史

106	您是否曾经尝试过以下药物？（可多选）： □1 无 → *(请跳到第 E 部分)* □2 香烟 □3 酒类 □4 K 粉.LSD.PCP 等 □5 蓝精灵 □6 伟哥 □7 大麻 □8 可卡因 □9 海洛因，吗啡等鸦片类 □10 止咳水 □11 摇头丸 □12 冰毒 □13 麻果 □14 其它_____
108	过去 6 个月您是否曾经使用过以下药物？（可多选） □1 无 □2 香烟 □3 酒类 → 是否在性交前或性交时饮用酒类？□1 每次□2 经常□3 偶尔 □4 从不 □4 K 粉.LSD.PCP 等 □5 蓝精灵 □6 伟哥 □7 大麻 □8 可卡因 □9 海洛因，吗啡等鸦片类 □10 止咳水 □11 摇头丸 □12 冰毒 □13 麻果 □14 其它，请注明_____ → 您是否在性交前或性交时使用这些药品？ □1 每次 □2 经常 □3 偶尔 □4 从不
110	您过去 6 个月内是否曾经注射过毒品？ □1 是 → 112 您注射吸毒时是否曾经与别人共用过针具(注射器)？ □1 每次 □2 经常 □3 偶尔 □4 从不 □2 否

G、性病既往史

过去一年内你有过下列症状吗?

113 □1 排尿痛或烧灼感 □2 尿道分泌物异常 □3 生殖器皮肤破损或增生物 □4 肛门皮肤破损或增生物 □5 肛门脓液 □6 没有

过去一年内你是否曾经被诊断患有以下疾病?

115 □1 梅毒 □2 尖锐湿疣 □3 疱疹 □4 淋病 □5 衣原体 □6 尿道炎 □7 无 □9 其它___

H、艾滋病相关信息

H3	120 您是否接受过艾滋病检测? □1 是→ 122 您最近一次 HIV 检测是什么时候? □2 否 □1 过去 6 个月内 □2 过去一年内 □3 1－2 年前 □4 2 年以前
126	最近一年，您是否接受过有关预防艾滋病的下列服务？（可多选） □1 安全套发放 □2 润滑剂发放 □3 同伴志愿者教育 □4 性病检查或治疗 □5 艾滋病咨询或检测 □6 艾滋病性病宣传材料（小册子、折页等）
127	您获得艾滋病知识信息的主要来源是什么?（可多选） □1 电视 □2 广播 □3 报刊 □4 书籍 □5 朋友 □6 医生 □7 咨询服务/电话咨询 □8 免费宣传材料 □9 宣传栏广告栏 □10 互联网 □11 学校教育 □12 其它_____
128	最近 6 个月内，您使用的安全套或润滑剂是从哪里获得的? □1 疾控中心/志愿者/场所老板等免费发放的 □2 性伴提供 □3 到药店/性用品商店/超市等处购买 □4 其他，_____
129	您认为自己目前已感染艾滋病的可能性有多大? □1 完全没有 □2 相当少 □3 有些 □4 相当大 □5 非常大 □6 已感染
130	请您凭感觉回答下面有关艾滋病知识的问题: 一个看上去健康的人有可能携带艾滋病病毒吗？…… □1 可能 □2 不可能 □3 不知道 输入带有艾滋病病毒的血液/血制品会感染艾滋病病毒? □1 会 □2 不会 □3 不知道 与艾滋病感染者或病人共用针具会不会感染艾滋病病毒? □会 □2 不会 □3 不知道 每次性行为时正确使用安全套能降低感染艾滋病病毒危险？□1 能 □2 不能 □3 不知道 保持一个未感染艾滋病病毒的性伴能降低感染艾滋病病毒危险？□1 能□2 不能□3 不知道
131	感染了艾滋病病毒的孕妇可能将病毒传染给她的孩子吗? □1 可能 □2 不可能 □3 不知道 与艾滋病病毒感染者/病人一起吃饭会感染艾滋病病毒? □1 会 □2 不会 □3 不知道 蚊虫叮咬会不会传播艾滋病病毒？…… □1 会 □2 不会 □3 不知道 肛交比口交更易传播艾滋病吗？ …… □1 是 □2 不是 □3 不知道
132	请判断下面有关安全套使用的问题是否正确? 安全套也有保质期，使用前需查看有效期……1 对 □2 错 □3 不知道 套上安全套时要捏住顶端的小囊，挤出空气，完全展开直至阴茎根部□1 对 □2 错□3 不知道 安全套在射精前戴上也可以预防性病/艾滋病…… □1 对 □2 错 □3 不知道 使用安全套时，应该在阴茎疲软前抽出……□1 对 □2 错 □3 不知道 性交后，安全套不可重复使用……□1 对 □2 错 □3 不知道

调查到此结束。非常感谢回答这些问题。谢谢你的帮助。

结果代码： 1)完成 2)没完成 3)部分完成(原因：_____) 是否采血：□是 □否

Appendix II (Example for VDT list, sampling frame and calendar)

Table 1: Sample universe of venues/update monthly sampling frame

No.	Mon	Tue	Wed	Thu	Fri	Sat	Sat	Sun	Sun
F001	(1)7-9	(2)7-9	(3)7-9	(4)7 9	(5)7-9	(6)4-6	(7)6 8	(8)4-6	(9)6 8
F002	(1)7-9	(2)7-9	(3)7-9	(4)7 9	(5)7-9	(6)4 6	(7)6-8	(8)4-6	(9)6-8
F003	(1)7-9	(2)7-9	(3)7-9	(4)7 9	(5)7-9	(6)4-6	(7)6 8	(8)4 6	(9)6 8
F004	(1)7-9	(2)7-9	(3)7-9	(4)7-9	(5)7-9	(6)4 6	(7)6-8	(8)4-6	(9)6-8
F005	(1)7-9	(2)7-9	(3)7-9	(4)7-9	(5)7 9	(6)4-6	(7)6 8	(8)4 6	(9)6 8
F006	(1)7-9	(2)7-9	(3)7-9	(4)7-9	(5)7-9	(6)4-6	(7)6-8	(8)4-6	(9)6-8
F007	(1)7-9	(2)7-9	(3)7-9	(4)7-9	(5)7-9	(6)4-6	(7)6-8	(8)4-6	(9)6 8
F008	(1)7-9	(2)7-9	(3)7-9	(4)7-9	(5)7-9	(6)4 6	(7)6-8	(8)4-6	(9)6 8
F009	(1)7 9	(2)7-9	(3)7-9	(4)7 9	(5)7-9	(6)4-6	(7)6 8	(8)4-6	(9)6-8
F010	(1)7-9	(2)7-9	(3)7-9	(4)7-9	(5)7-9	(6)4 6	(7)6-8	(8)4 6	(9)6 8
F011	(1)7 9	(2)7 9	(3)7-9	(4)7 9	(5)7-9	(6)4-6	(7)6-8	(8)4-6	(9)6-8
F012	(1)7-9	(2)7-9	(3)7-9	(4)7-9	(5)7-9	(6)4-6	(7)6-8	(8)4 6	(9)6-8
F013	(1)7-9	(2)7-9	(3)7-9	(4)7-9	(5)7-9	(6)4-6	(7)6 8	(8)4-6	(9)6-8
F014	(1)7-9	(2)7-9	(3)7 9	(4)7 9	(5)7 9	(6)4-6	(7)6 8	(8)4-6	(9)6-8
F015	(1)7-9	(2)7-9	(3)7-9	(4)7-9	(5)7-9	(6)4 6	(7)6-8	(8)4 6	(9)6-8
E025	(1)10-12	(2)10-12	(3)10-12	(4)10-12	(5)10-12		(6)10-12		(7)10-12
E026	(1)10 12	(2)10-12	(3)10 12	(4)10 12	(5)10-12		(6)10 12		(7)10-12
E027	(1)10-12	(2)10 12	(3)10-12	(4)10-12	(5)10-12		(6)10-12		(7)10-12
E028	(1)10-12	(2)10 12	(3)10-12	(4)10-12	(5)10-12		(6)10-12		(7)10-12
E029	(1)10-12	(2)10-12	(3)10 12	(4)10-12	(5)10 12		(6)10 12		(7)10-12
P030	(1)8-10	(2)8-10	(3)8-10	(4)8-10	(5)8-10		(6)8-10		(7)8 10
P031	(1)8 10	(2)8 10	(3)8 10	(4)8 10	(5)8-10		(6)8 10		(7)8-10
P032	(1)8-10	(2)8-10	(3)8-10	(4)8 10	(5)8-10		(6)8-10		(7)8-10
P033	(1)8-10	(2)8-10	(3)8-10	(4)8-10	(5)8-10		(6)8-10		(7)8-10
0034	(1)8-10	(2)8-10	(3)8 10	(4)8-10	(5)8 10		(6)8 10		(7)8-10
0035	(1)8-10	(2)8-10	(3)8-10	(4)8-10	(5)8-10		(6)8-10		(7)8-10
0036	(1)8-10	(2)8-10	(3)8 10	(4)8-10	(5)8-10		(6)8 10		(7)8-10
0037	(1)8-10	(2)8-10	(3)8-10	(4)8 10	(5)8-10		(6)8-10		(7)8 10
0038	(1)8-10	(2)8-10	(3)8 10	(4)8 10	(5)8-10		(6)8 10		(7)8-10
0039	(1)8-10	(2)8-10	(3)8-10	(4)8-10	(5)8-10		(6)8-10		(7)8 10
0040	(1)8-10	(2)8-10	(3)8 10	(4)8-10	(5)8 10		(6)8 10		(7)8-10
S041	(1)8-10	(2)8 10	(3)8-10	(4)8-10	(5)8 10		(6)8-10		(7)8 10
S042	(1)8-10	(2)8-10	(3)8-10	(4)8-10	(5)8-10		(6)8-10		(7)8 10
S043	(1)8 10	(2)8-10	(3)8-10	(4)8 10	(5)8-10		(6)8-10		(7)8-10
S044	(1)8-10	(2)8 10	(3)8 10	(4)8-10	(5)8-10		(6)8-10		(7)8-10
S045	(1)8-10	(2)8-10	(3)8-10	(4)8-10	(5)8-10		(6)8 10		(7)8-10

Table 2: Monthly sampling calendar

日	一	二	三	四	五	六
						26 F5 S2 F11 P3 **4—6** **8-10**
27 S1 O1 **8-10**	**28** B2 B5 **9—11**	**29** F1 F4 **7-9**				
			7 F3 F21 **7-9**		**9** F22 F13 **7-9**	
11 F19 F14 **6-8**	**12** F15 F8 **7-9**	**13**	**14** F24 F2 **7-9**	**15** P3 P1 **8-10**	**16** B4 B1 **9-11**	**17** F10 P2 F7 O5 **4-6** **8-10**
18 O2 S5 **8-10**	**19** O4 S4 **8-10**					

Appendix III (HIV infection and characteristics of MBs or OMSMs in 2008 or 2009)

Table 3 HIV prevalence and related risk factors for MB 2008 vs.2009

	2008				2009			
	Total(n=394) n (%)	HIV positive(n=21) n (%)	X^2	P	Total(n=456) n (%)	HIV positive(n=17) n (%)	X^2	P
Overall	394(100)	21(5.3)			456 (100)	17 (3.7)		
Tested Syphilis positive								
Yes	54(13.7)	11(20.4)	10.376	**0.001**	56(12.3)	8(14.3)	47.269	**<0.001**
No	402(102.0)	6(1.5)			338(74.1)	13(3.8)		
Venue type of recruitment								
Bar and massage center	235(59.6)	5(2.1)	12.728	**0.005**	262(57.5)	3(1.1)	23.601	**<0.001**
Family club	104(26.4)	11(10.6)			148(32.5)	7(4.7)		
Park	40(10.2)	3(7.5)			26(5.7)	4(15.4)		
Small venue in suburb	15(3.8)	2(13.3)			18(3.9)	3(16.7)		
Hometown								
Low HIV prevalence area	336(85.3)	12(3.6)	13.988	**<0.001**	379(83.1)	11(2.9)	4.264	**0.039**
High HIV prevalence area	58(14.7)	9(15.5)			77(16.9)	6(7.8)		
Self-identified sexual orientation								
Homosexual/gay	96(24.4)	12(12.5)	11.689#	**0.001**	157(34.4)	10(6.4)	6.776#	**0.009**
Bisexual	148(37.6)	6(4.1)			153(33.6)	6(3.9)		
Heterosexual or unsure	150(38.1)	3(2.0)			146(32.0)	1(0.7)		
Gender of first sex partner								
Male	109(27.7)	10(9.2)	4.414	**0.036**	143(31.4)	9(6.3)	3.821	**0.050**
Female	285(72.3)	11(3.9)			313(68.6)	8(2.6)		

	2008				2009			
	Total(n=394) n (%)	HIV positive(n=21) n (%)	X^2	P	Total(n=456) n (%)	HIV positive(n=17) n (%)	X^2	P
Female sex partner								
0	134(34.0)	14(10.4)	14.109	**0.001**	218(47.8)	13(6.0)	8.347	**0.015**
1	72(18.3)	5(6.9)			59(12.9)	3(5.1)		
>1	188(47.7)	2(1.1)			179(39.3)	1(0.6)		
No. of commercial male partners								
<=4	176.0(44.7)	5.0(2.8)	3.906	**0.048**	269.0(59.0)	7.0(2.6)	2.317	0.128
>4	218.0(55.3)	16.0(7.3)			187.0(41.0)	10.0(5.3)		
Sold sex to women								
Yes	126.0(32.0)	2.0(1.6)	5.142	**0.023**	128.0(28.1)	2.0(1.6)	2.325	0.127
No	268.0(68.0)	19.0(7.1)			328.0(71.9)	15.0(4.6)		
Male clients from Hong Kong								
No	89.0(22.6)	9.0(10.1)	5.211	**0.022**	119.0(26.1)	8.0(6.7)	4.023	**0.045**
Yes	305.0(77.4)	12.0(3.9)			337.0(73.9)	9.0(2.7)		
Unprotected vaginal or anal sex with female partners								
No	229(58.1)	14(6.1)	0.665	0.415	327(71.7)	16(4.9)	4.370	**0.037**
Yes	165(41.9)	7(4.2)			129(28.3)	1(0.8)		
History of HIV test								
Within 6 months	104(26.4)	13(12.5)	11.870#	**0.001**	138(30.3)	6(4.3)	0.745#	0.388
Before 6 months	65(16.5)	2(3.1)			128(28.1)	6(4.7)		
No	220(55.8)	6(2.7)			190(41.7)	5(2.6)		

#: trend test value; UAI: unprotected anal intercourse; STI: sexually transmitted infection.

Table 4 HIV prevalence and related risk factors for OMSM in 2008 vs.2009

	2008				2009			
	Total(n=334) n (%)	HIV positive(n=17) n (%)	X^2	P	Total(n=467) n (%)	HIV positive(n=28) n (%)	X^2	P
Overall					467(100)	28(6.0)		
Tested Syphilis positive								
Yes	85(25.4)	17(20.0)	12.510	**<0.001**	77(16.5)	14(18.2)	36.158	**<0.001**
No	382(114.4)	11(2.9)			257(55.0)	14(5.4)		
Venue type of recruitment								
Bar and massage center	60(18.0)	1(1.7)	6.878	0.142	55(11.8)	0(0.0)	7.142	0.129
Family club	16(4.8)	3(18.8)			53(11.3)	4(7.5)		
Park	126(37.7)	12(9.5)			120(25.7)	11(9.2)		
Small venue in suburb	23(6.9)	1(4.3)			90(19.3)	3(3.3)		
Sauna	109(32.6)	11(10.1)			149(31.9)	10(6.7)		
Age（Years）								
18-20	22(6.6)	3(13.6)	1.157#	0.282	21(4.5)	2(9.5)	0.039#	0.843
21-25	114(34.1)	4(3.5)			137(29.3)	6(4.4)		
26-30	90(26.9)	10(11.1)			129(27.6)	9(7.0)		
>30	108(32.3)	11(10.2)			180(38.5)	11(6.1)		
Hometown								
Low HIV prevalence area	295(88.3)	24(8.1)	0.202	0.653	401(85.9)	27(6.7)	2.738	0.098
High HIV prevalence area	39(11.7)	4(10.3)			66(14.1)	1(1.5)		
Marital status								
Unmarried	263(78.7)	21(8.0)	0.705	0.703	319(68.3)	19(6.0)	0.446	0.800
Married	71(21.3)	7(9.9)			148(31.7)	9(6.1)		

	2008				2009			
	Total(n=334) n (%)	HIV positive(n=17) n (%)	X^2	P	Total(n=467) n (%)	HIV positive(n=28) n (%)	X^2	P
Self-identified sexual orientation								
Homosexual/gay	179(53.6)	19(10.6)	1.818#	0.178	271(58.0)	21(7.7)	4.755#	**0.029**
Bisexual	108(32.3)	6(5.6)			151(32.3)	7(4.6)		
Heterosexual or unsure	47(14.1)	3(6.4)			45(9.6)	0(0.0)		
Gender of first sex partner								
Male	183(54.8)	15(8.2)	0.018	0.892	262(56.1)	21(8.0)	4.319	**0.038**
Female	151(45.2)	13(8.6)			205(43.9)	7(3.4)		
UAI in male sex								
No	153(45.8)	7(4.6)	5.331	**0.021**	267(57.2)	16(6.0)	0.000	0.997
Yes	181(54.2)	21(11.6)			200(42.8)	12(6.0)		
UAVI with female partners								
No	269(80.5)	21(7.8)	0.598	0.439	404(86.5)	28(6.9)	4.645	**0.031**
Yes	65(19.5)	7(10.8)			63(13.5)	0(0.0)		
STI, drug use, HIV-related knowledge and services								
Ever diagnosed for STI								
Yes	67(20.1)	8(11.9)	1.381	0.240	37(7.9)	7(18.9)	11.907	**0.001**
No	267(79.9)	20(7.5)			430(92.1)	21(4.9)		
Self-perception of HIV infection								
No chance	273(81.7)	18(6.6)	16.020	**<0.001**	417(89.3)	24(5.8)	0.893	0.640
Little chance	55(16.5)	7(12.7)			46(9.9)	4(8.7)		
High chance	6(1.8)	3(50.0)			4(0.9)	0(0.0)		

#: trend test value; UAI: unprotected anal intercourse; STI: sexually transmitted infection.