The Update of HIV-1 Prevalence and Incidence and Spatio-Temporal Analyses of HIV Recent Infection Among Four Sub-Groups in Sichuan, China During Surveillance Period Between 2016 and 2022

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Background: Sichuan Province has gradually become a hot-spot for HIV/AIDS. Little is known about the HIV-1 incidence and prevalence among four sub-groups since 2015. Meanwhile, the distributions of hot-spot areas related to recent infection cases that indicate current transmission among the four subgroups are also rare.

Objective: The main purpose of this study was to assess the HIV-1 prevalence and incidence and to visualize the distributions of hot-spot areas of current transmission among four subgroups (people who inject drugs, male STD clinic attendees, female sex workers and men who had sex with men) during the surveillance period in Sichuan province between 2016 and 2022.

Results: Of the 267,617 individuals, 2158 HIV-positive samples were tested with Lag-Avidity EIA, among which 493 samples were identified as recent infections. Among people who inject drugs (PWID), both HIV-1 prevalence (from 1.41% to 0.34%) and incidence (from 0.03% to 0.140%) showed a significant decreasing trend. Among men who had sex with men (MSM), female sex workers (FSWs), and male STD clinic attendees, HIV-1 prevalence indicated significant decreasing trends, whereas HIV-1 incidence showed no significant changes. Spatial analysis demonstrated the formation of hot-spots and clusters of current transmissions sharing regional differences, mainly concentrated in the southeast, and most of these were consecutive hot-spots.

Conclusion: The prevention and control were efficacious and persistent. However, among the other three subgroups, there is a need for a regional cooperative in prevention and control approaches and collaborative research in many aspects.

Keywords: human immunodeficiency virus, recent infection, hot spots, people who inject drugs, PWID, male STD clinic attendees, mSTD, female sex workers, FSWs
workers and tuberculosis (TB) patients; a web-based HIV/AIDS case reporting system established in 2005; special epidemiological surveys have been launched among various groups to augment surveillance data, and those data have been entered into annual joint assessment reports. HIV sentinel surveillance is a series of annual cross-sectional surveys on risk behaviors and sero-testing in representative areas and populations, including national sentinel surveillance sites and provincial sentinel surveillance sites, which is a necessary complement to the former.

Traditionally, the focus of analysis of data from sentinel surveillance was a trend of HIV prevalence and risk factors associated with HIV infection in a given region and population; however, there is a dearth of information regarding the hot-spot distribution of recent infection cases among the four sub-populations at the city level in Sichuan province. Moreover, the HIV prevalence and incidence after 2015 have remained unclear.

Thus, to address the paucity of relevant information regarding the hot-spot distribution and an update of HIV prevalence, incidence, we conducted a spatial analysis of HIV-1 recent infection cases among four sub-populations (people who inject drugs, male STD clinic attendees, female sex workers and men who had sex with men) and updated the HIV prevalence and incidence between 2016 and 2022.

**Methods**

**Sample Collection and Laboratory Testing**

Sentinel surveillance is a cross-sectional survey and conducted annually between 2016 and 2022, according to the National AIDS sentinel surveillance implementation program, the surveillance period is from April to June. If the sample size is still insufficient at the end of the surveillance period, it can be extended by up to one month. Between 2016 and 2021, 119 sentinel sites were set up for regular surveillance among PWID (39 sites), male STD clinic attendees (30 sites), FSWs (40 sites), and MSM (10 sites) in Sichuan Province (Table 1). By 2022, 4 PWID sites and three FSW sites were closed. Generally, the sample size is 400 per site; when the infection rate of a local high-risk population is higher

<table>
<thead>
<tr>
<th>Region</th>
<th>MSM</th>
<th>FSW</th>
<th>mSTD</th>
<th>PWID</th>
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<td>2</td>
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<tr>
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<tr>
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</tr>
</tbody>
</table>

(Continued)
than 10%, the sample size can be reduced to 250. If the sample size cannot meet the requirements until the end of the surveillance period, then the actual sample size should be reported. Anonymous interviews were dispensed to every participant to collect information on demographic features and HIV-1 transmission-related risk behaviors, and each participant provided 2–3 mL of whole blood at the local CDC. Plasma was separated from the whole blood and used for HIV-1 serological testing. Plasma samples were first screened twice by an enzyme immunoassay and confirmed by a Western blot assay (HIV-1 BLOT 2.2, MP Diagnostics, Singapore) or Recombinant Strip Immunoblot assay (recomLine HIV-1 and HIV-2 IgG, MIKROGEN DIAGNOSTIK, Germany). All HIV-1 tests were voluntary and informed of the study. Written consent was obtained from all the participants. The study was approved by the Ethics Committee of the Sichuan Center for Disease Control and Prevention and conducted in accordance with the Helsinki Declaration of 1964.

### Limiting Antigen Avidity Assay

The LAg-Avidity EIA Test\(^{13-20}\) is an in vitro 96-well format enzyme immunoassay that measures the increasing avidity of HIV antibodies from liquid serum/plasma or dried blood spot-eluted specimens after seroconversion. Antibody avidity reflects the relative binding strength, a functional property, of developing antibodies and is likely to be more robust in detecting recent HIV-1 infections than assays that detect antibody levels, which is a passive parameter.\(^{21}\) Specimens that are initially < 2.0 normalized optical density(ODn) in the assay are repeat tested in triplicate (confirmatory testing), and specimens that are confirmed < 1.5 normalized optical density(ODn) in the assay are considered to represent recent infection.

### Calculation of Prevalence and Incidence

HIV-1 prevalence is defined as the proportion of individuals currently living with HIV-1 infection.\(^{22,23}\) The prevalence can be estimated by \(p = yi/n\), where \(yi\) is the total number of all cases testing positive for HIV-1 and \(n\) is the total number of people who were tested.\(^{23}\)

The HIV-1 incidence is the rate at which HIV-1 infection occurs in a population.\(^{22}\) It is a quantitative index that measures the extent of ongoing HIV-1 transmission within a population.\(^{22}\) In this study, we calculated the prevalence and...
incidence as rates because sentinel surveillance is a cross-sectional survey conducted annually between 2018 and 2022 (the surveillance period was from April to June). For a reliable comparison of the incidence between this work and previous works, we continued to use the McDougal formula to calculate the incidence. A difference existed in the window period (the mean length of time individuals remained classified as “recently infected”). Previous studies have used BED-CEIA to test for evidence of recent infection, and in China, the window period was 168 days. Our study used the Limiting Antigen Avidity assay, and in China, the window period was 130 days.

According to the Operations Manual, when the number of recent infections identified by the LAg-avidity EIA is more than 10, the estimated incidence is reliable. In most cities, the recent infections for each year is less than 10, and a solution is to combine data from several years to calculate the incidence.

Spatial Analysis for HIV Recent Infections
The spatial analysis was conducted with ArcGIS 10.7 (ESRI, Redlands, CA, USA) to visualize the hot-spot areas of four sub-populations in sentinel surveillance. Local spatial auto-correlation was used to analyze the correlation between the distribution of recent HIV cases in a specific city and that in adjacent cities. The Getis-Ord Gi* statistic was used to calculate the z-scores and p-values; local clusters with a z-value >1.76 were defined as hot-spots, indicating that the distribution in those counties was highly clustered.

Statistical Analysis
Data analysis was conducted using SPSS (version 23.0; IBM, Armonk, New York, USA). Trend tests were performed using $\chi^2$ tests with linear-by-linear association. All tests were two-tailed, and statistical significance was set at $p <0.05$.

Results
Summarization
In total, there were 119 sentinel surveillance sites covering the four sub-group between 2016 and 2021. As 3 STD sites and 4 DUS sites were closed, there were 112 sentinel surveillance sites by 2022 [Table 1]. A total of 267,617 individuals participated in a sentinel surveillance program for HIV between 2016 and 2022, including 22,991 MSM, 62,725 male STD clinic attendees, 85,572 FSWs, and 96,329 PWID. HIV-1 antibodies were distinguished in 7876 samples by serum screening twice, of which 4426 samples were reported previously. All newly reported HIV-1 infected cases were immediately confirmed by Western blotting or Recombinant Strip Immunoblot assays. Excluding 168 samples whose volumes were not sufficient for LAg-avidity EIA, a total of 2158 samples (92.78%) were tested with LAg-Avidity EIA, among which 493 samples were identified as HIV-1 recent infection. Based on these results, the prevalence and estimated incidence were obtained for each of the four sub-populations (Table 2).

PWID
HIV prevalence and estimated incidence between 2016 and 2022 among STD are shown in Table 2. The estimated incidence showed a significant trend ($\chi^2=8.299$, $P=0.004$) and the prevalence showed a significant decreasing trend between 2016–2022 ($\chi^2=234.324$, $P=0.000$). Spatial analysis revealed a significant cluster in Liangshan (2016–2022, Figure 1A-C), while Panzhihua (2016–2017 and 2020–2022, Figure 1A and C) showed a secondary cluster.

Male STD Clinic Attendees
HIV prevalence and estimated incidence between 2016 and 2022 among STD are shown in Table 2. The estimated incidence did not show a significant trend ($\chi^2=2.605$, $P=0.107$); however, the prevalence showed a significant decreasing trend between 2016–2022 ($\chi^2=41.196$, $P=0.000$). Spatial analysis revealed a significant cluster in Deyang (2016–2017, Figure 2A), Yibin (2018–2019, Figure 2B) and Luzhou (2020–2022, Figure 2C), respectively, and Liangshan and Yibin (2020–2022, Figure 2C) showed a secondary cluster.
## Table 2 HIV-1 Prevalence Rate and Incidence Rate from Four-Groups Sentinel Surveillance

<table>
<thead>
<tr>
<th>Population</th>
<th>Year</th>
<th>The Number of Cases&lt;sup&gt;a&lt;/sup&gt;</th>
<th>The Number of HIV Antibody-Positive Cases&lt;sup&gt;b&lt;/sup&gt;</th>
<th>The Number of HIV Confirmed Antibody-Positive Cases&lt;sup&gt;c&lt;/sup&gt;</th>
<th>The Number of Previously Reported Cases&lt;sup&gt;d&lt;/sup&gt;</th>
<th>The Number of Newly Diagnosed Cases&lt;sup&gt;e&lt;/sup&gt;</th>
<th>The Number of Samples Tested with LAg-Avidity Assay&lt;sup&gt;f&lt;/sup&gt;</th>
<th>The Number of Recently Infected Cases&lt;sup&gt;g&lt;/sup&gt;</th>
<th>Prevalence Rate (%)</th>
<th>Estimated Incidence Rate (%, 95% CI)</th>
<th>( \chi^2 )</th>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td>MSM</td>
<td>2016</td>
<td>3236</td>
<td>305</td>
<td>287</td>
<td>130</td>
<td>157</td>
<td>157</td>
<td>53</td>
<td>4.95</td>
<td>3.22(2.55,3.90)</td>
<td>( \chi^2=72.825, )</td>
<td>P=0.000</td>
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<tr>
<td></td>
<td>2017</td>
<td>3291</td>
<td>316</td>
<td>308</td>
<td>142</td>
<td>166</td>
<td>157</td>
<td>34</td>
<td>3.41</td>
<td>4.07(3.27,4.87)</td>
<td>( \chi^2=0.059, )</td>
<td>P=0.808</td>
</tr>
<tr>
<td></td>
<td>2018</td>
<td>3203</td>
<td>238</td>
<td>232</td>
<td>108</td>
<td>124</td>
<td>118</td>
<td>59</td>
<td>2.46</td>
<td>3.47(2.88,4.07)</td>
<td>( \chi^2=2.605, )</td>
<td>P=0.107</td>
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<tr>
<td></td>
<td>2019</td>
<td>3283</td>
<td>222</td>
<td>220</td>
<td>123</td>
<td>97</td>
<td>97</td>
<td>40</td>
<td>0.92</td>
<td>0.31(0.19,0.42)</td>
<td>( \chi^2=2.605, )</td>
<td>P=0.107</td>
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<td>3208</td>
<td>222</td>
<td>219</td>
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<td>106</td>
<td>106</td>
<td>60</td>
<td>0.43</td>
<td>0.21(0.12,0.29)</td>
<td>( \chi^2=0.476, )</td>
<td>P=0.490</td>
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<tr>
<td></td>
<td>2021</td>
<td>3265</td>
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<td>83</td>
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<td>2022</td>
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<td>173</td>
<td>117</td>
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<td>56</td>
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<td>77</td>
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<td>0.31(0.19,0.42)</td>
<td>( \chi^2=2.605, )</td>
<td>P=0.107</td>
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<td>8775</td>
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<td>40</td>
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<td>13</td>
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<td>0.21(0.12,0.29)</td>
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<td>61</td>
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<td>28</td>
<td>28</td>
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*Continued*
Table 2 (Continued).

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<thead>
<tr>
<th>Population</th>
<th>Year</th>
<th>The Number of Cases</th>
<th>The Number of HIV Antibody-Positive Cases</th>
<th>The Number of HIV Confirmed Antibody-Positive Cases</th>
<th>The Number of Previously Reported Cases</th>
<th>The Number of Newly Diagnosed Cases</th>
<th>The Number of Samples Tested with LAg-Avidity Assay</th>
<th>The Number of Recently Infected Cases</th>
<th>Prevalence Rate (%)</th>
<th>Estimated Incidence Rate (% , 95% CI)</th>
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<tr>
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<td>13,700</td>
<td>695</td>
<td>641</td>
<td>441</td>
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<td>15</td>
<td>1.41</td>
<td>χ²=234.324, P=0.000</td>
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<td>675</td>
<td>636</td>
<td>443</td>
<td>193</td>
<td>163</td>
<td>7</td>
<td>0.99</td>
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<td>709</td>
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<td>200</td>
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<td>14</td>
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<td>2</td>
<td>1.16</td>
<td>χ²=132.65, P=0.000</td>
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</tbody>
</table>

Notes: aThe number of cases means individuals participated in HIV-1 sentinel surveillance program. bHIV antibody-positive cases indicate positive results in the two-enzyme immunoassay screening. cHIV-confirmed antibody-positive cases indicate positive results in Western blot assay or Recombinant Strip Immunoblot assay. dPreviously reported cases means those previously reported positive in confirmatory tests and were reported in the China HIV/AIDS case reporting system. eNewly diagnosed cases are those obtained positive results in confirmatory tests and have not yet been reported in the China HIV/AIDS case reporting system. fSamples tested with the LAg-avidity assay means that they were conducted in the LAg-avidity assay. gRecently infected cases means those were classified as LAg-Avidity positive.

Abbreviations: MSM, men who had sex with men; mSTD, male STD clinic attendees; FSW, female sex workers; PWID, people who inject drugs.
HIV prevalence and estimated incidence between 2016 and 2022 among STD are shown in Table 2. The estimated incidence did not show a significant trend ($\chi^2=0.476$, $P=0.490$); however, the prevalence showed a significant decreasing trend between 2016–2022 ($\chi^2=27.125$, $P=0.000$). Spatial analysis revealed a significant cluster in Mianyang (2018–2019, Figure 3B), Yibin (2016–2022, Figure 3A-C), and Luzhou (2020–2022, Figure 3C), respectively, and Luzhou (2018–2019, Figure 3B) and Deyang (2016–2017, Figure 3A) showed a secondary cluster.

**Discussion**

To our knowledge, this study is the first to report a combined application of spatial analysis and LAg-avidity EIA to illustrate hot-spot areas of recent HIV-1 cases at the city level among four sub-groups in China for seven years in a row, which will incentivize the focus of intervention efforts on relevant areas and populations. Furthermore, we first updated the HIV-1 prevalence and incidence among the four subgroups until 2022, continuing previous works.8
Overall, among the four subgroups, a relatively high level emerged in 2018–2019 and a relatively low level emerged in 2020–2022. We believe that the emergence of this phenomenon during 2018–2019 was related to China’s scale-up testing policies. In January 2017, the General Office of the State Council of the People’s Republic of China issued China’s 13th Five-Year Plan for HIV/AIDS prevention. This plan was first required to identify infected individuals and patients to a large extent. Accordingly, the plan required that medical institutions, maternity and child healthcare institutions, and disease prevention and control institutions at or above the county level should all have the capacity for HIV testing. Moreover, HIV testing and consultation should be included in voluntary premarital medical and physical examinations of the target population. In September 2019, the Chinese CDC issued an implementation plan for HIV/AIDS prevention (2019–2022) was issued by Chinese CDC. In the plan, it was required that community health service centers and township health centers should all be capable of rapid HIV testing and counseling. This suggests that HIV testing should be included in the health examinations of the elderly in basic public health services. Despite these scale-up testing policies, more HIV infection cases were found, resulting in a relatively higher incidence among the four subgroups. Between 2020 and 2022, the outbreak of SARS-CoV-2 led to an unprecedented disruption in life. To mitigate viral transmission, many governments have introduced prevention measures based on social and behavioral restrictions (eg, physical distancing, stay-at-home policies, or quarantine). Sexual behavioral changes, pathological disorders, and income reduction may result in a temporarily lower incidence among the four subgroups.

PWID was the only group in which the HIV incidence significantly decreased. In 1989, the first HIV epidemic in Yunnan was identified among PWID in Ruili County bordering Myanmar, and then further spread along major drug-trafficking roads to Guangxi, Sichuan, Xinjiang, Guangdong, and other provinces. Historically, HIV-1 infection has been largely confined to certain high-risk populations such as intravenous drug users. The relative importance of...
IDU in contributing to the total number of reported HIV/AIDS cases has been declining over the years as sexual transmission increases. In this study, both HIV-1 incidence and prevalence of PWID among the four sub-populations were lower than those reported in previous studies. A relatively higher HIV-1 incidence among PWID was observed between 2018 and 2019, for the reasons mentioned above. Among PWID, the significant decreasing trend in HIV-1 incidence could mainly result from the persistent implementation of needle exchange programs and methadone maintenance treatment. The decrease between 2020–2022, it was possibly due to the pandemic of SARS-Cov-2, with a reduction in drug supplies, different lockdown measures, and alteration of drug use patterns.

Spatial analysis illustrated that Liangshan Prefecture and Panzhihua City were hot-spot areas. Liangshan is an autonomous prefecture of Sichuan Province and consists of 17 counties, with 5 counties having a high HIV prevalence (>1%). In particular, long-term poverty and a long history of drug production and trade are the primary reasons for high HIV prevalence. In September 2017, the National Health Commission and Sichuan Provincial Government jointly issued the “First Phase Action Plan for HIV Prevention and Health Poverty Alleviation in Liangshan Prefecture, Sichuan Province, China (2017–2020)”, which led to positive challenges in HIV prevention and control.

With the implementation of the National Methadone Maintenance Treatment Program and the Needle Exchange Program in 2004, sexual contact has gradually become the primary transmission route for HIV/AIDS, particularly heterosexual transmission. Through commercial sex work, male STD clinic attendees and female sex workers represent a crucial “bridge population” driving HIV infection in the general population. In this study, STD and FSW had an observably lower HIV-1 prevalence than previous reports in Sichuan and Yunnan provinces; however, there were no significant trends in HIV-1 incidence. As previous works reported, consistent condom use was still low.

Figure 3 2016–2022 Hot spots of HIV recent cases among FSW at city level by year. (A) Sichuan province, 2016–2017. (B) Sichuan province, 2018–2019. (C) Sichuan province, 2020–2022.
both in heterosexual STD clinic patients and FSW. Without condom protection, the risk of HIV transmission among sexual behaviors would be greatly increased. Spatial analysis illustrated that the southeastern cities were consecutive hot-spot areas, such as Yibin and Luzhou. This result can be interpreted as follows. The southeast region of Sichuan has a high population density and accounts for a large migrant labor force in large cities across the country. Migrant workers showed a significant increase in engagement with commercial sex workers and a low percentage of consistent condom use during commercial sex. In addition, HIV infection is widely prevalent among older men (aged ≥ 50 years) in this region. Among older men, the primary mode of heterosexual transmission is commercial heterosexual contact due to sex needs, single, widowhood, and a stable financial situation. Besides, works on HIV-1 molecular epidemiology showed that commercial heterosexuality caused an aggregated HIV epidemic in this region, and HIV-1 genetic transmission networks suggested that there was more likely regional transmission in these areas. In summary, HIV prevention approaches in these areas may require joint interventions, both inside and outside (eg, information exchange for better launching joint interventions and collaborating with each other).

MSM had the highest HIV prevalence and incidence among the four subgroups in this study, and HIV incidence was estimated to be lower than the national level calculated by a meta-analysis and previous data reported in Sichuan. The low level is partly due to progress in diagnosing infections among people living with HIV-1 and ensuring that they have access to early, ongoing treatment and, in part, to the pandemic of SARS-CoV-2. Some studies have suggested a reduction in sexual activity among MSM during this period. Thus, future studies should monitor the HIV epidemic in this high-risk population. Spatial analysis demonstrated a significant and consecutive hot spot in Luzhou City, which confirmed a supposition about an HIV-1 epidemic spreading from the core city (like Chengdu) to the peripheral region.

Figure 4 2016–2022 Hot spots of HIV recent cases among MSM at city level by year. (A) Sichuan province, 2016–2017. (B) Sichuan province, 2018–2019. (C) Sichuan province, 2020–2022.
among MSM, as reported in our previous work.\(^{68}\) This spread may be derived from its geographical position neighboring other provinces with a high HIV-1 burden\(^ {67,72,73}\) and Luzhou’s policies,\(^ {74,75}\) which increased screening for HIV and AIDS, and more cases are being detected.\(^ {76}\) According to Yuan,\(^ {65}\) Luzhou does not have a strong HIV-1 transmission link with other cities within Sichuan. In these contexts, more intensive work should be conducted, concentrating on the identification of transmission networks of new infections, demographic characteristics, and spatial distribution, as well as the exploration of the relationship between molecular transmitted clusters and social covariates.

This study had some limitations. First, most sentinel surveillance systems have limited geographical coverage, with under-representation of rural and smaller areas; therefore, the results of this study were not entirely representative of the whole province. Second, the implementation of sentinel surveillance varies considerably between cities, and the quality of surveys might differ over time depending on available resources; therefore, the results of this study were undoubtedly affected by these factors. Third, given the nature of a cross-sectional survey, temporality between surveillance periods cannot be inferred. For example, we cannot claim the findings of this study, which represent the entire year. Fourth, considering the mis-classification of the LAg-avidity EIA, there is a possibility of overestimation of the incidence. Fifth, owing to stringent SARS-CoV-2 prevention measures, it is not yet clear whether the declining trends were actual.

**Conclusion**

In this work, we updated the data on HIV-1 prevalence and incidence among four subgroups until 2022, continuing and confirming the supposition of previous studies. HIV-1 prevalence and incidence among PWID showed a declining trend, and spatial analysis indicated that Liangshan Prefecture was still a consecutive hot-spot area among PWID, which suggests that the prevention and control of this population in this region were efficacious and should be persistent. However, among the three sub-groups belonging to heterosexual transmission routes, HIV-1 prevalence and incidence showed no significant changes, and spatial analysis indicated that the southeastern region in Sichuan province was a hot-spot area in the study period, which is referred to as a regional cooperative in prevention and control approaches and collaborative research in many aspects.

**Abbreviations**

PWID, people who inject drugs; STD, sexually transmitted diseases; FSWs, female sex workers; MSM, men who have sex with men; LAg-Avidity EIA, Limiting Antigen Avidity enzyme immunoassay; HIV, Human immunodeficiency virus; CDC, Centers for Disease Control and Prevention; ODn, normalized optical density.

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**Author Contributions**

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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**Disclosure**

The authors declare no conflicts of interest for this work.
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