An Assessment of Trends in HIV-1 Prevalence and Incidence and Spatio-Temporal Analyses of HIV-1 Recent Infection Among MSM During the Surveillance Period Between 2018 and 2022 in Sichuan, China

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Background: Men who have sex with men (MSM) is one main type of high-risk activities facilitating HIV-1 transmission in Sichuan province. Previous works on HIV-1 incidence and prevalence among MSM only concentrated before 2018, the situation after that is unknown. In addition, the distribution of hot-spots related to current HIV-1 epidemic is also rarely known among MSM in Sichuan.

Objective: To update trends of HIV-1 prevalence and incidence and to visualize hot-spots of ongoing transmission in Sichuan province during surveillance period among MSM between 2018 and 2022.

Methods: Limiting Antigen Avidity assay was performed to detect recent infection within new HIV-1 diagnoses founded during surveillance period among MSM. The HIV-1 prevalence and incidence were calculated according to an extrapolation method proposed by publications and guidelines. Trend tests were performed using χ² tests with linear-by-linear association. The spatial analysis was conducted with ArcGIS 10.7 to figure hot-spots of HIV-1 recent infections among MSM.

Results: Between 2018 and 2022, 16,697 individuals participated in HIV-1 MSM sentinel surveillance program, of which 449 samples (98.25%) were tested with LAg-Avidity EIA, and 230 samples were classified as recent infection. Respectively, the overall prevalence and incidence were 2.74% and 3.69% (95% CI: 3.21, 4.16) and both had significant declining trends (p < 0.001). Luzhou city had a highest HIV-1 incidence (10.74%, 95% CI: 8.39, 13.10) over the study period and was recognized as a hot-spot for recent HIV-1 infection among MSM.

Conclusion: During the surveillance period, both HIV-1 prevalence and incidence were declining. However, Luzhou city had an unusually high HIV-1 incidence and became an emerging hot-spot of recent HIV-1 infection among MSM. This finding suggested focused attention, cross-regional intervention strategies, and prevention programs are urgently required to curb the spread of ongoing transmission.

Keywords: human immunodeficiency virus type 1, HIV-1 recent infection, incidence, hot-spots, men who have sex with men

Introduction
Since the first AIDS case in China was reported in 1985,¹ China had struggled against HIV over the last four decades. Currently, new epidemic trends have emerged in China and some new HIV/AIDS “hot-spots” appeared, such as Sichuan Province.² Sichuan is a populous province in the southwest of mainland China and the most developed one in Southwest China (including Sichuan, Yunnan, Guizhou, Tibet and Chongqing province).³ The number of people living with HIV-1 in Sichuan increased from 13 thousands to 17 thousands between 2018 and 2020, ranked first in China.⁴ The number of
newly diagnoses HIV-1 cases attributable to homosexual contact gradually increased, representing 5.86% of all cases at 2018 to 10.8% at 2020, in Sichuan unpublished data.

Men who have sex with men is one main type of high-risk activities facilitating HIV-1 transmission both in China and Sichuan.\textsuperscript{6} The prevalence of HIV-1 among Chinese MSM has substantially increased from 1.4% in 2005 to 6.9% in 2018.\textsuperscript{7,8} In Sichuan, Chengdu city, HIV-1 prevalence progressively increased from 0.6% in 2003 to 14.4% in 2015 among MSM.\textsuperscript{9,10} People who got HIV-1 infection usually have no particular symptoms and no awareness of their infection, and once diagnosed, they may have been infected for years. Antiretroviral therapy (ART) can extend the life of people living with HIV-1 by durable and maximal suppression of plasma viremia.\textsuperscript{11} Accordingly, the prevalence data reflect the cumulative effect of HIV-1 infection in the population, which may have a time-lag effect and do not accurately reflect the features of transmission currently occurring.\textsuperscript{12} Instead, HIV-1 incidence, defined as a rate which new HIV-1 infection is acquired in a population within a year, is a quantitative index that measures the extent of ongoing HIV-1 transmission in the population.\textsuperscript{13} However, HIV-1 incidence among MSM both in Sichuan and China, were also particularly high. Previous works reported persistently high HIV-1 incidence among MSM in Sichuan province (5.16%, 95% CI: 4.65–5.66%) between 2011 and 2015, which was slightly higher than the national level (5.0%, 95% CI: 4.1–5.8%).\textsuperscript{10,14} However, data in the most recent years are scarce.

Use of spatial analysis has become progressively common in HIV-1 study, focusing on epidemiological trends and spatial distribution,\textsuperscript{5,6,15} the identification of geographic variation of infection,\textsuperscript{16} as well as exploration of spatial relationships between HIV-1 and social covariates.\textsuperscript{17} However, there exists a gap of work conducted on geospatial analysis to determine hot-spots of recent HIV-1 infection among MSM, while recent study focused on new diagnosed HIV-1 cases.\textsuperscript{12,18}

Thus, there is an urgent need to visualize the hot-spots areas of ongoing HIV-1 transmission and to update the trends of HIV-1 prevalence and incidence in Sichuan province during surveillance period among MSM after 2015. Both could provide technical guidance on better understanding of HIV/AIDS epidemics and conducting better HIV prevention interventions. So, we conducted a spatial analysis of recent HIV-1 infection cases among MSM sentinel surveillance between 2018 and 2022 in Sichuan province.

**Methods**

**Sample Collection**

According to the Operation Manual,\textsuperscript{19} 10 sentinel sites (Figure 1) were established for regular surveillance among MSM in Sichuan Province between 2018 and 2022. Men whose age were between 18 and 65 and had insertional oral or anal sex in the past 12 months were recruited through snowball sampling (Snowball sampling is where research participants recruit other participants for a test or study. It is used where potential participants are hard to find\textsuperscript{20–22}) at various sites, including the internet, bathing room, sauna, pub and park, etc. Sentinel surveillance, which is a cross-sectional survey, was conducted annually between 2018 and 2022, and the surveillance period was from April to June. Sample sizes were as following: Chengdu, Dazhou, Deyang, Luzhou, Mianyang and Yibin were all 400 per site; Guangyuan, Leshan, Liangshan and Zigong were all 200 per site. If the sample size was insufficient at the end of the surveillance period, it can be extended for one month. An anonymous interview was dispensed to every participant for collection of information on demographic features and HIV-1 transmission-related risk behaviors and each participant provided 2–3 mL of whole blood in local Center for Disease. Plasma was separated from whole blood and then used for HIV-1 testing. The study was approved by the Ethics Committee of the Sichuan Center for Disease Control and Prevention and conducted following the Helsinki Declaration of 1964.

**Laboratory Testing**

Plasma samples were first screened by an enzyme immunoassay twice and confirmed by a Western blot assay (HIV-1 BLOT 2.2, MP Diagnostics, Singapore). Excluded previously reported HIV-1-seropositive cases, blood samples confirmed by Western blot assay were tested for evidence of recent infection by the Sichuan CDC laboratory using the LAg-Avidity assay from the Maxim Biomedical, Inc, Rockville, USA.\textsuperscript{23} The tests were performed entirely in accordance with
the manufacturers’ instruction manuals. A normalized optical density (ODn) of <1.5 was considered to represent recent infection. All HIV-1 tests were informed and voluntary. Written consents were obtained from all participants.

Calculation of Prevalence and Incidence

The HIV-1 prevalence is the proportion currently living with HIV-1 infection. The prevalence can be estimated by $p = y_i/n$, where $y_i$ is the total number of all cases testing positive for HIV-1 and $n$ is the total number of people who were tested.

HIV-1 incidence is the rate at which HIV-1 infection is acquired in a population. It is a quantitative index that measures the extent of ongoing HIV-1 transmission in the population. For a reliable comparability of incidence between this work and previous work, we continued to use the same McDougal formula for calculation of incidence. The only difference existed in the window period (the mean length of time individuals remains classified as “recently infected”). Previous work used BED-CEIA to test for evidence of recent infection, and in China, the window period was 168 days. Our work used Limiting Antigen Avidity assay to test and in China, the window period was 130 days.

According to the Operations Manual, when the number of recent infections identified by LAg-Avidity EIA is more than 10, the estimated incidence is reliable. In most of cities, the number of recent infections for each year was less than 10, a solution was to combine the data of five years to calculate the incidence. For province level, the incidences for each year were calculated.

In this work, we calculated the incidence as a rate, due to the fact that the sentinel surveillance was a cross-sectional survey and was conducted annually between 2018 and 2022 (the surveillance period was from April to June).
Spatial Analysis for HIV-1 Recent Infections
The spatial analysis was conducted with ArcGIS 10.7 (ESRI, Redlands, CA, USA) to visualize hot-spots and to create map of distribution of MSM sentinel surveillance sites in Sichuan. Local spatial auto-correlation was used to analyze the correlation between the distribution of HIV recent 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 >1.86 were defined as a hot-spot, meaning the distribution in those counties was high clustering.

Statistical Analysis
Data analysis was conducted in 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 a p value <0.05 was considered statistically significant.

Results
Overall Prevalence and Incidence Rates in MSM
Between 2018 and 2022, a total of 16,697 individuals participated in HIV-1 MSM sentinel surveillance program. Except for 608 previous reported cases and 19 participants who refused blood collection, 457 newly reported HIV-1 positive infections were confirmed with Western blot assay. Except 8 samples whose volumes were not enough for LAg-Avidity EIA, a total of 449 samples (98.25%) were tested with LAg-Avidity EIA, among which 230 samples were identified as HIV-1 recent infection. Based on these, the prevalence was 2.74% and incidence was 3.69% (95% CI: 3.21, 4.16) obtained for MSM (Table 1).

HIV-1 MSM Prevalence Between 2018–2022
During the study period, the overall prevalence of new HIV-1 diagnosis in Sichuan province decreased from 7.63% in 2018 to 1.51% in 2022. The highest prevalence appeared in 2018 and showed a significant decreasing trend for 2018–2022 (trend test, $\chi^2$=24.031, p < 0.001) (Table 1 and Figure 2).

At a city level, there was a significant difference among cities ($\chi^2$=95.934, P < 0.0001). Chengdu and Luzhou was found with highest HIV-1 prevalence (4.26% vs 4.25%), and Deyang, Liangshan, Yibin was at a medium level with HIV-1 prevalence between 2.55% and 2.81%. The relatively lower HIV-1 prevalence appeared in Dazhou, Guangyuan, Leshan and Zigong between 0.11% and 1.65% (Table 2).

HIV-1 MSM Incidence Between 2018–2022
During the study period, the estimated HIV-1 incidence in Sichuan province remained stable at a relatively high level from 3.96% (95% CI: 2.95–4.98) in 2018 to 2.37% (95% CI: 1.54–3.21) in 2022. The highest estimated HIV-1 incidence appeared in 2020 (4.42%, 95% CI: 3.30, 5.53). Also, there was a significant decreasing trend for 2018–2022 (trend test, $\chi^2$=29.225, p < 0.001) (Table 1 and Figure 2).

At a city level, there was a significant difference among cities ($\chi^2$=129.588, P < 0.0001). Luzhou was definitely found with highest estimated HIV-1 incidence (10.74%, 95% CI: 8.39, 13.10). The next was Chengdu, with 4.60% (95% CI 3.07, 6.12) of the estimated HIV-1 incidence. Deyang, Mianyang and Yibin shared a third level of estimated HIV-1 incidence, from 3.09% (95% CI 1.85, 4.32) to 3.83% (95% CI 2.48, 5.17). Others (Dazhou, Guangyuan, Liangshan, Leshan and Zigong) remained with a relatively lower estimated HIV-1 incidence (Table 2).

Spatial Analysis
Local spatial correlation was applied to the year-wise distribution of hot-spots at city levels throughout Sichuan province (Figure 3). Hot-spots were mainly located in Luzhou city (Figure 3A–E) and occasionally emerged in Chengdu (Figure 3C). Luzhou city had been the hot-spot every year among MSM between 2018 and 2022. In 2020, Chengdu was another hot-spot beside Luzhou.
<table>
<thead>
<tr>
<th>Region</th>
<th>Year</th>
<th>The Number of Cases</th>
<th>The Number of HIV Antibody-Negative Cases</th>
<th>The Number of HIV 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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<td>10 sites</td>
<td>2022</td>
<td>3505</td>
<td>3330</td>
<td>173</td>
<td>120</td>
<td>53</td>
<td>53</td>
<td>31</td>
<td>1.51</td>
<td>$\chi^2=24.031, p &lt; 0.001$</td>
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<td></td>
<td>2021</td>
<td>3265</td>
<td>3074</td>
<td>188</td>
<td>111</td>
<td>77</td>
<td>76</td>
<td>40</td>
<td>2.36</td>
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<td></td>
<td>2020</td>
<td>3208</td>
<td>2986</td>
<td>219</td>
<td>113</td>
<td>106</td>
<td>106</td>
<td>60</td>
<td>6.83</td>
<td></td>
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<tr>
<td></td>
<td>2019</td>
<td>3286</td>
<td>3063</td>
<td>223</td>
<td>124</td>
<td>99</td>
<td>95</td>
<td>40</td>
<td>6.79</td>
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<td></td>
<td>2018</td>
<td>3433</td>
<td>3160</td>
<td>262</td>
<td>140</td>
<td>122</td>
<td>119</td>
<td>59</td>
<td>7.63</td>
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<tr>
<td></td>
<td>2018–2022</td>
<td>16,697</td>
<td>15,613</td>
<td>1065</td>
<td>608</td>
<td>457</td>
<td>449</td>
<td>230</td>
<td>2.74</td>
<td></td>
</tr>
</tbody>
</table>

Notes: aThe number of cases means individuals participated in HIV-1 MSM sentinel surveillance program. bHIV antibody-negative cases means those got negative result in twice enzyme immunoassay screening. cHIV antibody-positive cases means those got positive result in twice enzyme immunoassay screening. dPreviously reported cases means those previously reported positive in confirmatory tests and was reported in the China HIV/AIDS case reporting system. eNewly diagnosed cases means those got positive result in confirmatory tests and was not yet reported in the China HIV/AIDS case reporting system. fSamples tested with LAg-Avidity assay means those were test by the LAg-Avidity assay. gRecently infected cases means those were classified as LAg-Avidity positive.
Discussion

To our knowledge, the work first reported a joint application of LAg-Avidity EIA and the spatial analysis to illustrate hotspots in sentinel surveillance in China five years in a row, which will trigger intensive focus of intervention efforts on relevant areas. Furthermore, we first updated trends of HIV-1 prevalence and incidence among MSM during the surveillance period between 2018 and 2022 in Sichuan, which is a continuation and confirmed a supposition of our previous work.\(^{10}\)

Overall, the total prevalence and incidence of five years was 2.74% and 3.69% (95% CI:3.21, 4.16) between 2018 and 2022. Total HIV-1 incidence was estimated to be lower than the national level calculated by meta-analysis.\(^{28}\) Compared to past increasing trend in HIV-1 incidence between 2011 and 2015 among MSM in Sichuan province,\(^{10}\) there was a significant declining trend both in HIV-1 prevalence and estimated incidence (p < 0.001) for 2018–2022. The declines are due in part to progress in diagnosing infections among people living with HIV-1 and ensuring they have access to early, ongoing treatment,\(^{29–31}\) and on the other hand, it is possibly due to the pandemic of SARS-CoV-2. Some studies\(^{32,33}\) suggested a reduction in sexual activity among MSM during that time. But other studies\(^{34,35}\) suggested it may be a temporary reduction. Thus, the consistent, effective HIV-1 prevention strategies should still be taken in relevant areas.

As mentioned in our previous work,\(^{10}\) there was a distinct possibility of a HIV-1 epidemic spread from core city (like Chengdu) to peripheral region among MSM. And this work has confirmed the supposition. Spatial analysis (Figure 3A–E) demonstrated significant clustering in Luzhou city as a hot-spot of recent HIV-1 infection cases for five years with highest estimated HIV-1 incidence, instead of Chengdu. Luzhou, located in southeastern Sichuan Province, is a regional center in the combined region of Sichuan, Yunnan, Guizhou, and Chongqing.\(^{36}\) According to Tobler’s First Law of Geography,\(^{37}\) HIV-1 burden should be similar among neighboring districts than among non-neighbors.\(^{15}\) Surrounded by other high HIV-1 burden provinces\(^{28,38,39}\) may be one reason for its highest estimated HIV-1 incidence. According to Yuan’s report,\(^{40}\) Luzhou did not find strong HIV-1 transmission link with other cities within Sichuan. In these contexts, we supposed Luzhou’s HIV/AIDS epidemics might be more related to neighboring provinces. Therefore, more cross-regional work should be launched from now on, concentrating on the identification of transmission networks of new infection, demographic characteristics and spatial distribution, as well as exploration of the relationship between molecular transmitted cluster and social covariates. The another possible explanation for high estimated incidence was
<table>
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<tr>
<th>Region</th>
<th>Year</th>
<th>The Number of cases</th>
<th>The Number of HIV Antibody-Negative Cases</th>
<th>The Number of HIV 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>Prevalence Rate(%)</th>
<th>Estimated Incidence Rate(%, 95% CI)</th>
<th>Region</th>
<th>Year</th>
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<td>Chengdu</td>
<td>2018–2022</td>
<td>1920</td>
<td>98</td>
<td>12</td>
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<td>83</td>
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<td>4.26</td>
<td>χ²=95.934, P&lt;0.0001</td>
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<td>Dazhou</td>
<td>2018–2022</td>
<td>1863</td>
<td>137</td>
<td>104</td>
<td>33</td>
<td>32</td>
<td>10</td>
<td>1.65</td>
<td>χ²=129.588, P&lt;0.0001</td>
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<td>Deyang</td>
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<td>1876</td>
<td>113</td>
<td>57</td>
<td>56</td>
<td>56</td>
<td>24</td>
<td>2.81</td>
<td>χ²=2.109, 4.32</td>
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<tr>
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<td>2018–2022</td>
<td>1122</td>
<td>138</td>
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<td>14</td>
<td>14</td>
<td>6</td>
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<td>Leshan</td>
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<td>1</td>
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<td>χ²=0.11</td>
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<td>χ²=2.076, 3.23</td>
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<td>1849</td>
<td>147</td>
<td>62</td>
<td>85</td>
<td>84</td>
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<td>4.25</td>
<td>χ²=10.74, 13.10</td>
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<td>2018–2022</td>
<td>1959</td>
<td>92</td>
<td>13</td>
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<td>79</td>
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<td>3.85</td>
<td>χ²=3.83, 5.17</td>
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<td>Yibin</td>
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<td>39</td>
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<td>51</td>
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<td>2.55</td>
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<td>1.49</td>
<td>χ²=1.96, 3.24</td>
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</tbody>
</table>

Notes: *The number of cases means individuals participated in HIV-1 MSM sentinel surveillance program. **HIV antibody-negative cases means those got negative result in twice enzyme immunoassay screening. ***HIV antibody-positive cases means those got positive result in twice enzyme immunoassay screening. ****Previously reported cases means those previously reported positive in confirmatory tests and was reported in the China HIV/AIDS case reporting system. *****Newly diagnosed cases means those got positive result in confirmatory tests and was not yet reported in the China HIV/AIDS case reporting system. ******Samples tested with LAg-Avidity assay means those were test by the LAg-Avidity assay. *******Recently infected cases means those were classified as LAg-Avidity positive.
the policies expanding screening for HIV-1. Some “hidden” HIV-1/AIDS cases would be detected earlier because of scale-up screening. According to Ren’s study, the number of new HIV-1 diagnosed cases showed an increasing trend year by year, which created unique challenges for HIV-1 prevention and treatment, directly leading to potential transmission to others.

Chengdu city, which was once a core HIV-1 hot-spot district among MSM in Sichuan province, had become a secondary hot-spot in this work. Although there was a declining trend from 2018 to 2022 in comparison with data reported earlier, the incidence still remained at a higher level in Chengdu than that in other cities within Sichuan province. The steady decrease in HIV-1 incidence indicated an advance on HIV-1 prevention strategies, but MSM was still a vulnerable group for HIV-1 infection. Chengdu is a labor-importing mega-city. In 2020, Chengdu had 20.94 million resident population and 8.46 million migrant workers from outside, while in 2018, the data was 16.33 million and 2 million, respectively. In view of the fact, consistent, effective HIV-1 prevention and treatment should adequately reached those who could most benefit from them.

Furthermore, Yibin city, Mianyang and Deyang city found high estimated HIV-1 incidence. The geographical position of them made the choice. Transmission networks with strong links were identified among Deyang and Mianyang. So, HIV-1 prevention efforts in those regions may need joint interventions between cities.

The study still has several limitations. First, the result may be biased owing to the limited coverage of MSM sentinel surveillance sites. Sichuan province consists of 21 cities, but our sites were only located 10 cities where MSM is active and easily recruited. So, the results may not be generalized to the whole province without caution. Second, given the nature of a cross-sectional survey, temporality between surveillance period cannot be inferred. For instance, we cannot claim findings of this work representing the whole year. Third, the number of new diagnosed HIV-1 cases among MSM might be affected by the scale-up of HIV-1 antibody testing. In the past five years, HIV-1 testing has expanded greatly in Sichuan, especially in 2018. This expansion could create detection bias, as more cases can be diagnosed because there is more testing done. Fourth, in view of misclassification of the LAg-Avidity EIA, there is a possibility of an overestimate on the incidence. Fifth, due to the stringent SARS-CoV-2 prevention measures, it was not yet clear whether the decline trends of HIV-1 prevalence and incidence were actual or not.

Figure 3 Hot-spots of HIV-1 recent cases at city level by year among MSM between 2018 and 2022. (A) Sichuan province, 2018. (B) Sichuan province, 2019. (C) Sichuan province, 2020. (D) Sichuan province, 2021. (E) Sichuan province, 2022.
Conclusion
Our work identified Luzhou city who replaced Chengdu city as a new hot-spot of recent HIV-1 infection among MSM had an unusually high HIV-1 incidence compared with other sites. It suggested that focused attention, implementation of cross-regional intervention strategies and prevention programs targeting MSM in this key area are urgently required to control the spread of HIV-1. Moreover, this work updated the trends of HIV-1 prevalence and incidence during the surveillance period between 2018 and 2022 among MSM in Sichuan. Both HIV-1 prevalence and incidences among MSM showed a significant declining trend. However, there is an uncertainty regarding the effect of the SARS-CoV-2 pandemic on the trends of HIV prevalence and incidence. Thus, the consistent, effective HIV-1 prevention strategies should still be taken.

Abbreviations
LAg-Avidity EIA, Limiting Antigen Avidity enzyme immunoassay; HIV-1, Human immunodeficiency virus; Men who have sex with men; CDC, Center for Disease Control and Prevention.

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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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