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HIV infection and risk factors among Bangkok prisoners, Thailand: a prospective cohort study

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Abstract

Background: Incarceration has been associated with HIV infection among injection drug users. However, data on HIV risk factors of the inmates during incarceration are rarely reported from Thailand.

Methods: A prospective cohort of 689 male inmates in a Bangkok central prison was studied during 2001–2002. Follow up visits were conducted for 5 months, with testing for HIV and other infections and interviewing of demographics and risk behaviors.

Results: Among 689 male inmates, half (50.9 %) were drug injectors. About 49% of the injectors had injection during incarceration. Most (94.9%) of the injectors had shared injection paraphernalia with others. Successful follow up rate was 98.7% after 2,581 person-months observation. HIV incidence was 4.18 per 100 person – years among all inmates, and 11.10 per 100 person – years among the injection inmates. Multivariate analysis identified variables associated with HIV prevalence: history of injection [OR = 2.30, 95%CI: 1.91–2.77], positive urine opiate test [OR = 5.04, 95%CI: 2.63–9.67], history of attendance to drug withdrawal clinics [OR = 2.00, 95%CI: 1.19–3.35] and presence of tattoos on the body [OR = 1.23, 95%CI: 1.01–1.52].

Conclusions: The main HIV risk factors of Bangkok inmates were those related to drug injection. Harm reduction measures and HIV intervention strategies should be implemented to prevent more spread of HIV among the inmates and into the community.

Background

The problem of human immunodeficiency virus (HIV)

infection and acquired immunodeficiency syndrome (AIDS) is usually approached from a perspective of risk

behaviors and modes of transmission. In Thailand, this approach has been implemented since 1989 for HIV sentinel surveillance of certain high-risk people; e.g. commercial sex workers, injection drug users and male clients attending sexually transmitted diseases clinics [1]. As far as interventions are concerned, such an approach leaves certain target groups not presenting at the usual settings unaccounted for. For instance, men who have sex with men do not have "well-defined" physical places where they usually meet and are less likely to receive targeted HIV-1 related interventions. On the other hand, certain HIV high-risk groups in well-defined but restricted settings are also left out from interventions they deserve. Inmates are an example of the left-out population. HIV-1 seroprevalence of the inmates in the United States, Brazil and Scotland have been reported at 20.3, 13.7 and 5.8%, respectively [2-4]. No such data of Thai inmates have been reported so far, however. The lack of information from inmates may be due to several reasons. First, inmates are sensitive people to study and most investigators find it difficult to gain public acceptance and access to this group. Second, inmates are marginalized people and generally receive less attention than other main stream groups. Third, public health officials believe that interventions towards this group are generally less effective and, sometimes, less efficient than other groups because of their tendencies to resist interventions and/or their lack of freedom to choose healthy environments to live. Under these conditions, not only do the inmates not receive appropriate interventions they deserve, but the general public also loses the benefits they may learn from studies in the inmates. We therefore carried out a study in a prison in central Thailand. We determined factors associated with HIV seropositivity among the inmates, studied other concomitant infections, verified if there were continued uses of illegal drugs and continued homosexual practices in the prison, and explored new HIV infections in the prison.

Setting

The study was conducted in the Medical Correctional Institution of the Klong Prem Central Prison, Bangkok, Thailand. The prison is the largest one in Thailand and served 7,177 inmates in 2002 [5]. The 300-bed hospital of the prison treats approximately 250 inmates daily. Most of them seek treatments for common respiratory tract infections.

Methods

Participants and Follow-ups

Over the period of 15 months (between June 2001 and August 2002), about 1,000 male inmates were approached and invited to join the study. A total of 689 male inmates was eligible and agreed to be part of the study. To be eligible for the study, the inmates must be

between 20-50 years old at the enrolment, have at least 5 years of remaining sentence term from the date of study, not be seriously ill, and pass a test on basic knowledge about HIV/AIDS. The test on basic HIV knowledge was required as a part of participant's enrolment to ensure that the study did not enroll those who are totally ignorant about HIV and might not understand the significance of their participation in the project. The staff of the project explained the objectives and the nature of the study to the potential participants. If they fitted the eligibility criteria and agreed to join the study, a consent form was signed. They were then interviewed about their demographics and information related to their incarceration. There were no potential participants who did not pass the basic test on HIV knowledge. HIV risk behaviors before and during incarceration were ascertained at the date of enrolment. The risk behaviors were verified whether they were presented before the incarceration (Yes or No) and whether they were continued or initiated during the incarceration (Yes or No). The responses were then categorized into "No/No" (never), "Yes/No" (before incarceration only), "Yes/Yes" (continued into incarceration), and "No/Yes" (initiated during incarceration).

Blood was taken for determination of HIV-1 and other infectious profiles, complete blood count and blood chemistry. Urine specimens were collected for determination of opiates and metamphetamine. Pre-test and post-test counseling for anti-HIV testing was given to all inmates by experienced nurses.

The follow-up period was 5 months. As a part of experimentation of follow-up schedules, 500 inmates (72.6%) were followed up only once at the end of 5 months. The other 189 (27.4%) were followed up monthly for 5 times during the study period. The rationale behind more frequent visits in a part of the participants is to enable us to detect early HIV seroconverters (if any) and to study these early seroconverters in more details. However, financial limitation of the project did not allow us to follow up all participants on a monthly basis. For those who were followed up on a monthly basis, they were interviewed, counseled, physically examined, and blood tested at each follow-up visit as they were at baseline. Risk behaviors were assessed at the baseline and at the 5-month visit only. In this study, the results of anti-HIV testing were kept confidential and were not used to separate anti-HIV positives from those who were negative. At the time of the study, there were no programs that provide prophylaxis for opportunistic infections or offer anti-retroviral for HIV-infected prisoners.

There were no reported incidents of adverse consequences associated with notifying HIV seropositives or seroconverters of their status. We also specifically asked, at the end

of the study, if the participants experienced such adverse consequences during their participation in the study and none reported such experiences.

Data management and statistical analysis

All personal data of the inmates including their identification, demographics, risk behaviors and laboratory findings were kept confidential. Codes were used to identify these subjects. All data were double entered by the investigators, using Microsoft Access version 97 (Microsoft Corporation, New York, USA). Potential HIV risk factors were examined using univariate and multivariate analyses. For association of categorical variables, Yate's corrected chi square test was used, except where the expected frequency is less than 5 and Fisher's exact test is recommended. Variables that were found statistically significant in the univariate analysis and biologically plausible, as determined by prior knowledge and suggested by literature, were included in the multivariate analysis. Multiple logistic regression analysis was used with anti-HIV status as the outcome variable (Intercooled version 6, Stata Corporation College Station, Texas, USA). Maximum likelihood ratio estimation was used to estimate the parameters and the goodness of fit was applied to assess various models during the model-fitting process. Likelihood ratio (LR) test was applied to assess statistical significance. Both crude odds ratio and adjusted odds ratios (ORs), with associated 95% confidence intervals (CIs) and p-values, were presented.

Drug use risks were assessed based on the inmates' injection history, history of attending drug abuse treatment clinic, and urine test for opiates at the time of enrolment. Injection history was further assessed based on duration of injection, presence or absence of infection scar(s), and history of sharing drug injection paraphernalia.

HIV seroconversion rate was also calculated among the inmates who were HIV negative at enrolment and turned HIV-positive during the follow-up period. Poisson estimation was used to determine the rate and it's associated 95% confidence interval.

Laboratory tests

At the enrolment, the subjects were blood tested for Anti-HIV antibody by HIV EIA (Uni-Form II plus O, Organon Techinka, Boxtel, The Netherlands) with HIV-1 confirmation by Western blot (HIV Blot 2.2, Gene Lab Diagnostic Pte Ltd., Science Park, Singapore). The hepatitis B profiles included HBsAg, HBc and HBs antibody EIA (ETI-MAK-4, ETI-AB COREK-2 and ETI-AB-AUK-3, Diasorin s.r.1, Vercelli, Italy). The hepatitis C antibody was determined by an EIA kit (ETI-AB-HCVK-3, Diasorin s.r.1, Vercelli, Italy). The syphilis serology included VDRL, and TPHA (VDRL, Syphscreen RPR, Porton Cambridge, Kennett, United

Table 1: Demographic characteristics and laboratory test results of 689 male inmates at the enrolment, Bangkok Thailand, 2001–2002.

Characteristics	Number (%)*
Age (years)	
Mean ± SD	32.3 ± 6.7
Median (Range)	32(20–50)
Marital status	
Single	293 (42.6)
Married	237 (34.5)
Divorced, separated and widowed	157 (22.9)
Home town	
Bangkok and its suburbs	334 (48.5)
Other locations in Thailand	310 (45.0)
Outside Thailand (foreigners)	45 (6.5)
Highest education level	
Primary	354 (52.3)
Secondary or Higher	323 (47.7)
Occupation before incarceration	
No occupation	44 (6.4)
Labor	463 (67.2)
Private business	116 (16.8)
Others	66 (9.6)
Monthly income (Baht) excluding the unemployed ^{†**}	
Mean ± SD	12,788 ± 25,157
Median(Range)	6,000 (330–250,000)
Type of offense	
Narcotic-related	298 (45.9)
Life- or sex-offended	179 (27.6)
Theft or robbery	172 (26.5)
Term of sentence (years)	
Mean ± SD	18.9 ± 8.4
Median (Range)	18(5.7–30.0)
Duration of sentence already served (years)	
Mean ± SD	5.8 ± 3.9
Median(Range)	5.0(1.0–18.0)
Laboratory profiles (% positivity)	
Anti HIV antibody	175 (25.4)
Anti HCV antibody	309 (44.9)
HBs Antigen	77 (11.2)
Anti HBc antibody	442 (64.2)
Anti HBs antibody	286 (41.5)
Anti Chlamydia IgM antibody	140 (20.3)
VDRL with TPHA confirmation	22 (3.2)
Anti HSV2 IgM antibody	72 (10.4)
Urine opiates	78 (11.3)
Urine metamphetamines	42 (6.1)

Note: *The total numbers may not add up to 689 because of no responses from some participants. **1 US dollar = 43 Baht (approximately)

Kingdom and Syphilis TPHA tests, Human, Wiesbaden, Germany). In addition, anti HSV-2 IgM EIA (Capita HSV-2 IgM, Trinity Biotech, New York, USA) and anti-chlamydia IgM EIA (Sero ELISA chlamydia true IgM, Savyon Diagnostics Ltd., Ashdod, Israel) were also carried out.

Urine was tested for opiates and metamphetamine (Capita EIA test kit, Trinity Biotech, New York, USA). Complete blood count was analyzed by an automated analyzer (Counter-HmX, Florida, USA). Blood chemistry profiles included liver function test, lipid and renal profiles and were tested by an automated analyzer (Cobas Mira, Roche Boehringer Mannheim, Mannheim Germany). All laboratory tests were performed only at the enrolment, except for HIV and HCV serology that were tested at every visit.

Ethical Review

The Ethical Review Committee for Research in Human Subjects of the Ministry of Public Health of Thailand approved the study protocol in March 2001.

Results

Demographic characteristics, risk behaviors and laboratory results

During the study period, there were 689 male inmates who were eligible and agreed to join the study. Most of them are young with the median age of 32 years. Nearly half are single and are residents of Bangkok and its suburbs. Only half have highest education at primary level. Before incarceration, 62.7% of the inmates were labor workers with the median monthly income of 6,000 Thai Baht (about 136 USD). About 43% were incarcerated for narcotic-related offenses, with the median sentence term of 18 years. About 62.4% of the inmates had been incarcerated for 5 years or more. Details of their demographic data are illustrated in table 1.

HIV risk behaviors before and during incarceration

Potential HIV risk behaviors of the inmates are described in table 2. Among the 689 inmates, 351 (50.9%) reported drug injection before or during incarceration or both. Out of the 351 injectors, 83 (15.6%) injected drug before incarceration only, 80 (15.9%) initiated injection while incarcerated and 188 (53.6%) continued drug injection into the incarceration. Nearly all (333 or 94.9%) injectors had an experience of sharing injection paraphernalia with other injectors. Among the 333 inmates who shared injection, 74 (22.2%) shared equipment before incarceration only, 92 (27.6%) initiated sharing equipment during incarceration and 167 (50.2%) continued sharing behaviors while incarcerated. Nearly half (161 or 45.9%) of the 351 injectors had injection scars on their bodies and 78 (22.2%) of the injectors had positive urine opiate test. Tattooing and sharing razor blade were also common risk behaviors in the inmates. Receiving blood transfusion was not very frequent (7.8%) among the inmates.

Sexual risks of the 689 male inmates before incarceration were mainly heterosexual ones. Most of them (81.4%) visited prostitutes at least once. About 80% reported having sex with non-wife women. More than 95% reported experiences of unprotected sex. More than one quarter of the inmates reported sex with men. Among those who had sex with men, more than 80% continued into the incarceration or initiated during the incarceration. Since condom is practically unavailable in the prison, most of this kind of sex is likely to be unprotected.

Table 2: HIV risk factors before and during incarceration of 689 male inmates, Bangkok Thailand, 2001 – 2002.

Risk factors	Risk before and during incarceration, Number (%)				p-values
	No/No	Yes/No	No/Yes	Yes/Yes	
Heroin injection	338(49.1)	83(12.0)	80(11.6)	188 (27.3)	<0.001
Injection risk among 351 heroin injectors					
- Sharing injection paraphernalia	18 (5.1)	74(21.1)	92(26.2)	167 (47.6)	0.007
- Presence of injection scar(s) on the body*	190 (54.1)	161(45.9)			
- Positive urine opiates*	273 (77.8)	78(22.2)			
- Having attended withdrawal clinics*	240 (63.4)	111(36.6)			
- Injection duration >7 years*	205 (58.4)	144(41.0)			
Presence of tattoos	228 (33.1)	127 (18.4)	168 (24.4)	166(24.1)	0.003
Sharing razor blades with others	150 (21.8)	31 (4.5)	211 (30.6)	297(43.1)	<0.001
Ever receiving blood transfusion	635 (92.2)	51 (7.4)	1 (0.2)	2(0.3)	0.017**
Ever visiting prostitute*	128 (18.6)	561 (81.4)			
Having sex with non-wife women*	144 (20.9)	545 (79.1)			
Having sex without condom	21(3.8)	531(79.1)	7(1.0)	130(18.9)	0.652
Having sex with men	507(73.6)	40 (5.8)	78(11.3)	64 (9.3)	<0.001
Having sexually transmitted diseases	393 (57.0)	288(41.8)	1 (0.2)	7 (1.0)	0.024**

Note: No/No = Never, Yes/Yes = Continued into incarceration, Yes/No = Before incarceration only, No/Yes = Initiated during incarceration
 *These risk behaviors were ascertained at the time of enrolment. **Fisher's exact test

Table 3: Univariate analysis of HIV risk factors among 689 male inmates, Bangkok Thailand, 2001–2002.

Factors	Anti-HIV positivity N (%)	Unadjusted OR	95 % CI	p-value
Demographics				
Age (years, median)				
≤ 32	93/378 (24.6)	1.00		
>32	82/311 (26.4)	1.10	0.78–1.55	0.60
Marital status				
Single	74/293 (25.3)	1.00		
Ever married	101/396 (25.5)	1.01	0.72–1.43	0.94
Home town				
Bangkok/suburbs	81/334 (24.3)	1.00		
Other places	94/355 (26.5)	1.12	0.80–1.56	0.50
Highest education level				
Primary	89/347 (25.7)	1.00		
Secondary or higher	79/323 (24.5)	0.94	0.66–1.33	0.72
Employment				
Unemployed	13/44 (29.6)	1.00		
Employed	162/645 (25.1)	0.80	0.41–1.55	0.51
Monthly income excluding the unemployed (Baht, median)				
≤ 6000	72/326 (22.1)	1.00		
> 6000	81/297 (27.3)	1.32	0.92–1.91	0.13
Type of offense				
Narcotic-related	82/298 (27.5)	1.00		
Others	93/391 (23.8)	0.82	0.58–1.16	0.26
Term of sentence (years, median)				
≤ 18	45/150 (30.0)	1.00		
> 18	44/148 (29.7)	1.00	0.60–1.62	0.96
Duration of sentence already served (years, median)				
≤ 5	59/185 (31.9)	1.00		
>5	30/111 (27.0)	0.79	0.47–1.33	0.78
Blood-related risk				
History of heroin injection				
- Never	13/338 (3.9)	1.00		
- Before incarceration only	20/83 (24.1)	7.94	3.80–16.58	<0.001
- Continued into incarceration	99/188 (52.7)	27.81	15.00–51.48	<0.001
- Initiated during incarceration	43/80 (53.8)	29.05	14.42–58.49	<0.001
Urine opiate test				
Non-reactive	117/611 (19.2)	1.00		
Reactive	58/78 (74.4)	12.24	7.12–21.05	<0.001
Ever attending withdrawal clinic				
Never	109/578 (18.9)	1.00		
Ever	66/111 (59.5)	6.31	4.10–9.71	<0.001
Tattooing				
- Never	17/228 (7.5)	1.00		
- Before incarceration only	34/127 (26.8)	4.54	2.43–8.47	<0.001
- Continued into incarceration	62/166 (37.4)	7.40	4.14–13.21	<0.001
- Initiated during incarceration	62/168 (36.9)	7.26	4.06–13.00	<0.001
Sharing razor blades				
Never	27/150 (18.0)	Referent		
Ever	139/538 (25.8)	1.72	1.09–2.72	0.019
Receiving blood transfusion				
Never	162/635 (25.5)	Referent		
Ever	13/54 (24.1)	0.93	0.49–1.76	0.816
Risk factors among 351 injectors only				
Sharing injection paraphernalia				
No	5/19 (26.32)	Referent		
Yes	157/332 (47.29)	2.51	0.92–6.85	0.074
Injection scars				
No	59/190 (31.05)	Referent		
Yes	103/161 (63.98)	3.94	2.53–6.15	<0.001

Table 3: Univariate analysis of HIV risk factors among 689 male inmates, Bangkok Thailand, 2001–2002. (Continued)

Injection duration (years, median)				
≤ 7	73/207 (35.27)	Referent		
>7	89/144 (61.81)	2.97	1.91–4.61	<0.001
Sexual risks				
Visiting prostitute before incarceration				
Never	39/128 (30.5)	Referent		
Ever	136/561 (24.2)	0.73	0.48–1.11	0.144
Sex with non-wife women				
Never	44/143 (30.8)	Referent		
Ever	131/546 (24.0)	0.71	0.47–1.06	0.098
Unprotected sex (sex without condom)				
Never	14/20 (70.0)	Referent		
Ever	16/1669 (24.1)	0.14	0.05–0.35	<0.001
Sex with men				
- Never	123/506 (24.3)	Referent		
- Before incarceration only	13/40 (32.5)	1.50	0.76–2.97	0.249
- Continued into incarceration	20/64 (31.2)	1.42	0.81–2.48	0.228
- Initiated during incarceration	19/79 (24.0)	0.99	0.57–1.71	0.960
Having sexually transmitted diseases				
Never	102/393 (26.0)	Referent		
Ever	73/296 (24.7)	0.93	0.66–1.32	0.700

HIV-1 positivity and risk factors

The overall anti-HIV positivity was 175/689 or 25.4% (95%CI: 22.0 – 28.6%). In the univariate analysis as shown in table 3, demographic characteristics, incarceration-related factors, and most sexual risk behaviors (except for unprotected sex) were not statistically associated with HIV positivity.

Strong association with HIV positivity was observed in the inmates who engaged in drugs injection, had reactive urine opiate test, attended withdrawal clinics, had tattoos and shared razor blade and had unprotected sex (p< 0.05). Higher HIV positivity was observed more among the inmates who initiated or continued such risks during the incarceration than those who declared no such risks at all or had risks only before incarceration.

Among the 351 injectors, HIV positivity was associated with history of sharing injection equipment, presence of injection scar(s), and injection duration more than 7 years.

After being adjusted for age in the multiple logistic regression analysis, variables found to remain statistically significant are injection history, history of attending drug withdrawal clinic, urine opiates, and body tattoos. The adjusted odds ratios and associated confidence intervals are shown in table 4.

HIV seroconversion rate

Out of the 689 inmates, 166 (25.4%) were anti-HIV positive at the enrolment. The remaining 523 inmates were

Table 4: Multiple logistic regression analysis of HIV risk factors among 689 male inmates, Bangkok Thailand, 2001–2002.

Factors	Adjusted OR	95% CI	p-values
Age	0.99	0.96–1.02	0.480
Injection history*			
No	1.00		
Yes	2.30	1.91–2.77	<0.001
History of attending withdrawal clinic			
No	1.00		
Yes	2.00	1.19–3.35	0.009
Positive urine opiate test			
No	1.00		
Yes	5.04	2.63–9.67	<0.001
Presence of tattoos on the body			
No	1.00		
Yes	1.23	1.01–1.52	0.048

* Injection history included information of injection before or after incarceration, presence or absence of injection scars, history of sharing injection paraphernalia and injection duration.

followed-up for a period of 5 months. Successful follow-up rate was 98.7%. During the follow up period (2,581 person-months), 9 inmates got HIV seroconversion, corresponding to the estimated HIV-1 incidence of 4.18 per 100 person-years (95% CI: 4.11 – 4.26 per 100 person-years). All 9 HIV incident cases were injectors. When we restricted the calculation only to the injectors, the HIV-1 incidence would be 11.10 per 100 person-years (95% CI:

10.89–11.31 per 100 person-years) during 973 person-months of observation.

Other infectious markers and drug uses

The seroprevalence of certain infectious diseases and drugs are shown in table 1. Among the 689 inmates, high prevalence of hepatitis viral infections was observed as follows: 309 (44.85%) for anti HCV, 77 (11.18%) for HBV s antigen, 286 (41.51%) for anti HBs, and 442 (64.15%) for anti HBc. There were 140 inmates (20.32%) who were reactive for Chlamydia IgM.

Other sexually transmitted diseases than HIV-1 were also studied, e.g. 72 (10.45%) reactive for HSV-2 IgM and only 22 (3.19%) reactive for Syphilis with TPHA confirmation.

In addition, continued use of illegal drugs inside the prison was observed, e.g. 78 (11.82%) for opiates and 42 (6.1%) for metamphetamaine as determined by urine testing.

Discussion and conclusion

HIV-1 prevalence of these Bangkok inmates was 25.4% which is quite high but comparable to that of 20% in the US and South African prisons but higher than that of 13.7% in Brazilian prisons [2,6,3]. HIV prevalence of the inmates with drug injection and sharing injection equipment while incarcerated was reported to be as high as 53–57% in several studies including the rate of 53.4% in central Thailand that was reported in HIV-1 sentinel surveillance in the year 2000 [1].

The overall HIV-1 seroconversion rate of the inmates in this study is 4.18 per 100 person years, which is comparable to that of Bangkok injection drug user [7]. However, HIV-1 incidence rate of 11.10 per 100 person years among the inmates with injection risk was observed. This is about one-third of the rate of 35 per 100 person-years in Bangkok injection drug users who continued drug injection while incarcerated [8] and 31.3 per 100 person-years among injection drug users in the north Thailand [9]. Such substantially lower HIV-1 incidence rates in this study are probably due to scarcity of the abuse drugs and injection equipment, differences in sentence term, prohibition of all kinds of sexual behaviors inside the prison and limitation on follow-up time and number of participants. More studies may be needed to clarify these issues.

Despite the limitations imposed on the inmates, some of them continued risk behaviors into the incarceration or initiated such behaviors during the incarceration. Such practices remain them at the high risk for HIV and other infections.

In this study, high burdens of infections other than HIV-1 among the inmates were also observed, e.g. HCV and HBV infections. Such high risk of hepatitis viral infections were consistent with previous reports in Brazilian prisons (41% for HCV and 68.1% for HBV) and in Spanish prisons (42.5% for HCV and HBV co-infections), but the rates were considerably lower than those in Danish prisons (64% for HCV and 87% for HBV) and among Austrian imprisoned injection drug users (75% for HCV and 68% for HBV) [3,10–12].

Inmates are still at high risk for HIV and need more attention. Firstly, inmates are still at risk of sex transmission of HIV despite their physical containment. As a matter of fact, high heterosexual risks and sexual transmitted diseases among injection drug users are recently reported in Vancouver injection drug users [14]. Secondly, inmates will be part of the outside society once they finish their term in prison. Understanding their risks of HIV and injection drug use and providing proper interventions offer benefits not only to the inmates, their families and their partners, but also to the public health of the communities to which they will return. Finally, these inmates are neglected people. Drug treatment and HIV intervention programs for the inmates received only 5,910,000 Thai Baht (about 137,405 USD) for 247,415 inmates for the whole country [15]. With the estimated number of about 60,000 HIV-positive inmates and almost 100,000 inmates who continue use of drug during incarceration, this amount of budget is certainly fall short of need.

Competing interests

None declared.

Authors' contributions

All authors read and approved the final manuscript and contributed equally to the manuscript.

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