HHS Public Access

Author manuscript

AIDS Behav. Author manuscript; available in PMC 2019 July 01.

Published in final edited form as:

AIDS Behav. 2018 July ; 22(Suppl 1): 70–75. doi:10.1007/s10461-018-2052-2.

Characterization of HIV Recent Infection Among High-Risk Men at Public STI Clinics in Mumbai

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Abstract

We examined associations with HIV recent infection and estimated transmitted drug resistance (TDR) prevalence among 3345 men at sexually transmitted infection clinics in Mumbai (2002–2005). HIV seroincidence was 7.92% by the BED-CEIA and was higher at a clinic located near brothels (12.39%) than at a hospital-based clinic (3.94%). HIV recent infection was associated with a lifetime history of female sex worker (FSW) partners, HSV-2, genital warts, and gonorrhea. TDR prevalence among recent infection cases was 5.7%. HIV testing services near sex venues may enhance case detection among high-risk men who represent a bridging population between FSWs and the men's other sexual partners.

Keywords

HIV; Recent infection; Sexually transmitted infections; High-risk men; Female sex workers; Transmitted drug resistance

Introduction

In India, more than 2 million persons are estimated to be living with HIV [1, 2]. More than 80% of HIV infections are believed to be transmitted through sexual exposure [2]. In Mumbai, HIV prevalence was 36.8% among female sex workers (FSWs) and 21.2% among sexually transmitted infection (STI) clinic patients in 2006 [3]. Previous studies suggest that many male patients at STI clinics acquire HIV infection from FSWs [4, 5].

Laboratory methods for the characterization of HIV recent infection can help target prevention activities among key populations such as FSWs and their male clients.

Compliance with Ethical Standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

"Serological testing algorithm for recent HIV seroconversion" (STARHS) and BED immunoglobulin G capture EIA (BED- CEIA) are laboratory approaches that distinguish between recent and long-standing infections based on immunological changes in HIV antibody levels during the first few months following infection [6, 7].

HIV drug resistance testing can guide the selection of antiretroviral drugs effective against the HIV strain(s) a patient carries. The predominant circulating HIV strains in India are subtype C viruses, which account for more than 95% of infections [8]. Most algorithms for HIV drug resistance interpretation were developed from analyses of subtype B isolates; however, natural polymorphisms between sub-types can affect interpretations [9].

In the present analysis, we examined the demographic, behavioral, and clinical factors associated with HIV recent infection and estimated prevalence of transmitted drug resistance (TDR) among men who presented at STI clinics in Mumbai.

Methods

We performed a secondary analysis using baseline data from an HIV/STI behavioral intervention trial conducted at 2 Mumbai public STI clinics. Men who presented at the Bombay Municipal Clinic (BMC) and the Sion Hospital Outpatient Dermatology Clinic (Sion) between 2002 and 2005 were enrolled if they were aged ≥ 16 years, complained of STI symptoms, reported unprotected sex in the past 3 months, or requested an HIV test. BMC is a stand-alone STI clinic located near many brothels, and Sion is a dermatology clinic located at a major public health teaching hospital that provides care mainly for men from the nearby slum.

Participants received HIV testing, pre- and post-test counseling, a physical exam, and evaluation and treatment for STIs. They also completed an interviewer-administered questionnaire on demographic characteristics and sexual history. HIV-1 infection was evaluated at the STI clinics by enzyme immunoassays (EIA) (Biokit Elisa, Lab-systems, Helsinki, Finland) or by a rapid-test algorithm based on national guidelines (Biokit, Werfen Group, Barcelona, Spain; Tri-Dot, J. Mitra & Co Pvt. Ltd, New Delhi, India); positive samples were confirmed by Western blot (Chiron RIBA*HIV-1/HIV-2 SIA, Ortho Clinical Diagnostics, Emeryville, California). STI diagnoses based on an algorithm combining test results, clinical history, and physical exam have been described in an earlier publication [10]. In brief, laboratory testing for STIs included serologic detection of syphilis and HSV-2, and polymerase chain reaction (PCR) detection of gonorrhea, chlamydia, and chancroid. Primary syphilis was defined based on the presence of a genital ulcer on exam, with a clinical diagnosis of a primary chancre confirmed by laboratory testing. Secondary syphilis was defined based on signs of secondary disease or by laboratory test result. Latent syphilis was defined as a positive laboratory test result in the absence of clinical manifestation. Incident HSV-2 was defined based on the presence of a genital ulcer or vesicles on exam, a positive PCR result, and the absence of HSV-2 IgG antibodies. Recurrent HSV-2 was defined based on the presence of a genital ulcer or vesicles on exam, a positive PCR or serology result, and the presence of HSV-2 IgG antibodies. Chronic HSV-2 was defined based on the presence of HSV-2 IgG antibodies in the absence of a clinical diagnosis and, if

in the presence of a genital ulcer, a negative PCR result. The study received approval from the University of California, San Francisco (UCSF) and Sion Municipal Medical College institutional review boards.

BED-CEIA testing was conducted retrospectively on frozen HIV-positive serum specimens, applying a 0.8 optical density threshold and the recommended 197-day conversion window period for subtype C viruses [7]. Seroincidence estimates were calculated using an adjustment formula that corrects for the misclassification of duration of infection as a result of low antibody levels during very early infection and late-stage disease, persons with long-standing infections who never evolve high antibody levels, and HIV-positive samples not available for recent infection testing [7].

HIV viral genotyping was conducted using frozen serum samples from treatment-naive recent infection cases (Trugene HIV-1 Genotyping Kit) to estimate TDR prevalence. Sequence results were interpreted using guidelines from the manufacturer, the HIV Drug Resistance Database, and the International Antiviral Society-USA, as there can be variations between guideline versions [11, 12].

Participants with available questionnaire data, baseline HIV results, and stored specimens were included in this analysis. HIV recent infection cases were compared with HIV-negative cases. Bivariate logistic regression models were used to assess associations between risk factors and HIV recent infection, and to generate odds ratios (OR) and 95% confidence intervals (CI). A purposeful selection technique was used to create the multivariable model. All covariates in the bivariate analyses associated with recent infection with Wald test $p < 0.25$ were included initially. Covariates then were excluded in a backwards stepwise fashion, retaining those associated with $p \leq 0.10$. Any covariates initially excluded were individually re-included in this model, and their association reassessed. Covariates associated with $p \leq 0.10$ were retained in the multivariable model. Model fit was assessed using standardized Pearson Chi square test.

Results

There were 3345 participants enrolled, of which 47 men did not have baseline HIV testing results, and 142 men who tested HIV-positive did not have specimens available for BED-CEIA testing; these participants were excluded from this analysis. Of the remaining 3156 men, BED-CEIA testing was conducted on 353 specimens identified as HIV- positive at baseline by the local laboratories. At the initial testing phase, 31 specimens had optical density values < 0.3, and confirmatory retesting by standard EIA determined that 15 specimens were false positives; these cases were re-classified as HIV-negative. Thus, 338 cases were classified as HIV-positive for this analysis: 207 from BMC and 131 from Sion.

Ninety-eight of the 338 HIV-positive cases were characterized as recent infections: 70 at BMC and 28 at Sion. The calculated HIV-1 seroincidence was 7.92% [95% CI 5.66, 10.18] and was higher at BMC than Sion (12.39% [95% CI 8.40, 16.38] vs. 3.94% [95% CI 1.94, 5.94]; $p < 0.001$).

The final analysis included 2916 men, of whom 2818 were HIV-negative and 98 were recently-infected. Associations between HIV recent infection and demographic, clinical, and behavioral characteristics are presented in Table 1. Being a BMC patient ($p = 0.01$), having > 10 life-time FSW partners ($p = 0.01$), moderate/high perceived risk for HIV infection ($p = 0.02$), recurrent and chronic HSV-2 ($p < 0.01$), genital warts ($p < 0.01$), and gonorrhea ($p < 0.01$) were associated with higher likelihood of HIV recent infection; being Muslim was associated with lower likelihood ($p < 0.01$).

Sixty-two recent infection cases had sufficient specimen volume for HIV-1 drug resistance testing, and 35 cases yielded interpretable sequences. Two cases had major resistance mutations detected: RT M184V in one case, and RT K101E in the other. TDR prevalence was estimated to be 5.7% (2 of 35 cases) using the 3 drug resistance interpretation guidelines. The most common minor mutations detected were those conferring possible resistance to protease inhibitors, specifically H69K (97%), I93L (94%), L89M (91%), and M36I (69%). The mutation combination of K20R, M36I, and H69K was present in 6 cases, which was interpreted as conferring possible resistance to the protease inhibitor tipranavir, based on the Trugene and the International Antiviral Society-USA guidelines that would have been used at the time of the trial.

Discussion

HIV seroincidence among men seeking care at the 2 STI clinics in Mumbai was high. The 7.92% seroincidence detected is similar to the 7.2% found among male STI clinic attendees in Pune a few years prior to this study [13]. The association between HIV recent infection and HSV-2 supports the premise that vesicular and ulcerative lesions during HSV-2 reactivation increase susceptibility to HIV [14]. Men who perceived themselves at moderate or high risk for HIV were more likely to be recently infected, suggesting an awareness of their risky behaviors. A high proportion of men reported a lifetime history of sex with FSWs, consistent with data from other studies [13].

TDR prevalence was low among HIV recent infection cases, which may be due to the limited availability of antiretroviral drugs in India from 2002 to 2005, when participants were enrolled for the study. Two of 3 drug resistance interpretation algorithms regard mutations at positions 20, 36, and 69 to be associated with resistance to tipranavir, even though these polymorphisms are wild-type for subtype C viruses. In this setting, where subtype C viruses are pre-dominant, common natural polymorphisms adversely impact HIV-1 drug resistance interpretations, an observation found in previous studies [15]. This highlights the importance of considering the genetic background and prevalent wild-types when applying drug resistance algorithms to non-B viruses to ensure accurate interpretations.

Data from the STI clinic attendees in this study may not be generalizable to men seeking care at other STI clinics or within the general population. There were limitations in the availability and quality of specimens for recent infection and drug resistance testing. For recent infection testing, we had access to specimens for only 70% of cases originally classified as HIV-positive, and therefore used the correction formula to adjust for

misclassifications due to missing specimens. Other studies reporting similar challenges with missing specimens also used the correction formula [16, 17]. There was insufficient specimen volume available to conduct drug resistance testing for one-third of recent infection cases. Additionally, frozen serum specimens were used for drug resistance testing, as plasma specimens were not available, which likely contributed to the high percentage of non-interpretable sequences.

Although participants were recruited in the mid-2000s, these findings are still relevant. HIV prevalence among FSWs in the state of Maharashtra has declined markedly, from the high levels at the time of the trial (54% in 2003, 42% in 2004, 24% in 2005) to 7% in the 2010–2011 sentinel surveillance report; however, this key population still represents a risk for onward HIV transmission [18]. Although antiretroviral drugs used in current treatment regimens may differ from those used during the study period, the interpretation of resistance patterns still needs to consider subtype- specific natural polymorphisms.

Conclusions

HIV seroincidence and STI prevalence were higher among the men at the STI clinic located near brothels than at the hospital-based clinic. High-risk men seeking care at STI clinics likely represent a bridging population between the key population of FSWs and other sexual partners of the men. Increasing HIV testing access would help identify previously undiagnosed FSWs and their male clients, thereby furthering efforts to reach the UNAIDS 90–90-90 treatment targets [19]. Prevention efforts should therefore consider providing HIV testing services near commercial sex venues and promoting frequent testing by FSWs and their male clients in order to diagnose HIV cases at an early stage of infection, which would enable earlier linkage to care and treatment as well as prevention of onward transmission.

Acknowledgments

Funding This study was funded by the National Institute of Allergy and Infectious Diseases (NIH R01 AI043914, PI: C.P. Lindan), UCSF AIDS Research Institute and the Carl L. Gaylord Estate (PI: H.M. Truong) and UCSF International Traineeships in AIDS Prevention Studies (NIH R25 MH064712; PI: G. Rutherford).

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Table 1

Associations between HIV recent infection and demographic characteristics, risk behaviors and sexually transmitted infections (STIs) among men at the Bombay Municipal Clinic (BMC) and Sion Hospital Outpatient Dermatology Clinic (Sion), Mumbai, 2002–2005

	All ***N*** **(%)**	**HIV-negative** ***N*** **(%)**	**HIV recent infection** ***N*** **(%)**	**Odds ratio (95% CI)**	***p*** **value**	**Adjusted odds ratio (95% CI)**	***p*** **value**
Overall	2916	2818	98				
Site							
Sion	1519 (52.1)	1491 (52.9)	28 (28.6)	ref		ref	
BMC	1397 (47.9)	1327 (47.1)	70 (71.4)	2.81 (1.80,4.38)	< 0.01	1.90 (1.15, 3.14)	0.01
Age							
16–25 years	1470 (50.5)	1431 (50.8)	39 (40.2)	ref		-	
26–35 years	919 (31.6)	880 (31.3)	39 (40.2)	1.63 (1.04,2.55)	0.04	-	
≥ 36 years	523 (18.0)	504 (17.9)	19 (19.6)	1.38 (0.79,2.42)	0.25	-	
Time residing in Mumbai							
0–5 years	1061 (36.7)	1038 (37.1)	23 (24.2)	ref		-	
≥ 6 years	1830 (63.3)	1758 (62.9)	72 (75.8)	1.85 (1.15,2.97)	0.01	-	
Living situation							
Flat/apartment/chawl	928 (31.9)	903 (32.1)	25 (25.8)	ref		-	
Slum/other	1983 (68.1)	1911 (67.9)	75 (74.2)	1.36 (0.86,2.16)	0.19	-	
Marital status							
Married	893 (30.7)	863 (30.7)	30 (30.9)	ref		-	
Single	1804 (62.0)	1744 (62.0)	60 (61.9)	0.99 (0.63, 1.55)	0.96	-	
Divorced/separated/ widowed	214 (7.4)	207 (7.4)	7 (7.2)	0.97 (0.42, 2.25)	0.95	-	
Current employment status							
Employed	2822 (96.9)	2728 (96.9)	94 (96.9)	ref		-	
Unemployed	90 (3.1)	87 (3.1)	3 (3.1)	1.00 (0.31, 3.22)	1.00	-	
Religion							
Hindu/Sikh	1873 (64.4)	1793 (63.8)	80 (82.5)	ref		ref	
Muslim	819 (28.2)	811 (28.9)	8 (8.3)	0.22 (0.11, 0.46)	< 0.01	0.19 (0.08, 0.45)	< 0.01
Buddhist/Jain/Christian	215 (7.4)	206 (7.3)	9 (9.3)	0.98 (0.48, 1.98)	0.95	1.07 (0.49, 2.32)	0.87
Education (grades completed)							

	All *N* (%)	HIV-negative *N* (%)	HIV recent infection *N* (%)	Odds ratio (95% CI)	*p* value	Adjusted odds ratio (95% CI)	*p* value
< 4	1030 (35.4)	994 (35.3)	36 (37.1)	ref		-	
4–9	1257 (43.2)	1212 (43.1)	45 (46.4)	1.03 (0.66, 1.60)	0.91	-	
≥ 10	625 (21.5)	609 (21.6)	16 (16.5)	0.73 (0.40, 1.32)	0.29	-	
Circumcision status							
Circumcised	794 (27.2)	783 (27.8)	11 (11.2)	ref		-	
Uncircumcised	2122 (72.8)	2035 (72.2)	87 (88.8)	3.04 (1.62, 5.73)	< 0.01	-	
Number of sex partners, lifetime							
≤ 10	1315 (45.4)	1287 (46.0)	28 (29.5)	ref		-	
> 10	1579 (54.6)	1512 (54.0)	67 (70.5)	2.04 (1.30, 3.19)	< 0.01	-	
Number of female sex work partners, lifetime							
0	292 (10.0)	289 (10.3)	3 (3.1)	ref		ref	
1–10	1390 (47.8)	1352 (48.1)	38 (39.2)	2.71 (0.83, 8.83)	0.10	6.77 (0.90, 50.64)	0.06
> 10	1229 (42.2)	1173 (41.7)	56 (57.7)	4.60 (1.43, 14.80)	0.01	12.74 (1.72, 94.45)	0.01
Condom use with female sex workers							
Not applicable[a]	292 (10.0)	289 (10.3)	3 (3.1)	ref		-	
Never	1001 (34.4)	961 (34.2)	40 (41.2)	4.01 (1.23, 13.06)	0.02	-	
Sometimes	1205 (41.4)	1157 (41.2)	48 (49.5)	4.00 (1.24, 12.92)	0.02	-	
Always	411 (14.1)	405 (14.4)	6 (6.2)	1.43 (0.35, 5.75)	0.62	-	
Alcohol use with female sex workers							
Not applicable[a]	292 (10.0)	289 (10.3)	3 (3.1)	ref		-	
Never	1042 (35.8)	1016 (36.2)	26 (26.8)	2.47 (0.74, 8.20)	0.14	-	
Sometimes	831 (28.6)	794 (28.3)	37 (38.1)	4.49 (1.37, 14.67)	0.01	-	
lways	742 (25.5)	711 (25.3)	31 (32.0)	4.20 (1.27, 13.85)	0.02	-	
Any female sex work partners, past 3 months							
No	882 (30.3)	859 (30.5)	23 (23.7)	ref		-	
Yes	2030 (69.7)	1956 (69.5)	74 (76.3)	1.41 (0.88, 2.27)	0.15	-	
Any unprotected sex with female sex workers, past 3 months							
No	1413 (48.6)	1379 (49.0)	34 (35.1)	ref		-	
Yes	1496 (51.4)	1433 (51.0)	63 (65.0)	1.78 (1.17, 2.72)	< 0.01	-	
Alcohol use with female sex workers, past 3 months							

	All *N* (%)	HIV-negative *N* (%)	HIV recent infection *N* (%)	Odds ratio (95% CI)	*p* value	Adjusted odds ratio (95% CI)	*p* value
Not applicable[b]	882 (30.3)	859 (30.5)	23 (24.0)	ref		-	
Never	947 (32.6)	916 (32.6)	31 (32.3)	1.26 (0.73, 2.18)	0.40	-	
Sometimes	338 (11.6)	325 (11.6)	13 (13.5)	1.49 (0.75, 2.98)	0.26	-	
Always	742 (25.5)	713 (25.4)	29 (30.2)	1.52 (0.87, 2.65)	0.14	-	
Perceived risk for HIV infection							
None/slight risk	1759 (64.0)	1712 (64.3)	47 (55.3)	ref		ref	
Moderate/high risk	990 (36.0)	952 (35.7)	38 (44.7)	1.45 (0.94, 2.25)	0.09	1.81 (1.12, 2.91)	0.02
Syphilis, by stage of infection							
No infection	2398 (82.2)	2330 (82.7)	68 (69.4)	ref		-	
Primary	68 (2.3)	65 (2.3)	3 (3.1)	1.58 (0.48, 5.16)	0.45	-	
Secondary	11 (0.4)	11 (0.4)	0 (0.0)	-	-	-	
Latent	439 (15.1)	412 (14.6)	27 (27.6)	2.25 (1.42, 3.55)	< 0.01	-	
HSV-2, by stage of infection							
No infection	1696 (58.2)	1673 (59.4)	23 (23.5)	ref		ref	
Incident	28 (1.0)	28 (1.0)	0 (0.0)	-	-	-	-
Recurrent	250 (8.6)	224 (8.0)	26 (26.5)	8.44 (4.74, 15.05)	< 0.01	8.50 (4.43, 16.30)	< 0.01
Chronic	942 (32.3)	893 (31.7)	49 (50.0)	3.99 (2.42, 6.59)	< 0.01	3.14 (1.79, 5.51)	< 0.01
Chancroid							
No	2890 (99.2)	2794 (99.2)	96 (98.0)	ref		-	
Yes	24 (0.8)	22 (0.8)	2 (2.0)	2.65 (0.61, 11.41)	0.19	-	
Chlamydial urethritis							
No	2864 (98.4)	2769 (98.4)	95 (96.9)	ref		-	
Yes	47 (1.6)	44 (1.6)	3 (3.1)	1.99 (0.61, 6.51)	0.26	-	
Genital warts							
No	2856 (97.9)	2767 (98.2)	89 (90.8)	ref		ref	
Yes	60 (2.1)	51 (1.8)	9 (9.2)	5.49 (2.62, 11.49)	< 0.01	5.73 (2.54, 12.95)	< 0.01
Gonorrhea							
No	2642 (91.2)	2560 (91.4)	82 (84.5)	ref		ref	
Yes	256 (8.8)	241 (8.6)	15 (15.5)	1.94 (1.10, 3.42)	0.02	3.14 (1.64, 6.03)	< 0.01

Totals may not always sum due to missing data; percentages may not always sum to 100% due to rounding

[a] Not applicable because they had no lifetime FSW partners

[b] Not applicable because they had no FSW partners in the past 3 months