Open Forum Infectious Diseases

MAJOR ARTICLE

Antiretroviral Drug Resistance in HIV Sequences From People Who Inject Drugs and Men Who Have Sex With Men Across 21 Cities in India

Steven J. Clipman,[1] Sunil S. Solomon,[1] Aylur K. Srikrishnan,[2] Allison M. McFall,[3] Selvamurthi Gomathi,[2] Shanmugam Saravanan,[2] Santhanam Anand,[2] Canjeevaram K. Vasudevan,[2] Muniratnam S. Kumar,[2] David D. Celentano,[3] Shruti H. Mehta,[3] and Gregory M. Lucas[1]

[1]Division of Infectious Diseases, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA, [2]YR Gaitonde Centre for AIDS Research and Education, Chennai, India, and [3]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA

Background. Drug resistance testing is limited in public-sector human immunodeficiency virus (HIV) care in India, and there are few systematic samplings for prevalent drug resistance mutations (DRMs), particularly among men who have sex with men (MSM) and people who inject drugs (PWID).

Methods. We conducted genotypic resistance testing on 915 HIV sequences sampled from viremic self-reported antiretroviral therapy (ART) experienced and naive PWID and MSM recruited from 21 cities across India in 2016–2017. We analyzed factors associated with resistance using logistic regression and evaluated evidence for transmitted resistance using phylogenetic analyses.

Results. Of the 915 participants sequenced, median age was 31, 436 were MSM, and 191 were ART experienced. Overall, 62.8% of ART-experienced participants and 14.4% of ART-naive participants were found to have low-level resistance or higher to 1 or more classes of drugs. Prevalence of tenofovir disoproxil fumarate resistance was 25.7% in ART-experienced participants and 1.11% in ART-naive participants. The highest proportion of drug resistance was seen across nucleoside reverse transcriptase inhibitors and nonnucleoside reverse transcriptase inhibitors, and resistance was significantly more common among MSM participants than PWID. Phylogenetic analyses revealed that 54.6% of ART-naive participants with resistance who clustered had shared DRMs, suggesting transmitted resistance may have occurred.

Conclusions. Patients experiencing virologic failure on first-line therapy switched blindly to tenofovir/lamivudine/dolutegravir may effectively be receiving dolutegravir monotherapy due to resistance to tenofovir and lamivudine. While dolutegravir is expected to have full activity in the majority of patients in India, follow-up is needed to understand how resistance may affect long-term outcomes.

Keywords. DRM; India; LMIC; MSM; phylogenetics; PWID.

Antiretroviral therapy (ART) has revolutionized the treatment of human immunodeficiency virus (HIV); however, HIV remains incurable, necessitating lifelong therapy. Over the course of treatment, antiretroviral drug resistance mutations (DRMs) can emerge and accumulate in the setting of inconsistent use of ART or insufficiently potent regimens. Data on the prevalence and the extent of drug resistance among samples of people living with HIV can inform clinical guidelines for the use of second-line therapies or beyond, particularly in low- and middle-income settings, where resistance testing is infrequently used in clinical practice due to prohibitive cost or lack of availability.

The National AIDS Control Programme in India provides free ART for all people living with HIV. First-line ART regimens available for adults and adolescents include zidovudine (ZDV), tenofovir disoproxil fumarate (TDF), abacavir (ABC), lamivudine (3TC), efavirenz (EFV), and nevirapine (NVP) [1]. More recently, dolutegravir (DTG), an integrase strand transfer inhibitor (INSTI), has been adopted as the preferred first-line treatment. EFV and NVP, both of which have a low genetic barrier to resistance, have been the only nonnucleoside reverse transcriptase inhibitors (NNRTIs) available in the program since its inception in 2004 [1, 2]. Individuals failing these regimens often exhibit cross-resistance to the second-generation NNRTIs etravirine and rilpivirine (RPV) [3, 4]. Until around 2018, people living with HIV and receiving ART in India were monitored clinically and immunologically with $CD4^+$ cell counts every 6 months, but not with routine viral load measurements [1]. Yet, immunological failure usually succeeds virologic failure, and the accumulation of DRMs by the time of detection

Received 20 June 2022; editorial decision 12 September 2022; accepted 15 September 2022; published online 17 September 2022

Correspondence: Steven J. Clipman, PhD, MSPH, Johns Hopkins University School of Medicine, 1830 E Monument St, Baltimore, MD 21287 (sclipman@jhmi.edu).

Open Forum Infectious Diseases®

https://doi.org/10.1093/ofid/ofac481

is common [5]. Earlier studies have reported a low prevalence of DRMs in India [2, 6–9], but these have been limited by small sample sizes and have not focused on key populations. In the absence of routine drug testing and a centralized repository of HIV sequences and resistance patterns, reports of DRMs among research participant samples, particularly key populations such as people who inject drugs (PWID) and men who have sex with men (MSM), are critical to monitoring evolving trends. In this manuscript, we report on the results of HIV genotypic resistance testing conducted among PWID and MSM from cities across India.

METHODS

Study Design and Participants

We conducted genotypic resistance testing on stored plasma samples that were provided by participants from the evaluation survey of a cluster-randomized trial that was conducted at 22 sites across India [10]. The trial compared a structural intervention (integrated care centers, targeted to either PWID or MSM) with the standard care and spanned 21 cities in India; 1 city (New Delhi) had both an MSM and PWID site. To assess the effectiveness of the intervention in increasing HIV testing uptake, we conducted a baseline survey (2012–2013) before the intervention roll-out and an evaluation survey (2016–2017) at study conclusion. The samples evaluated for DRMs in this manuscript pertain to this evaluation survey (2016–2017). We conducted the surveys using respondent-driven sampling (RDS), a sampling method that is widely used for "hidden populations" in which it is difficult to obtain a traditional sampling frame [11, 12].

As described previously [13], we initiated surveys with 2 or 3 seed participants who were influential and well-connected in their networks. Seed participants were given 2 coupons to recruit other PWID or MSM in the city. Recruits returned to the field site with a coupon and, if eligible, were enrolled and given 2 new coupons to recruit others. We continued recruitment through successive RDS waves until a target of 1000 participants was enrolled at each site. We tracked who recruited whom with the coupon system and asked participants to estimate their network size, defined as the number of key population members (PWID or MSM) they had seen in the prior 30 days. We used a fingerprint biometric to prevent people from participating more than once.

Participants were eligible to participate in the surveys if they (1) were 18 years or older; (2) presented a valid recruitment coupon, except "seeds"; (3) spoke Hindi, English, or the local language; and (4) were competent to provide informed consent. Additionally, in PWID sites, participants needed to self-report injection drug use in the prior 2 years, and in MSM sites, participants had to self-identify as male and report oral or anal intercourse with a man in the prior year. Participants provided a blood sample and completed an interviewer-administered electronic survey, which covered demographics, risk behaviors, and access to HIV testing and services. For the present analysis, stored samples from participants with HIV who had HIV RNA ≥1000 copies/mL and adequate specimen volume were eligible for drug resistance testing. HIV prevalence varied widely across cities [10]. In sites with ≤100 eligible participants with HIV, we attempted resistance testing on all eligible samples. In sites with >100 eligible participants with HIV, we randomly selected 100 samples for resistance testing (Table 1). Of 1659 samples in which resistance testing was attempted, amplification was successful in 915 (55.2%). Samples that could and could not be amplified only differed significantly by viral load, and prior use of the samples for other assays and repeated freeze-thaw cycles reduced testing yield.

Patient Consent Statement

All participants provided oral informed consent, and the study was approved by institutional review boards at the Johns Hopkins University School of Medicine (Baltimore, Maryland), Johns Hopkins Bloomberg School of Public Health (Baltimore, Maryland), and the YR Gaitonde Centre for AIDS Research and Education (Chennai, India). Additionally, trial activities were reviewed by a data and safety monitoring board and an advisory board for the PWID and MSM strata, respectively. The study protocol is publicly available [13].

HIV Testing and Laboratory Procedures

We provided pretest counseling and rapid on-site HIV testing using 3 rapid test kits. Participants received their HIV test results and posttest counseling after completing the survey. Participants with HIV who were newly diagnosed or not in care were provided with referrals to government ART centers. Samples were shipped to a central laboratory in Chennai, India, for additional testing and plasma storage at −80° C.

In participants who were HIV positive, we measured $CD4^+$ cell counts with the FlowCARE PLG CD4 assay (Beckman Coulter, Brea, California), plasma viral load using the RealTime HIV-1 assay (Abbott Molecular, Abbott Park, Illinois), and HIV antibody avidity percentage using the Johns Hopkins University–modified Bio-Rad Avidity assay based on the Genetic Systems HIV-1/HIV-2 PLUS O enzyme immunoassay (EIA) kit (Bio-Rad Laboratories, Hercules, California) [14]. We further tested serum samples from participants with HIV using the Limited Antigen (LAg)–Avidity EIA (Maxim Biomedical, Rockville, Maryland).

We characterized participants as recently infected if they had CD4 counts of >50 cells/μL, viral loads of >400 copies per mL, a LAg-Avidity normalized optical density value <2.9, and Bio-Rad HIV antibody avidity index <80% [15]. The mean time duration for which individuals are classified as recently infected by this recent infection testing algorithm (RITA) is 0.52 years [16]. This RITA has been validated against observed

Table 1. Sample Flow (Left to Right) of Participants Recruited by Respondent-Driven Sampling Satisfying the Criteria for, and Ultimately Completing, Drug Resistance Testing Across 21 Indian Cities, 2016–2017

City	Population	Total Recruited (N = 21 725)	HIV-Positive (n = 4280)	HIV RNA >1000 Copies/mL (n = 2356)	Sample Volume ≥ 1 mL (n = 1659)	Resistance Testing Successful (n = 915)
Aizawl	PWID	1000	287	219	150	62
Amritsar	PWID	1000	221	173	130	66
Bilaspur	PWID	1000	199	174	141	67
Chandigarh	PWID	1000	81	57	39	22
Churachandpur	PWID	1000	239	91	59	38
New Delhi[a]	PWID	1000	399	371	115	84
Dimapur	PWID	1000	144	68	50	35
Imphal	PWID	1000	304	85	52	17
Kanpur	PWID	999	260	227	132	51
Ludhiana	PWID	1000	201	105	41	21
Lunglei	PWID	1000	104	25	20	8
Mumbai	PWID	722	78	60	53	8
Bengaluru	MSM	1000	134	65	62	44
Belgaum	MSM	1000	102	36	34	25
Bhopal	MSM	1002	163	101	94	41
Chennai	MSM	1001	61	29	29	25
Coimbatore	MSM	1000	142	55	55	39
New Delhi[a]	MSM	1000	194	55	47	31
Hyderabad	MSM	1000	208	77	74	64
Madurai	MSM	1000	186	57	56	31
Vijayawada	MSM	1001	320	141	141	61
Visakhapatnam	MSM	1000	253	85	85	75

Data are presented as No. In sites with >100 participants with HIV RNA >1000 copies/mL and ≥1 mL of sample volume, we randomly selected 100 samples for resistance testing. Repeated freeze/thaw cycles and plasma viral load were the primary factors affecting successful sequencing.

Abbreviations: HIV, human immunodeficiency virus; MSM, men who have sex with men; PWID, people who inject drugs.

[a]New Delhi had both a PWID and an MSM site.

seroconversions within longitudinal cohorts in HIV subtype C epidemic settings [14, 15, 17].

Sequencing

We obtained HIV-1 partial *pol* gene sequences (HXB2, NC_001802; nucleotides 1816–2772) covering the full-length protease (amino acids 1–99) and the first 230 amino acids of reverse transcriptase codons using reverse-transcription polymerase chain reaction amplification and Sanger sequencing on samples meeting eligibility criteria (n = 915) [18, 19]. We removed primer sequences with Sequencher version 5.4.6 (Gene Codes, Ann Arbor, Michigan) and trimmed sequences by quality and to remove leading and trailing ambiguous bases. We assembled consensus contigs from forward and reverse reads with a minimum overlap of 20 bp and an 85% minimum match percentage. Sequences are available in GenBank under the accession numbers ON423719–ON424633.

Subtyping and Drug Resistance Testing

We determined HIV subtypes using the REGA HIV-1 subtyping tool version 3.0 (http://dbpartners.stanford.edu:8080/RegaSubtyping/stanford-hiv/typingtool/) [20]. We interpreted antiretroviral susceptibility and DRMs using the Stanford University HIVdb Program version 9.0 [21] (https://hivdb.stanford.edu), which classified DRMs according to their ability to confer resistance to nucleoside reverse transcriptase inhibitor (NRTI), NNRTI, and protease inhibitor (PI) drug classes. We characterized samples with DRMs as "resistant" in downstream analyses if they were determined to have low-level resistance or higher to any of these drug classes. We quantified total resistance across drug regimens using HIVdb DRM penalty scores (penalty score ≥15), consistent with the World Health Organization (WHO) HIV Drug Resistance Report [22]. We analyzed factors associated with drug resistance using univariable and multivariable logistic regression in Stata 15 software (StataCorp, College Station, Texas). We used a random forest feature selection algorithm [23] to explore candidate factors and considered variables for inclusion in multivariable models if they held biological/epidemiological significance or had significant associations in univariable models ($P < .05$).

Phylogenetic Analysis

To further examine potential evidence for transmitted resistance, we inferred phylogenetic trees and conducted a molecular network analysis. We aligned HIV sequences with the HXB2 reference (GenBank K03455.1) using multiple sequence comparison by log-expectation (MUSCLE). We determined the most appropriate nucleotide substitution model for phylogenetic

Table 2. Characteristics of 915 People Living With Human Immunodeficiency Virus Across India Sequenced for Drug Resistance Testing

Characteristic	All Participants (N = 915)	ART-Experienced (Ever)		ART-Naive	
		With Resistance (n = 120)	No Resistance (n = 71)	With Resistance (n = 104)	No Resistance (n = 620)
Age, y, median (IQR)[a]	31 (26–38)	39 (32–44)	35 (30–43)	29 (25–36)	30 (25–36)
Highest level of education[a]					
No schooling	179 (20)	28 (23)	12 (17)	20 (19)	119 (19)
Primary school (grades 1–5)	147 (16)	16 (13)	15 (21)	16 (15)	100 (16)
Secondary school (grades 6–10) or above	589 (64)	76 (63)	44 (62)	68 (65)	401 (65)
Marital status[a]					
Unmarried	486 (53)	36 (30)	21 (30)	59 (57)	370 (60)
Married or cohabitating	429 (47)	84 (70)	50 (70)	45 (43)	250 (40)
Ever previously tested for HIV[a]	658 (72)	120 (100)	71 (100)	72 (69)	395 (64)
Aware of HIV status[a]	399 (61)	120 (100)	71 (100)	27 (38)	181 (46)
Key population[a]					
MSM	436 (48)	98 (82)	45 (63)	55 (53)	238 (38)
PWID	479 (52)	22 (18)	26 (37)	49 (47)	382 (62)
Cisgender women	33 (7)	7 (32)	2 (8)	4 (8)	20 (5)
Cisgender men	446 (93)	15 (68)	24 (92)	45 (91)	362 (95)
$CD4^+$ count, cells/μL					
<200	324 (36)	65 (54)	41 (58)	38 (37)	180 (29)
≥200	587 (64)	55 (46)	30 (42)	66 (64)	440 (71)
$CD4^+$ count, cells/μL, median (IQR)	254 (157–378)	184 (96–293)	173 (113–302)	232 (145–345)	277 (188–404)
Plasma viral load, copies/mL, median (IQR)	55446 (18170–159202)	43318 (14084–166655)	69236 (11630–166849)	72284 (22980–181706)	55253 (20527–149240)
Recent infection[b]	159 (17)	12 (10)	8 (11)	15 (14)	124 (20)
Ever taken ART[a]	191 (21)	120 (100)	71 (100)	0 (0)	0 (0)
Ever taken ART drug[a,c]					
ATV/r	5 (3)	4 (3)	1 (1)	…	…
ZDV	79 (41)	46 (38)	33 (46)	…	…
d4T	84 (44)	48 (40)	36 (51)	…	…
3TC	110 (58)	63 (53)	47 (66)	…	…
TDF	49 (26)	30 (25)	19 (27)	…	…
EFV	35 (18)	22 (18)	13 (18)	…	…
NVP	88 (46)	51 (43)	37 (52)	…	…
Currently taking ART[a]	162 (85)	112 (93)	50 (70)	0 (0)	0 (0)
% ART taken in past month, median (IQR)[a]	95 (80–100)	96 (80–100)	93 (75–100)	…	…

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: 3TC, lamivudine; ART, antiretroviral therapy; ATV/r, atazanavir/ritonavir; d4T, stavudine; EFV, efavirenz; HIV, human immunodeficiency virus; IQR, interquartile range; MSM, men who have sex with men; NVP, nevirapine; TDF, tenofovir disoproxil fumarate; ZDV, zidovudine.

[a]Self-reported.

[b]Determined using a limiting antigen avidity algorithm.

[c]Participants could select >1 drug.

analysis using jModelTest version 2.1.10, [24] with scores determined using hierarchical likelihood ratio test and Akaike information criterion. We found that a general time-reversible (GTR) model with 4 categories of assumed rate heterogeneity (Γ_4) and invariant sites (I) was the most appropriate evolutionary model. We inferred maximum likelihood phylogenetic trees using RAxML with 500 bootstrap replications under the GTR + Γ_4 + I model. We identified putative transmission clusters using the "Max Clade" algorithm in TreeCluster version 1.0.2 [25] (github.com/niemasd/TreeCluster) with a distance threshold of 0.015 and support threshold of 0.9; sensitivity analysis was performed using Cluster Picker [26, 27]. A benefit of identifying clusters using TreeCluster is that it leverages the full phylogenetic tree and therefore uses more robust evolutionary model corrected tree-based distances rather than genetic distances computed from a multiple sequence alignment file [25].

RESULTS

Participant Characteristics

Of 915 samples sequenced for DRMs, 48% (436) came from MSM sites, and 52% (479) came from PWID sites. Among

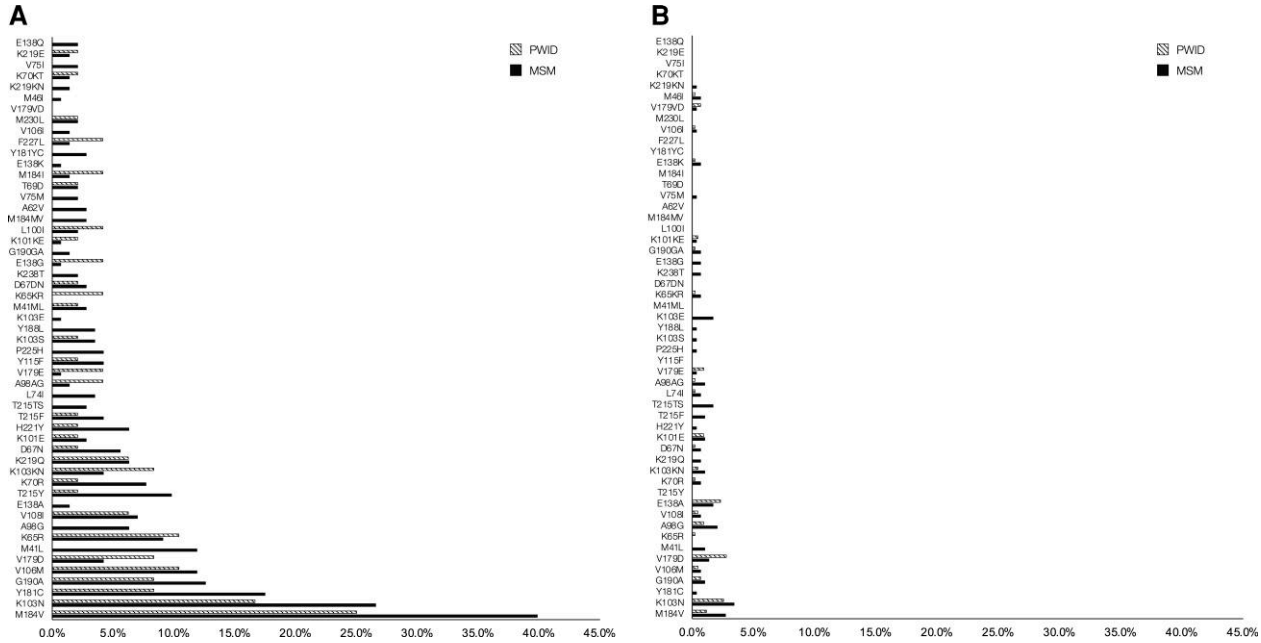

Figure 1. Prevalence of drug resistance mutations among self-reported antiretroviral therapy (ART)–experienced people who inject drugs (PWID) (n = 48) and men who have sex with men (MSM) (n = 143) (*A*) and self-reported ART-naive PWID (n = 431) and MSM (n = 293) (*B*) across 21 cities in India, 2016–2017. Only major protease inhibitor mutations and mutations present in >2 samples are shown.

PWID, 93% (446) were male, which is reflective of the epidemiology of drug use in these cities. Overall median age was 31 (interquartile range [IQR], 26–38) years, 64% (589) completed secondary school (grades 6–10) or above, and median $CD4^+$ cell count was 254 (IQR, 157–378) cells/μL, with 36% (324) having $CD4^+$ counts <200 cells/μL (Table 2). Overall, 72% (658) of sequenced participants reported being aware of their status prior to participation in the study, and 21% (191) of participants reported ever taking ART: 10% (48) of PWID participants, and 33% (143) of MSM participants. The majority of participants were infected with HIV-1 subtype C (883/915 [97.6%]). Six sequences (0.66%) were classified as subtype A, 7 (0.77%) as a recombinant of C and A1, 4 (0.44%) as subtype B, 4 (0.44%) as recombinant of C and B, and 1 (0.11%) as CRF 08_BC.

Drug Resistance Mutations

Of the 915 participant sequences analyzed, 28.5% (261/915) contained 1 or more DRM, representing 65.5% (125/191) of ART-experienced participants and 18.8% (136/724) of ART-naive participants. Within reverse transcriptase, 61.8% (118/191) of ART-experienced participants and 15.2% (110/724) of ART-naive participants harbored NNRTI resistance mutations and 44.5% (85/191) and 3.45% (25/724) of ART-experienced and naive participants, respectively, had NRTI resistance mutations. Within the viral protease, 1.05% (2/191) of ART-experienced participants and 0.97% (7/724) of ART-naive participants contained major PI resistance mutations. The most prevalent mutations in ART-experienced participants were M184V (69/191 [36.1%]; NRTI), K103N (46/191 [24.1%]; NNRTI), and Y181C (29/191 [15.2%]; NNRTI). Among ART-naive participants, the most prevalent mutations were K103N (21/724 [2.90%]; NNRTI), V179D (16/724 [2.21%]; NNRTI), and E138A (15/724 [2.07%]; NNRTI) (Figure 1). Dual-class NNRTI and NRTI mutations were seen in 42.4% (81/191) and 2.49% (18/724) of ART-experienced and naive participants, respectively. Triple-class resistance was seen in 1.05% (2/191) of ART-experienced participants and 0.28% (2/724) of ART-naive participants. The most frequent combination of NNRTI and NRTI dual-class mutations were K103N and M184V (38/99 [38.4%]). Of the 4 samples with NNRTI, NRTI, and PI triple-class mutations, 3 were seen among K103N, M184V, and M46I, and the fourth was among V108I, M184V, and L90M.

Resistance to ART Drugs

Overall, 62.8% (120/191) of ART-experienced participants and 14.4% (104/724) of ART-naive participants were found to have low-level resistance or higher to 1 or more drugs in 1 or more classes. A total of 16 (8.38%) ART-experienced and 1 (0.14%) ART-naive participants had resistance across all NRTI and NNRTI drugs (Figure 2; Supplementary Table 1). Drug resistance was more prevalent among MSM participants (35.1%) than among PWID (14.8%), and overall levels of resistance varied significantly by geography (1-way analysis of variance;

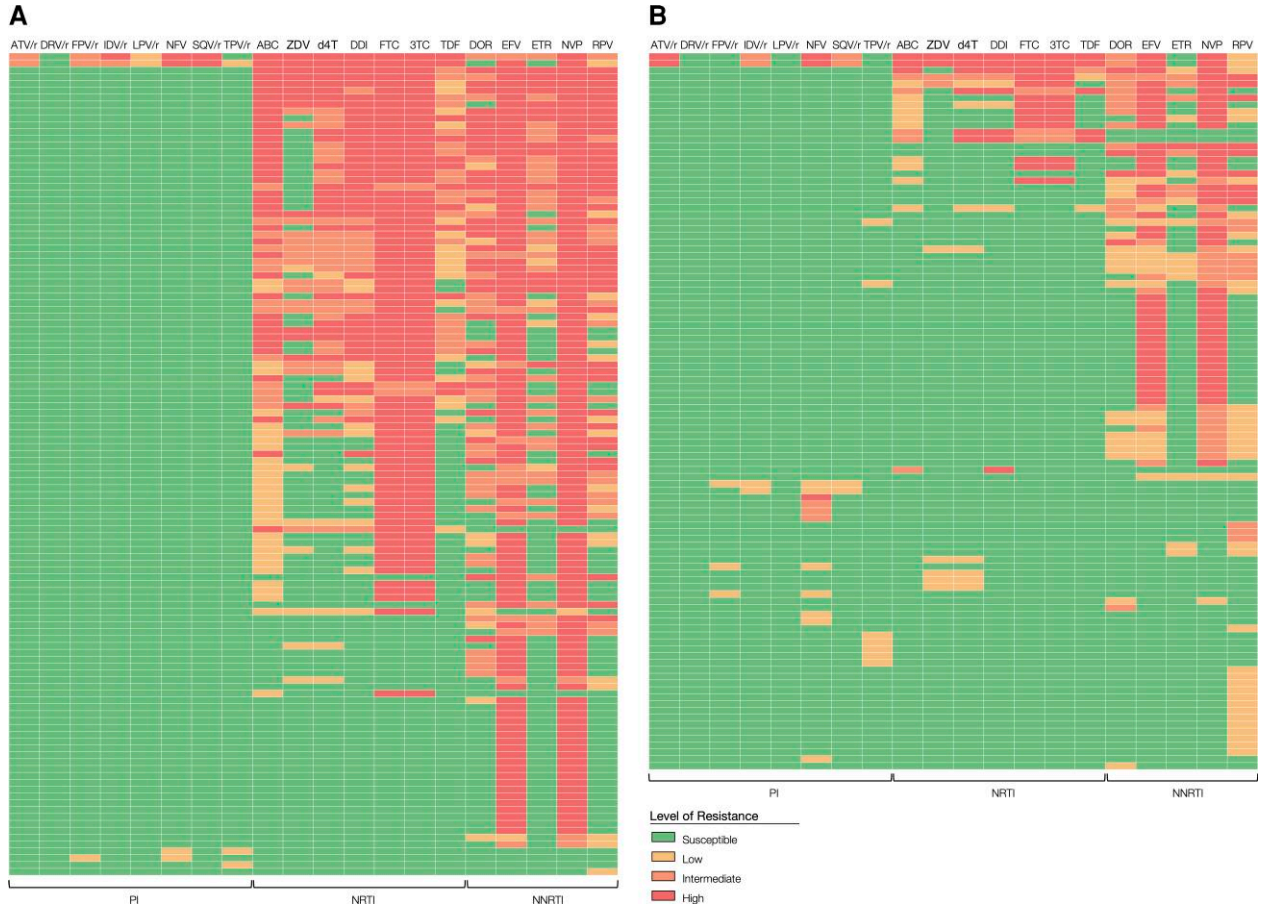

Figure 2. Human immunodeficiency virus antiretroviral (ARV) drug resistance among 120 ART-experienced (*A*) and 104 ART-naive (*B*) men who have sex with men and people who inject drugs with low-level drug resistance or higher across 21 cities in India, 2016–2017. Each row represents an individual participant sample. Columns represent ARV drugs, and colors denote drug resistance levels. The figure is oriented from top to bottom, depicting descending overall resistance. Abbreviations: /r, ritonavir; 3TC, lamivudine; ABC, abacavir; ATV, atazanavir; d4T, stavudine; DDI, didanosine; DOR, doravirine; DRV, darunavir; EFV, efavirenz; ETR, etravirine; FPV, fosamprenavir; FTC, emtricitabine; IDV, indinavir; LPV, lopinavir; NFV, nelfinavir; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NVP, nevirapine; PI, protease inhibitor; RPV, rilpivirine; SQV, saquinavir; TDF, tenofovir disoproxil fumarate; TPV, tipranavir; ZDV, zidovudine.

$P < .01$), with the highest levels seen in Vijayawada and Visakhapatnam (Supplementary Figure 1). Of the 14.4% (104/724) of self-reported ART-naive participants with drug resistance, 52.9% (55/104) were MSM, and 14.4% (15/104) were characterized as having recent HIV infection. Of the total 159 participants with recent infections, 17.0% (24) exhibited drug resistance, 14.6% (14/96) among PWID participants with recent infections, and 20.6% (13/63) among MSM participants with recent infections.

The highest proportion of drug resistance was seen with NNRTI class drugs, namely NVP (114/191 [59.7%] ART experienced; 60/724 [8.29%] ART naive) and EFV (113/191 [59.2%] ART experienced; 59/724 [8.15%] ART naive). Prevalence of TDF resistance was 6.23% (57/915) overall, 25.7% in ART-experienced participants, and 1.11% in ART-naive participants. Among NRTI-class drugs, 10.8% (99/915) of participants had resistance to ABC, 10.6% (97/915) to emtricitabine (FTC), and 10.6% (97/915) to 3TC. PI drugs contained the least resistance, which was primarily seen with nelfinavir at 1.9% (17/915). Self-reported ART-naive participants who were unaware of their HIV status had a lower proportion of resistance than ART-naive participants who were aware of their HIV status (Figure 3).

Factors Associated With Drug Resistance

Factors significantly associated with drug resistance in univariable models included being ≥40 years of age (odds ratio [OR], 2.22 [95% confidence interval {CI}, 1.50–3.29]), self-identifying as a cisgender female PWID (OR, 3.22 [95% CI, 1.49–6.97]) or MSM (OR, 3.48 [95% CI, 2.49–4.87]) compared to cisgender male PWID, being married or cohabitating (OR, 1.77 [95% CI, 1.31–2.40]), having ever taken ART (OR, 10.1 [95% CI, 7.03–14.4]) and in the past month (OR, 12.8 [95% CI, 8.69–18.9]), having a $CD4^+$ cell count <200 cells/μL (OR, 1.81 [95% CI, 1.33–2.46]), and being characterized as having a recent infection (OR, 0.58 [95% CI, .37–.91]) (Table 3). In a

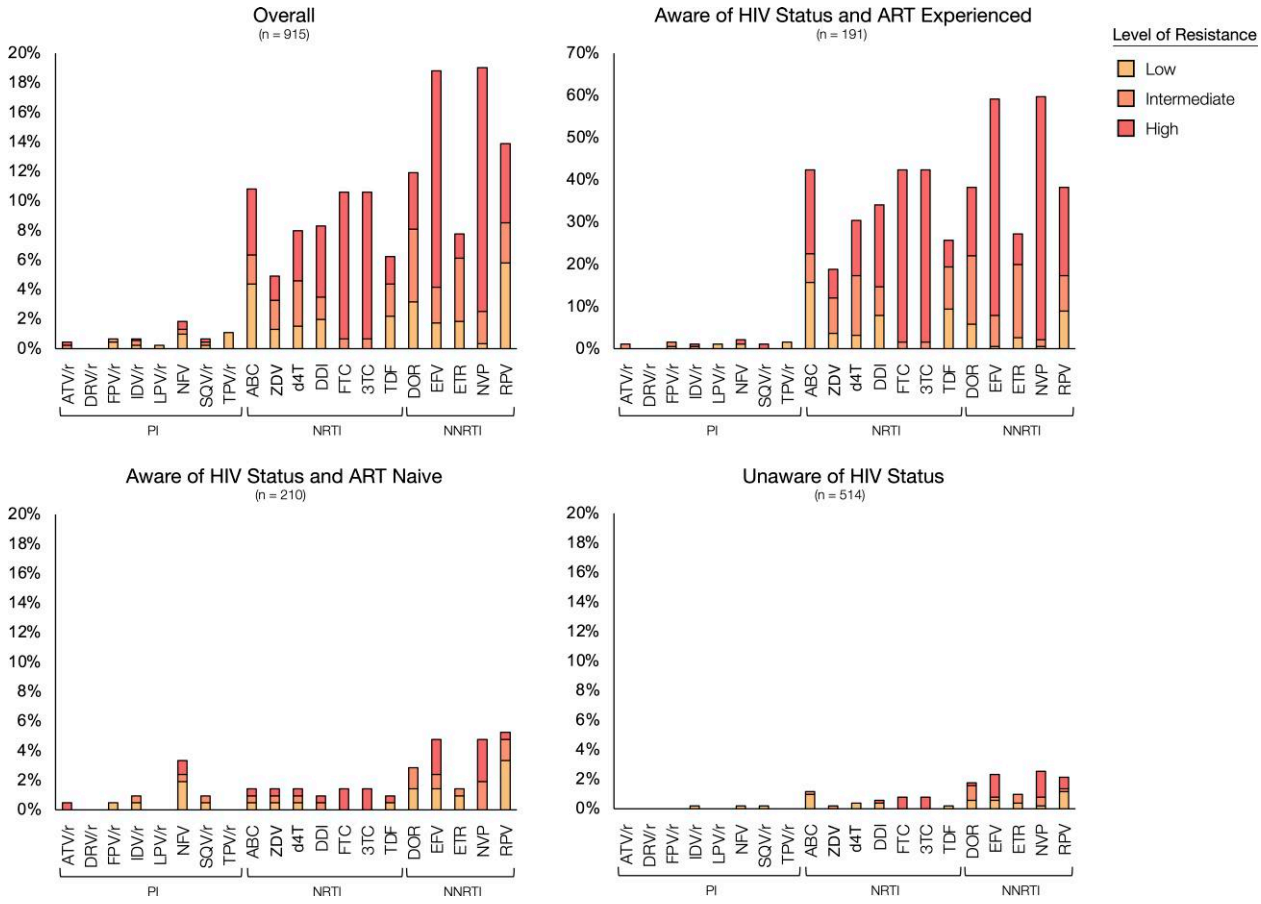

Figure 3. Prevalence and level of antiretroviral drug resistance among 915 representative human immunodeficiency virus (HIV) type 1 sequences from men who have sex with men and people who inject drugs sampled in 2016–2017 across 21 cities in India (*A*), as well as among 191 participants aware of their HIV status with self-report of ever taking antiretroviral therapy (ART) (*B*), among 210 participants aware of their HIV status and no self-reported history of ever taking ART (*C*) and among 514 participants unaware of their HIV status (and ART-naive) (*D*). Abbreviations: /r, ritonavir; 3TC, lamivudine; ABC, abacavir; ART, antiretroviral therapy; ATV, atazanavir; d4T, stavudine; DDI, didanosine; DOR, doravirine; DRV, darunavir; EFV, efavirenz; ETR, etravirine; FPV, fosamprenavir; FTC, emtricitabine; HIV, human immunodeficiency virus; IDV, indinavir; LPV, lopinavir; NFV, nelfinavir; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NVP, nevirapine; PI, protease inhibitor; RPV, rilpivirine; SQV, saquinavir; TDF, tenofovir disoproxil fumarate; TPV, tipranavir; ZDV, zidovudine.

multivariable model that included age, key population, marital status, ART use, $CD4^+$ cell count, and recent infection status, self-identifying as MSM (adjusted odds ratio [AOR], 2.06 [95% CI, 1.39–3.05]), currently taking ART (AOR, 9.74 [95% CI, 6.35–15.0]), and recent infection status (AOR, 0.13 [95% CI, .08–.19]) remained significantly associated with resistance (Table 3). In both univariable and multivariable models, recent infection status was the only significant factor inversely associated with drug resistance.

Phylogenetic Clustering

Overall, 11% (99) of participant sequences had a molecular link with another sequence at a 1.5% distance threshold and fell into 49 clusters (Figure 4). With the exception of 1 cluster of 3 participants, all were dyads. There were no instances where clustered participants were also adjacent in the RDS recruitment chain. Sequences also clustered exclusively by key population, that is, there were no instances of an MSM and PWID participant sequence falling into the same cluster, and the majority (94%) of sequences clustered by city. There were 3 dyads (2 PWID-PWID and 1 MSM-MSM) where participants were recruited from different cities. Intercity clustering was seen between Coimbatore and Madurai, Churachandpur and Imphal, and Churachandpur and Lunglei; all 6 of these participants reported travel in the prior 12 months. Of 104 ART-naive participants with resistance, 17 (16.4%) clustered with another participant; 12 (70.6%) clustered with another ART-naive participant (6/49 total clusters), and the remaining 5 clustered with an ART-experienced participant (5/49 total clusters). About a quarter (23.5%) of these 17 naive participants with resistance who clustered were also characterized by the RITA as being infected within the last 6 months. Of the 11 dyads containing an ART-naive participant, 6 dyads (54.6%) contained participants with shared DRMs (Table 4).

Table 3. Factors Associated With Human Immunodeficiency Virus (HIV) Drug Resistance by Univariable and Multivariable Logistic Regression Among 915 Representative HIV-1 Sequences From Across India

Variable	OR (95% CI)	AOR (95% CI)
Age, y		
<30	Ref.	Ref.
30–39	1.32 (0.93–1.89)	0.90 (0.59–1.35)
≥40	2.22 (1.50–3.29)	0.98 (0.60–1.61)
Key population		
PWID (cisgender men)	Ref.	Ref.
PWID (cisgender women)	3.22 (1.49–6.97)	2.08 (0.88–4.91)
MSM	3.48 (2.49–4.87)	2.06 (1.39–3.05)
Education		
No schooling	Ref.	…
Primary school	0.78 (0.46–1.27)	…
Secondary school or above	0.88 (0.60–1.29)	…
Marital status		
Unmarried	Ref.	Ref.
Married or cohabitating	1.77 (1.31–2.40)	0.92 (0.63–1.36)
Ever taken ART	10.1 (7.03–14.4)	…
Currently taking ART	12.8 (8.69–18.9)	9.74 (6.35–15.0)
$CD4^+$ cell count, cells/μL		
≥200	Ref.	Ref.
<200	1.81 (1.33–2.46)	1.34 (0.92–1.92)
Recent infection	0.58 (0.37–0.91)	0.13 (0.08–0.19)

The multivariable model included variables with values in the AOR column.

Abbreviations: AOR, adjusted odds ratio; ART, antiretroviral therapy; CI, confidence interval; MSM, men who have sex with men; OR, odds ratio; PWID, people who inject drugs.

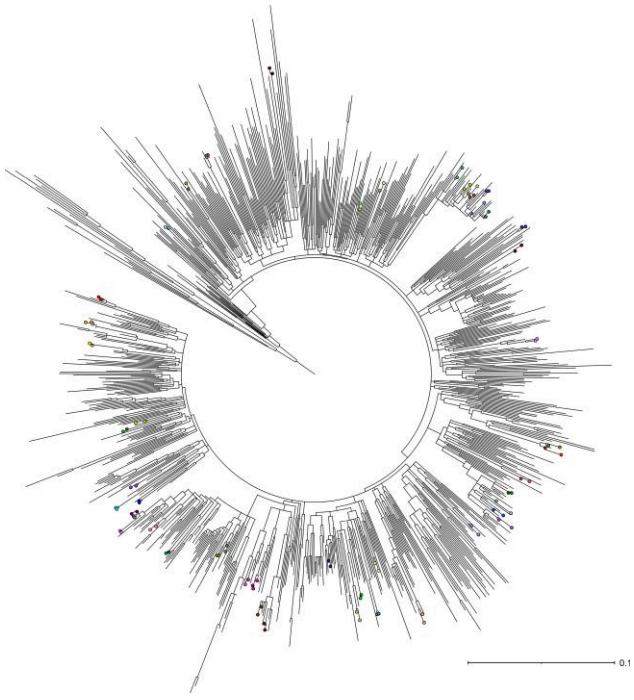

Figure 4. Maximum likelihood phylogenetic tree of 915 human immunodeficiency virus type 1 partial *pol* sequences isolated from men who have sex with men and people who inject drugs across 21 cities in India inferred under a general time-reversible + Γ4 + I evolutionary model. Branches with like-colored tip shapes denote transmission clusters at a 1.5% genetic distance threshold. Branches without tip shapes denote sequences that did not cluster.

DISCUSSION

Among 915 HIV sequences from an RDS sample of PWID and MSM across 21 cities in India, we saw resistance among 62.8% of ART-experienced participants and 14.4% of ART-naive participants. We saw resistance primarily within NNRTI-class drugs, namely NVP and EFV, and to a lesser extent, the NRTI-class drugs 3TC and FTC. Data on DRMs among PWID and MSM in India and other low- and middle-income countries (LMICs) are limited; however, the levels of resistance seen in these drugs are comparable to those reported among ART-experienced and -naive patients in other populations and settings [22]. For example, we saw NVP resistance among 59.7% and 8.2% of ART-experienced and -naive participants, respectively. The 2021 WHO HIV Drug Resistance Report found the prevalence of NVP resistance among ART-experienced and ART-naive individuals in the South-East Asia Region to be around 58% and 6%, respectively [22]. Similarly, although sample sizes were small, a systematic review of HIV DRMs across 23 studies in India found that the proportion of sequences with any DRM, any NRTI DRM, and any NNRTI DRM was 78.4%, 68.8%, and 73.1%, respectively [2].

The most frequent DRM we observed in ART-experienced participants was M184V. This DRM reduces susceptibility to 3TC/FTC by >100-fold. In contrast, M184V increases susceptibility to ZDV, stavudine (d4T), and TDF and slows the emergence of ZDV, d4T, and TDF resistance [28–30]. It also has been shown to affect viral fitness [31]. K103N was the second most frequent DRM seen in ART-experienced participants and the most frequent DRM in ART-naive participants. This nonpolymorphic mutation is typically selected for in patients taking NVP and EFV NNRTIs [32–34] and reduces NVP and EFV susceptibility by approximately 50- and 20-fold, respectively [35–37]. NNRTI resistance would not be expected to impact the effectiveness of INSTI-based regimens; however, resistance to TDF, which we found in 25.7% of ART-experienced participants but only 1.11% of naive participants, could affect efficacy of the commonly co-formulated regimen of DTG, TDF, and 3TC (i.e., TLD). Over the past few years, consistent with recommendations from WHO, INSTI-based therapy has become the predominant regimen used in India and other countries. INSTI-based regimens are used in patients newly starting ART, but also many patients previously taking NNRTI- or PI-based regimens (some failing their current regimen) have switched to INSTI-based regimens. The resistance profiles we observed suggest that patients prescribed TLD may effectively be receiving DTG monotherapy due to resistance to TDF and 3TC. However, of importance, there is evidence to suggest that even DTG monotherapy or DTG in the presence of recycled NRTIs may be sufficient to

Table 4. Participant Antiretroviral Therapy (ART) Status and Drug Resistance Mutations by Drug Class Among 11 Phylogenetic Clusters Containing ART-Naive Participants With Resistance

Cluster	ART Status	NRTI	NNRTI	PI Major	PI Accessory
1	Naive	None	E138A	None	None
1	Naive	None	E138A	None	None
2	Naive	None	K103N	None	None
2	Naive	None	K103N	None	None
3	Naive	None	V106VA	None	None
3	Experienced	None	None	None	None
4	Naive	None	P236PL	None	None
4	Experienced	None	None	None	None
5	Naive	None	K103E, E138A	None	None
5	Naive	None	E138A	None	None
6	Experienced	None	None	None	None
6	Naive	None	K101KE	None	None
7	Naive	M41L, D67N, K70R, L74I, M184V, T215F, K219Q	A98G, K103N, V108I, K238T	M46I, N88S	K20T
7	Naive	M41L, D67N, K70R, L74I, M184V, T215F, K219Q	A98G, K103KN, V108I, K238T	M46I, N88S	K20T
8	Experienced	None	None	None	None
8	Naive	None	None	M46MI	None
9	Naive	None	E138K	None	None
9	Naive	None	E138K	None	None
10	Naive	None	A98G	None	None
10	Naive	None	A98G	None	None
11	Experienced	None	None	None	None
11	Naive	None	K103N	None	None

Abbreviations: ART, antiretroviral therapy; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

maintain viral suppression, thereby highlighting the importance of routine viral load monitoring to identify failure early [38, 39]. Furthermore, the resistance observed to RPV also suggests that long-acting injectable cabotegravir (CAB) plus RPV should not be used without confirming the absence of NNRTI-associated DRMs as was required in registration trials. The efficacy of long-acting CAB + RPV in the presence of RPV-associated mutations is unknown.

Examining resistance among ART-naive participants and evidence for transmitted resistance, we found that resistance was slightly higher among self-reported treatment-naive participants who were aware of their HIV status compared with those who were unaware of their status, suggesting underreporting of ART use in the former group. Nonetheless, recency testing and phylogenetic data support that transmitted resistance may have occurred. Among ART-naive participants with resistance who phylogenetically clustered, shared DRMs were seen in more than half, and about a quarter were also identified as a recent infection. While limited, these data support transmitted resistance.

Comparing resistance by key population, MSM participants had >2-fold greater odds of resistance compared to PWID, even when accounting for self-reported ART use and other factors. This is consistent with the fact that ART initiation and persistence are higher among MSM with HIV than PWID [10, 40]. While there was not substantial variation or significant differences in self-reported ART adherence between participants with and without drug resistance, ART adherence remains a concern.

These findings should be interpreted in the context of key limitations. Namely, all responses related to ART regimens were self-reported and clinical records were unavailable. Questions focused on ART use in the prior 30 days, so we could not confirm whether a participant was on ART at the time of sampling, and since some mutations (e.g., M184V) disappear rapidly, they may have been missed. HIV sequencing did not cover the full *pol* region or genome; therefore, we were unable to assess resistance to integrase inhibitor drugs. This further limits phylogenetic inference due to selective pressure on the *pol* region and potential confounding from convergent evolution. Consequently, phylogenetic analyses should be interpreted as supporting rather than primary evidence for transmitted resistance. Nonetheless, existing studies of drug resistance in key populations, such as MSM and PWID, in LMICs are extremely limited. Finally, while the samples presented here were collected in 2016–2017, before both routine viral load monitoring and the introduction of INSTI drugs in public-sector HIV treatment, the patterns of resistance still hold particular relevance in the transition to INSTI drugs in India and consideration and planning around new and long-acting formulations.

CONCLUSIONS

These data provide valuable insight into drug resistance among key populations in India, particularly given that resistance testing is not currently part of routine care, and resistance data from India are not included in the WHO HIV Drug Resistance Report [22]. To progress toward the Joint United Nations Programme on HIV/AIDS 95-95-95 targets for 2030, committed to ensuring that 95% of people living with HIV know their status, 95% of people who know their status are receiving treatment, and 95% of people on HIV treatment have a suppressed viral load, public health programs in India must aim to get more MSM and PWID on ART and ensure proper adherence. Given the high levels of resistance seen among ART-experienced participants and the potential challenges these resistance patterns pose in the transition to INSTI drugs or future long-acting regimens, drug resistance testing may be increasingly needed to inform treatment regimens and achieve viral suppression in India.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the

posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. Conceptualization: S. J. C., S. S. S., S. H. M., D. D. C., G. M. L. Methodology: S. J. C., S. S. S., A. M. M., G. M. L. Formal analysis: S. J. C., A. M. M. Investigation: S. J. C., S. H. M., S. G., S. S., S. S. S., G. M. L. Data curation: S. J. C., A. M. M. Writing–original draft: S. J. C., S. S. S., S. H. M., G. M. L. Visualization: S. J. C. Supervision: S. S. S., S. H. M., G. M. L. Project administration: A. K. S., S. S., S. G., S. A., C. K. V., M. S. K. Funding acquisition: S. J. C., G. M. L., S. H. M., S. S. S. All authors have read and approved the final manuscript.

Acknowledgments. We thank the study participants who have made this research possible. We also thank Dr Eric Nawrocki of the National Center for Biotechnology Information for his gracious assistance with VADR, a suite of tools for GenBank submissions.

Data availability. All sequences in this study have been deposited in GenBank under accession numbers ON423719–ON424633.

Disclaimer. The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; or decision to submit the manuscript for publication.

Financial support. This study was supported by the National Institutes of Health (grant numbers R01DA041034, K24DA035684, DP2DA040244, DP2DA056130) and the Elton John AIDS Foundation.

Potential conflicts of interest. S. H. M. reports personal fees from Gilead Sciences outside the submitted work. S. S. S. reports grants/products and advisory board fees from Gilead Sciences and grants/products from Abbott Diagnostics, outside the submitted work. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. National AIDS Control Organization. National technical guidelines on antiretroviral treatment. In: Ministry of Health and Family Welfare, ed. New Delhi, India; **2018**.
2. Karade S, Chaturbhuj DN, Sen S, et al. HIV drug resistance following a decade of the free antiretroviral therapy programme in India: a review. Int J Infect Dis **2018**; 66:33–41.
3. Anquetil D, Deshpande A, Zongo D, Le Bihan L, Pinson PR, Fleury HJ. Susceptibility to etravirine of HIV type 1 subtype C isolates from nevirapine/efavirenz-experienced patients: comparative interpretation of ANRS and STANFORD algorithms. AIDS Res Hum Retroviruses **2012**; 28:1793–7.
4. Neogi U, Shet A, Shamsundar R, Ekstrand ML. Selection of nonnucleoside reverse transcriptase inhibitor-associated mutations in HIV-1 subtype C: evidence of etravirine cross-resistance. AIDS **2011**; 25:1123–6.
5. Cozzi-Lepri A, Phillips AN, Martinez-Picado J, et al. Rate of accumulation of thymidine analogue mutations in patients continuing to receive virologically failing regimens containing zidovudine or stavudine: implications for antiretroviral therapy programs in resource-limited settings. J Infect Dis **2009**; 200: 687–97.
6. Chauhan CK, Lakshmi PVM, Sagar V, Sharma A, Arora SK, Kumar R. Primary HIV drug resistance among recently infected cases of HIV in north-west India. AIDS Res Treat **2019**; 2019:1525646.
7. Pachamuthu B, Shanmugam S, Nagalingeswaran K, Solomon SS, Solomon S, Solomon S. HIV-1 drug resistance among untreated patients in India: current status. J Postgrad Med **2006**; 52:183–6.
8. Sinha S, Ahmad H, Shekhar RC, et al. Prevalence of HIV drug resistance mutations in HIV type 1 isolates in antiretroviral therapy naïve population from northern India. AIDS Res Treat **2012**; 2012:905823.
9. Arora SK, Gupta S, Toor JS, Singla A. Drug resistance-associated genotypic alterations in the pol gene of HIV type 1 isolates in ART-naive individuals in north India. AIDS Res Hum Retroviruses **2008**; 24:125–30.
10. Solomon SS, Solomon S, McFall AM, et al. Integrated HIV testing, prevention, and treatment intervention for key populations in India: a cluster-randomised trial. Lancet HIV **2019**; 6:e283–96.
11. Heckathorn DD. Respondent-driven sampling II: deriving valid population estimates from chain-referral samples of hidden populations. Soc Probl **2002**; 49: 11–34.
12. Salganik MJ, Heckathorn DD. Sampling and estimation in hidden populations using respondent-driven sampling. Sociol Methodol **2004**; 34:193–239.
13. Solomon SS, Lucas GM, Celentano DD, et al. Design of the Indian NCA study (Indian National Collaboration on AIDS): a cluster randomized trial to evaluate the effectiveness of integrated care centers to improve HIV outcomes among men who have sex with men and persons who inject drugs in India. BMC Health Serv Res **2016**; 16:652.
14. Laeyendecker O, Konikoff J, Morrison DE, et al. Identification and validation of a multi-assay algorithm for cross-sectional HIV incidence estimation in populations with subtype C infection. J Int AIDS Soc **2018**; 21:e25082.
15. Klock E, Wilson E, Fernandez RE, et al. Validation of population-level HIV-1 incidence estimation by cross-sectional incidence assays in the HPTN 071 (PopART) trial. J Int AIDS Soc **2021**; 24:e25830.
16. Patel EU, Solomon SS, Lucas GM, et al. Temporal change in population-level prevalence of detectable HIV viraemia and its association with HIV incidence in key populations in India: a serial cross-sectional study. Lancet HIV **2021**; 8:e544–53.
17. Laeyendecker O, Kulich M, Donnell D, et al. Development of methods for cross-sectional HIV incidence estimation in a large, community randomized trial. PLoS One **2013**; 8:e78818.
18. Rodgers MA, Vallari AS, Harris B, et al. Identification of rare HIV-1 group N, HBV AE, and HTLV-3 strains in rural south Cameroon. Virology **2017**; 504:141–51.
19. Rodgers MA, Holzmayer V, Vallari A, et al. Hepatitis C virus surveillance and identification of human pegivirus 2 in a large Cameroonian cohort. J Viral Hepat **2019**; 26:30–7.
20. Pineda-Peña AC, Faria NR, Imbrechts S, et al. Automated subtyping of HIV-1 genetic sequences for clinical and surveillance purposes: performance evaluation of the new REGA version 3 and seven other tools. Infect Genet Evol **2013**; 19: 337–48.
21. Rhee SY, Gonzales MJ, Kantor R, Betts BJ, Ravela J, Shafer RW. Human immunodeficiency virus reverse transcriptase and protease sequence database. Nucleic Acids Res **2003**; 31:298–303.
22. . World Health Organization (WHO). HIV drug resistance report. Geneva, Switzerland: WHO; **2021**.
23. Kursa MB, Rudnicki WR. Feature selection with the Boruta package. J Stat Softw **2010**; 36:1–13.
24. Darriba D, Taboada GL, Doallo R, Posada D. jModelTest 2: more models, new heuristics and parallel computing. Nat Methods **2012**; 9:772.
25. Balaban M, Moshiri N, Mai U, Jia X, Mirarab S. TreeCluster: clustering biological sequences using phylogenetic trees. PLoS One **2019**; 14:e0221068.
26. Ragonnet-Cronin M, Hodcroft E, Hué S, et al. Automated analysis of phylogenetic clusters. BMC Bioinformatics **2013**; 14:317.
27. Rose R, Lamers SL, Dollar JJ, et al. identifying transmission clusters with Cluster Picker and HIV-TRACE. AIDS Res Hum Retroviruses **2017**; 33:211–8.
28. Miller V, Ait-Khaled M, Stone C, et al. HIV-1 reverse transcriptase (RT) genotype and susceptibility to RT inhibitors during abacavir monotherapy and combination therapy. AIDS **2000**; 14:163–71.
29. Averbuch D, Schapiro JM, Lanier ER, et al. Diminished selection for thymidine-analog mutations associated with the presence of M184V in Ethiopian children infected with HIV subtype C receiving lamivudine-containing therapy. Pediatr Infect Dis J **2006**; 25:1049–56.
30. Miller MD, Margot N, Lu B, et al. Genotypic and phenotypic predictors of the magnitude of response to tenofovir disoproxil fumarate treatment in antiretroviral-experienced patients. J Infect Dis **2004**; 189:837–46.
31. Wainberg MA. The impact of the M184V substitution on drug resistance and viral fitness. Expert Rev Anti Infect Ther **2004**; 2:147–51.
32. Bacheler LT, Anton ED, Kudish P, et al. Human immunodeficiency virus type 1 mutations selected in patients failing efavirenz combination therapy. Antimicrob Agents Chemother **2000**; 44:2475–84.
33. Gulick RM, Ribaudo HJ, Shikuma CM, et al. Triple-nucleoside regimens versus efavirenz-containing regimens for the initial treatment of HIV-1 infection. N Engl J Med **2004**; 350:1850–61.
34. Reuman EC, Rhee SY, Holmes SP, Shafer RW. Constrained patterns of covariation and clustering of HIV-1 non-nucleoside reverse transcriptase inhibitor resistance mutations. J Antimicrob Chemother **2010**; 65:1477–85.
35. Melikian GL, Rhee SY, Varghese V, et al. Non-nucleoside reverse transcriptase inhibitor (NNRTI) cross-resistance: implications for preclinical evaluation of novel NNRTIs and clinical genotypic resistance testing. J Antimicrob Chemother **2014**; 69:12–20.
36. Rhee SY, Liu T, Ravela J, Gonzales MJ, Shafer RW. Distribution of human immunodeficiency virus type 1 protease and reverse transcriptase mutation patterns in 4183 persons undergoing genotypic resistance testing. Antimicrob Agents Chemother **2004**; 48:3122–6.

37. Zhang Z, Xu W, Koh YH, et al. A novel nonnucleoside analogue that inhibits human immunodeficiency virus type 1 isolates resistant to current nonnucleoside reverse transcriptase inhibitors. Antimicrob Agents Chemother **2007**; 51:429–37.
38. Mulenga LB, Fwoloshi S, Mweemba A, et al. Dolutegravir with recycled NRTIs is noninferior to PI-based ART: VISEND trial. Abstract 135. In: Conference on Retroviruses and Opportunistic Infections, Virtual, **2022**.
39. Paton N, Musaazi J, Kityo C, et al. Nucleosides and Darunavir/Dolutegravir in Africa (NADIA) trial: outcomes at 96 weeks. Abstract 137. In: Conference on Retroviruses and Opportunistic Infections, Virtual, **2022**.
40. Mehta SH, Lucas GM, Solomon S, et al. HIV care continuum among men who have sex with men and persons who inject drugs in India: barriers to successful engagement. Clin Infect Dis **2015**; 61:1732–41.