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HIV Drug Resistance Surveillance Among Jamaican Men Who Have Sex with Men Should Be Prioritized for Reducing HIV Transmission

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Abstract

The prevalence of human immunodeficiency virus type 1 (HIV-1) is highest among men who have sex with men (MSM) in Jamaica but no genotypic data are available on the virus strains that are responsible for the epidemic among this key population. HIV-1 polymerase (*pol*) genes from 65 MSM were sequenced and used to predict drug resistance mutations. An HIV drug resistance prevalence of 28% (minimum 13%) was observed among this cohort, with the most frequent mutations conferring resistance to efavirenz, nevirapine, and lamivudine. Phylogenetic analysis of the sequences revealed 10 times the number of linked HIV infections among this cohort than respondent reporting. HIV treatment and prevention efforts in Jamaica could benefit significantly from Pol genotyping of the HIV strains infecting socially vulnerable MSM prior to initiating antiretroviral therapy (ART), as this would guide suppressive ART and unearth HIV transmission clusters to enable more effective delivery of treatment and prevention programs.

The human immunodeficiency virus (HIV) infects approximately 35 million people globally and accounted for 1.6 million deaths in 2012. The Caribbean region has an HIV prevalence of 1%, which is the second highest after sub-Saharan Africa. The severity of the epidemic varies among individual countries in the region, as Cuba has the lowest HIV prevalence in the Caribbean (<0.1%) while Bahamas has the highest prevalence of 3.3%.[1] Heterosexual transmission accounts for the greatest number of new HIV cases globally, but men who have sex with men (MSM) often have a higher HIV prevalence than other population groups.[2] In Jamaica, HIV prevalence among the general adult population is 1.8% while the HIV prevalence among MSM is estimated as 38%.[1] Adverse social experiences such as rape, incarceration, homelessness, and unemployment contribute to the high HIV prevalence among Jamaica's MSM.[3]

In mixed HIV epidemics, such as Jamaica's, key populations must be specially targeted in order to achieve a significant reduction in population level HIV incidence. The World Health Organization recommends scaling up the use of antiretroviral (ARV) drugs for treating HIV-infected individuals and preventing transmission.[4] However, scaling up of antiretroviral use necessitates increased surveillance of HIV drug resistance.[4,5] A transmitted drug resistance of 12–29% has been reported for HIV-infected heterosexuals in Jamaica,[6,7] but estimates of drug resistance among the island's MSM is lacking. Integrating the use of ARV drugs in HIV prevention programs for Jamaica's MSM should be considered since HIV prevalence among this risk group has remained high since 1995, despite HIV educational interventions for behavior change.[8] Knowledge of the drug susceptibility of HIV strains in the target population could enable the most effective implementation of any ARV-based prevention strategy; consequently, this should be obtained for HIV strains infecting MSM in Jamaica.

Jamaica's HIV response scarcely uses genotypic data on the circulating HIV strains, despite evidence showing its usefulness in guiding effective antiretroviral therapy and more recent demonstrations that it can reveal transmission events and therefore sexual networks.[9] Since sequencing of the HIV polymerase gene can identify both drug resistance and HIV transmission networks, it is a valuable tool for understanding epidemics among stigmatized populations, such as MSM, as well as sex workers and their clients.[10]

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Identifying and understanding HIV transmission networks can support the successful design and implementation of treatment strategies.

This study investigates the polymerase gene of HIV strains infecting MSM in Jamaica to detect drug resistance mutations and identify transmission networks among this key population, so as to gain insights that could contribute to the development of effective interventions to reduce HIV transmission in Jamaica.

Plasma was previously collected from HIV-positive MSM who participated in a survey of Men's Health in Jamaica from 2010 to 2011. Participant recruitment, sample collection, participant characteristics, and ethical approval were previously described.[3]

RNA was extracted from plasma of 141 HIV-seropositive MSM using the QiaAmp Viral RNA Mini kit (Qiagen) according to the manufacturer's instructions. The RNA was reverse transcribed using SuperScript III RT (Life Technologies) and used as template in nested PCR targeting the complete protease (PR) and the majority of the reverse transcriptase (RT) gene. Nested PCR was done using the Expand High Fidelity PCR System (Roche).

First round primers were MPOLUP3 (5′-TTCAGAGCAG ACCAGAGCCAACAGC) and BPOLDN1 (5′-ATAAATCT GACTTGCCCAATTCAATTTTCC) and second round primers were BPOLUP4 (5′-ACAACAACTCCCTCTCAG AAGCAGG) and CPOLDN2 (5′-CCACTAACTTCTGTAT ATCATTGACAGTCC). The second round primers plus two internal primers, MBP006 (5′-CCTCCTTTTCCATTTCTG) and MBP008 (5′-CAGAAATGGAAAAGGAAG), were used for population sequencing through the University of North Carolina Genomic Analysis Core Facility. When necessary, two other internal primers were also used, MBP001 (5′-CTAATACTGTACCTATAGC) and MBP010 (5′-TCAAAGTAAGACAGTATGATC). Sequences were aligned and assembled into a single contiguous consensus for each individual using Sequencher software (Gene Codes Corporation). The subtype and drug resistance profile of the HIV PR/RT sequences were determined *in silico* using the REGA Subtyping tool (www.bioafrica.mrc.ac.za/rega-genotype/html/subtypinghiv.html) and the Calibrated Population Resistance (http://cpr.stanford.edu/cpr/index.html) tool, respectively. The two most identical homologs to each of the Jamaican MSM sequences were retrieved from the GenBank database.

A multiple alignment of the Jamaican MSM sequences and their homologs was constructed and included all GenBank HIV-1 sequences from Jamaica, the HXB 2 subtype B reference sequence, and consensus sequences from other HIV subtypes. Codon alignment of the sequences was performed with the Codon Alignment v.1.1.0 tool (www.hiv.lanl.gov/content/sequence/CodonAlign/codonalign.html) and the multiple alignment used for maximum likelihood phylogenetic reconstruction with a general time reversible model. The original alignment was stripped of 36 protease and 32 reverse transcriptase codons associated with drug resistance[5,11] and phylogenetic reconstruction was repeated on this edited alignment. Bootstrap analysis was performed on 1,000 replicates to assess nodal support. Putative transmission clusters were defined as ≥2 closely related sequences (≤1.5% mean intracluster difference in genetic distance) with high bootstrap support (≥98%) at the node-defining clade.

The HIV-1 polymerase (Pol) gene segment spanning coordinates 2252–3252 of HXBc2 and containing the complete 99 codons of PR and the first 234 codons of RT was successfully amplified from 46% (65/141) of samples and deposited in GenBank (accession nos.: KP159440 and KP159449–KP159508). All 65 sequences were HIV-1 subtype B and had an average sequence distance of 2.6% (range 0–5.4%).

The antiretroviral therapy (ART) treatment status of the study participants was not ascertained in the survey; however, major drug resistance mutations (DRMs) were observed in 28% (18/65) of the sequences. The prevalence of drug resistance in the entire HIV-positive MSM dataset will be no less than 13% (18/141). All 18 sequences with major DRMs were predicted to be resistant to nonnucleoside reverse transcriptase inhibitors (NNRTIs), with 67% (12/18) having the K103N mutation that gives high level resistance to nevirapine and efavirenz.[12] Dual resistance to both nucleoside reverse transcriptase inhibitors (NRTIs) and NNRTIs was predicted for 55% (10/18) of samples showing DRMs, and M184V, which confers high level resistance to lamivudine,[12] was the most common NRTI mutation in the dataset (Table 1). From the survey, MSM reported characteristics such as age, sexual orientation, socioeconomic status, literacy, ever raped, marital status, and number of partners. There was no significant difference in the surveyed characteristics between MSM who had drug-resistant HIV (drHIV) and MSM without drHIV or HIV-seropositive MSM from whom virus amplification failed.

Phylogenetic analyses with the DRM stripped alignment revealed that 31% (20/65) of the MSM HIV-1 sequences formed eight clusters of two or more sequences with 100% bootstrap support and a mean intracluster genetic distance ≤ 1.5% (Fig. 1). Cluster H (Fig. 1) contained a Jamaican reference sequence that was isolated from a Jamaican MSM in 2007; this was the only cluster containing any reference sequence. Cluster G, which is a dyad, is the only cluster containing DRM. Both members of Cluster G had the K103N mutation and one member additionally had M41L, M184V, and T215Y, which provide resistance to NRTIs. The majority (80%) of MSM whose HIV sequences clustered were 16–24 years old while only 44% of men whose sequences remained unclustered were in this younger age group. Neither age nor any of the other surveyed characteristics were statistically different between clustered and unclustered HIV sequences.

Table 1. Frequency of Different Types of Drug Resistance Mutations Among Men Who Have Sex with Men in Jamaica

NNRTI	N *(%)*	*NRTI*	N *(%)*
K103N	12 (67%)	M184V	9 (50%)
Y181C	5 (28%)	T215Y	4 (22%)
G190A/S	2 (11%)	M41L	4 (22%)
K101E	1 (6%)	K65R	2 (11%)
Y188L	1 (6%)	L74I	2 (11%)
P225H	1 (6%)	K70R	1 (6%)
M230L	1 (6%)	L210W	1 (6%)

NNRTI, nonnucleoside reverse transcriptase inhibitors; NRTI, nucleoside reverse transcriptase inhibitors.

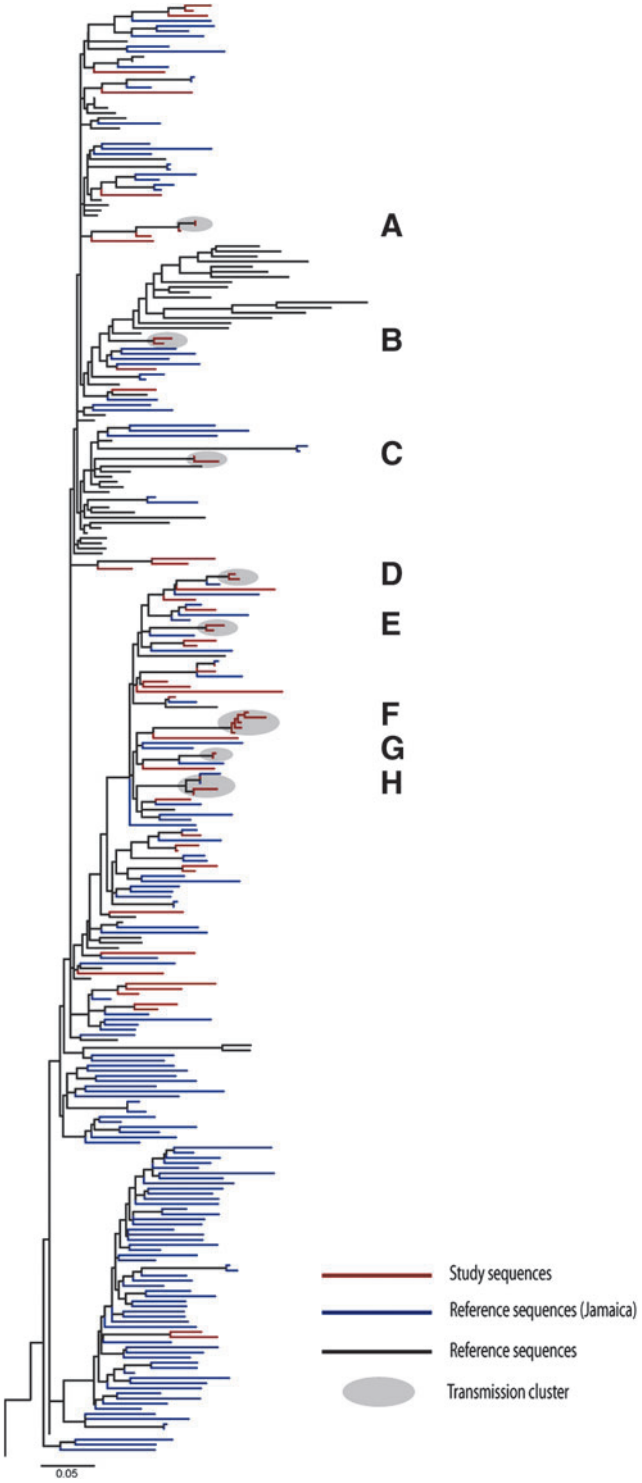

FIG. 1. Maximum-likelihood phylogenetic tree of 65 study sequences obtained from Jamaican men who have sex with men from 2010 to 2011, with closely related reference sequences available in GenBank (references sampled in Jamaica are indicated in *blue*). Study sequences ($n=21$; 30%) were identified in eight transmission clusters (indicated by *gray shading* and letters A–H).

In this study, the majority (54%) of samples indicated to be HIV positive based on antibody tests failed to generate amplicons in PCR. Control reactions confirm the integrity and viability of the RNA extraction and PCR reactions. It is therefore likely that the samples are at fault; however, limited sample volumes prevented further investigation of this. The ART status of the study participants was not ascertained during the survey, but 6.4% (9/141) of the HIV-seropositive survey participants reported never receiving an HIV test before, indicating that the overwhelming majority of these men could have been aware of their HIV-positive status. If participants were receiving ART, then this could have suppressed their circulating HIV RNA to undetectable levels and consequently contribute to 54% of the samples failing to produce amplicons.

The 28% drug resistance prevalence observed among this sample set is consistent with that reported for treatment-experienced (35%) and treatment-naive (29%) heterosexual Jamaicans[6,7] and higher than the 7% reported in Cuba.[13] None of the samples had major protease inhibitor mutations, in contrast to findings among a Jamaican heterosexual cohort.[7] The epidemiology survey through which these samples were obtained did not ask respondents to self-report whether they previously received ARV treatment; it is therefore unclear whether the 26% prevalence of drug resistance is acquired, transmitted, or due to both. The M184V mutation readily emerges during suboptimal lamivudine treatment, but reduces the ability of the virus to replicate. The result is that M184V is poorly maintained in the absence of lamivudine, such as during primary infection, soon after HIV transmission.[14] M184V occurred in 50% of the drug-resistant sequences obtained. Considering the poor replicative fitness of M184V, men possessing viruses with this mutation are more likely to have been drug exposed rather than acquiring these mutant HIV strains by horizontal transmission. Efavirenz, nevirapine, and lamivudine are among the ARV drugs used in first line ART in Jamaica.[15]

The presence of resistance mutations against efavirenz, nevirapine, and lamivudine suggests that similar to the heterosexual population, a subset of MSM will fail or are failing first line antiretroviral therapy. Individual ART failure prevents the realization of population level protection against HIV and erodes the future usefulness of ARV drugs. The inconsistent condom use reported by MSM in general[8] suggests the likelihood for transmission of resistant strains. There is therefore an urgent need for Jamaica to initiate drug resistance surveillance.

This study provides insight into drug resistance among Jamaican MSM and provides the first look at HIV drug resistance among MSM in a Caribbean nation other than Cuba. The detection of any statistically significant behavioral or demographic association with drug resistance or clustering was constrained by the small sample size that resulted from only 46% (65/141) of the PR/RT being amplifiable from the HIV-seropositive MSM in the survey. There is strong social stigma against MSM in Jamaica, which drives the HIV epidemic underground and contributes to the spread of HIV. The men from whom these samples were obtained are unlikely to be representative of the MSM population as a whole because they were primarily of lower socioeconomic status with a history of adverse life events and had high rates of transactional and commercial sex.[8] Consequently, the 26% drug

resistance prevalence among this sample set is not generalizable to the wider HIV-seropositive MSM community in Jamaica. Nevertheless, the drug resistance profiles unveiled here suggest an urgent need to scale up HIV testing and HIV drug resistance monitoring to acquire concrete insights that can lead to effective prevention and treatment of HIV infection among MSM.

Phylogenetic analyses revealed that 31% of the sequences obtained in this study clustered closely with at least one other study sequence, which is similar to other studies with convenience-based samples.[9] Phylogenetic clusters with low genetic distance are likely to represent linked transmission, especially when DRM sites are excluded from analyses[9] and unsampled third parties may be part of the transmission chain. From the survey, 2.9% (2/70) of men from whom HIV sequences were obtained reported having been invited into the study by a sexual partner. Despite the stringent criteria utilized in demarcating clusters (≤0.015 genetic distance and 100% bootstrap support), the phylogenetic analyses detected 10 times the number of linked HIV transmissions than that reported by MSM. The discrepancy between the two methods may arise from respondent misreporting, anonymous partners, indirect transmissions, as well as the fact that current sexual partners are not necessarily the transmission partners and highlights a limitation when using sexual contact reporting to reconstruct transmission networks. Jamaica could benefit greatly by using HIV Pol genotyping for both drug resistance surveillance as well as identifying transmission clusters. Transmission clusters could then be characterized to better understand the epidemic and design appropriate strategies for improving HIV treatment and prevention.

This study suggests that the prevalence of HIV drug resistance among MSM in Jamaica is comparable to that among the general population. It is therefore necessary to concomitantly scale up genotypic testing of HIV strains among MSM and the general population for effective HIV treatment and prevention to ensure the continued success of Jamaica's HIV response.

Sequence Data

Sequences generated in this study were deposited in the NCBI GenBank database under the accession numbers KP159440 and KP159449–KP159508.

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Author Disclosure Statement

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