HIV-1 Antiretroviral Resistance in Cuba, 2009–2014

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ABSTRACT

INTRODUCTION By the end of 2017, there were more than 28,000 individuals living with HIV in Cuba, over 80% receiving antiretroviral therapy, which dramatically reduces viral replication, improves immune status and decreases risk of transmission. These results could be jeopardized by emergence of HIV-1 drug resistance. In 2009, a test for HIV-1 genotypic resistance was introduced in routine clinical practice in Cuba.

OBJECTIVE Investigate antiretroviral resistance and its relation to subtype distribution in HIV-1 treatment-naïve and previously treated patients in Cuba.

METHODS Resistance and HIV-1 subtype distribution were determined in 342 antiretroviral treatment-naïve patients and 584 previously treated for HIV-1 whose blood specimens were sent to the Pedro Kourí Tropical Medicine Institute during 2009–2014. Transmitted drug resistance was determined using the Calibrated Population Resistance Tool v.6. Drug resistance analysis was conducted using the algorithm Rega v9.1.0.

RESULTS Prevalence of transmitted drug resistance was 11.4%, and 41% of mutated viruses exhibited dual-class resistance to nucleoside reverse transcriptase inhibitor and non-nucleoside reverse transcriptase inhibitor. Overall, 84.9% of patients had ≥1 resistance mutation, 80% had ≥1 nucleoside reverse transcriptase inhibitor mutation, 71.4% had ≥1 non-nucleoside reverse transcriptase inhibitor mutation and 31.7% had ≥1 protease inhibitor mutation. K65R and K101E mutations were significantly more frequent in subtype C, L210W in CRF19_cpx, and M47V/I in CRF BGs (20, 23, 24). Full class resistance to nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, protease inhibitors and multidrug resistance were detected in 21.2%, 32.4%, 8% and 4.1% of patients, respectively. Average percentage resistance to nucleoside reverse transcriptase inhibitor, protease inhibitor, full class resistance to nucleoside reverse transcriptase inhibitor, protease inhibitor and multidrug resistance increased in patients failing two or more regimens. Nevertheless, after 2011, a declining trend was observed in the frequency of multidrug resistance and full class resistance to nucleoside reverse transcriptase inhibitors and protease inhibitors.

CONCLUSIONS Detected levels of transmitted drug resistance highlight the need for a national surveillance study in treatment-naïve patients. Resistance prevalence is high in previously treated patients but appears to be decreasing over time. The frequency of resistance mutations in recombinant forms of HIV in Cuba needs further study.

KEYWORDS Antiretroviral therapy, highly active antiretroviral therapy, HIV, anti-HIV agents, drug resistance, multiple drug resistance, Cuba

INTRODUCTION

There is a global and regional commitment to reach the Joint United Nations Programme on HIV/AIDS' 90–90–90 target in 2020, and to end AIDS by 2030.[1] The 90–90–90 target is that 90% of all people living with HIV will have been diagnosed, 90% of all people with known HIV infection will be receiving antiretroviral therapy (ART), and 90% of all people receiving ART will have a suppressed viral load. Latin America and the Caribbean region face major challenges in meeting this target. PAHO has reported substantial progress (continuing decline in AIDS-related deaths and mother-to-child HIV transmission, increasing numbers of people who know their HIV status and receive treatment), but the annual number of new infections in the Caribbean has remained static since 2012 and HIV incidence remains high in key populations, mainly men who have sex with men (MSM) and transgender women.[1,2]

IMPORTANCE This study shows high levels of resistance to antiretroviral drugs used in Cuba up to 2014, indicating an urgent need for changes in first-line therapy. It also reinforces the necessity of resistance testing for all patients failing antiretroviral therapy.

In 2001, Cuba's Ministry of Public Health (MINSAP) decided to produce generic drugs for treatment of HIV. Efforts to provide access to ART have accelerated since then and have resulted in decreased AIDS mortality and incidence of opportunistic infections.[3,4] By the end of 2017, >28,000 individuals were living with HIV in Cuba, >90% of infected individuals were aware of their HIV status and approximately 80% were on ART. However only half of patients in treatment were virally suppressed (information from MINSAP's National HIV Registry, 2016), a major gap for Cuba in meeting the third 90–90–90 target.

ART dramatically reduces viral replication, improves immune status and decreases risk of HIV transmission, but these outcomes could be jeopardized by HIV-1 resistance. A 2004 study that explored ART resistance in Cuba found low levels of resistance.[4] In 2009 the Pedro Kourí Tropical Medicine Institute (IPK) introduced an in-house HIV-1 genotyping system for routine assessment of drug resistance in Cuban patients.[5]

The aim of this research was to investigate the frequency and profile of antiviral drug resistance in HIV-1 treatment-naïve and previously

treated patients and estimate the prevalence of specific resistance mutations among HIV-1 variants circulating in Cuba.

METHODS

Population IPK is the reference center for HIV care and therapy in Cuba, thus samples from all over Cuba are sent to IPK for genotypic drug resistance testing. A total of 926 viral sequences were collected of all HIV-1 genotypic drug resistance testing carried out at IPK's laboratory as part of routine clinical care from April 2009 to December 2014. One sample per patient was analyzed from 584 previously treated patients and 342 treatment-naïve individuals. Only epidemiologic, demographic, clinical, virological and immunological data were collected; no patient identifying information was retained.

Viral load and CD4 count Plasma HIV-1 viral loads were determined using the Nuclisens Easy Q HIV-1 kit v2.0 (Biomérieux, France) or COBAS Ampliprep/COBAS Taqman HIV-1 test v2.0 for use with the High Pure System (Roche, Germany). CD4 cell counts were determined using a Becton Dickinson counter (Bio-Sciences, USA).

Genotypic drug resistance testing For HIV-1 genotyping, 1 mL plasma was ultracentrifuged and the suspended pellet extracted using QIAamp Viral RNA Kit (QIAGEN, Germany) manually, or automatically on QIAcube (QIAGEN, Germany), per manufacturer's protocol. HIV-1 RNA reverse transcription, amplification and population-based bidirectional Sanger sequencing of pol fragments were carried out as described elsewhere.[5] Sequences obtained covering a fragment of 1302 bp that overlaps with codons 1–99 of protease and 1–335 of reverse transcriptase were edited and assembled using Sequencher, v4.1 (Gene Codes Corporation, USA).

Data analysis HIV subtype was determined using Rega subtyping tool version 3 and confirmed by manual phylogenetic analysis, using MEGA v6 (Kimura's 2-parameter correction, bootstrap 1000).[6]

Therapeutic failure was defined as ART failure to reduce and maintain viral load at <200 copies/mL. Information about treatment compliance was unavailable.

Prevalence of genotypic drug resistance mutations in treatment-naïve patients was analyzed using the Calibrated Population Resistance Tool v6 and based on WHO's 2009 surveillance of drug-resistant mutations.[7]

Drug resistance interpretation in previously treated patients was conducted using the resistance interpretation algorithm Rega v9.1.0. Resistance to drug classes was calculated by averaging the percentage of resistance (R) and intermediate resistance (I) for each drug class. Full-class resistance (FCR) was defined as lack of full susceptibility to any antiviral drug in a given drug class.[8] Multidrug resistance (MDR) was scored if the virus strain was susceptible to no more than one drug belonging to the three commonly available drug classes in Cuba.[8] For statistical analysis, chi square with Yates correction, Fisher exact test and odds ratios (OR) were calculated using Epidat v3.0.10.[9]

Ethics The study was approved by the IPK Ethics Committee and complies with the Declaration of Helsinki.[10] At time of collection, all subjects included in the study gave written informed consent for their specimens to be used for research purposes.

RESULTS

Study population Participants were predominantly male (83.3%), MSM (76.8%) and resided in Havana (66.1%). Median age was 32.4 years (interquartile range, IQR: 24.6–41.3) and 40.5 years (IQR: 33.6–46.6) for treatment-naïve and previously treated patients, respectively. Median CD4 cell count in treatment-naïve patients was higher than in previously treated patients (349 cells/mm^3 vs 208 cells/mm^3), but viral loads were similar in both groups (18,966 copies/mL and 21,264 copies/mL, respectively) (Table 1).

Mean time since ART initiation was 3 years (IQR: 1.1–5.6). All patients had received nucleoside reverse transcriptase inhibitors (NRTI); 90.1% had received ≥1 non-nucleoside reverse transcriptase inhibitors (NNRTI) and 62.7% had received ≥1 protease inhibitor (PI). Only 12.8% of patients had received mono- or dual therapy regimens. At the time of drug resistance testing, the most commonly prescribed drugs were lamivudine (3TC), 92.5%; zidovudine (AZT), 44.7%; and nevirapine (NVP), 44.2%.

Subtype distribution In the study period, 30.9% of HIV-1 strains were subtype B, 22% were BG recombinants (CRF20_BG, CRF23_BG and CRF24_BG), 18.3% CRF19_cpx, 9.8% CRF18_cpx, 6.5% URF, 5.5% subtype C, 2.3% subtype G and 4.7% were other subtypes with frequencies <1% (subtypes A, F, J, H; CRF02_AG, CRF06_cpx, CRF14_BG and CRF31_BC). There were no significant differences between HIV-1 subtypes identified in samples from treatment-naïve and those from treatment–experienced patients (Table 1).

Drug resistance in treatment-naïve *patients* Overall, 11.4% (39/342) of treatment-naïve HIV-1 patients showed evidence of transmitted drug resistance (TDR). The frequency of single TDR against NRTI was 20.5%, against NNRTI 12.8% and against PI 17.9%, for a total of 51.2% single drug class resistance. High prevalence of dual-class resistance was observed (43.6%), mainly to NRTI+NNRTI (41%). Triple drug class resistance was observed in 2 patients (5.1%) (Table 2).

The most common mutations related to NRTI resistance were M184V/I (46.2%), T215Y/I/S/D (25.6%) and K219Q/E/N/R (20.5%); for NNRTI were K103N (23.1%) and Y181 C/I (28.2%) while for PI was M46I/L (15.4%) (Table 2).

No significant differences were observed in overall TDR mutation frequency between chronically infected patients (48.7%) and recently diagnosed individuals (51.3%). However, TDR to NRTI was higher in chronically infected individuals. In contrast, TDR against NNRTI and PI was higher in recently diagnosed individuals. Mutation M184V/I was more frequently detected among chronically infected individuals ($p = 0.0390$, OR 4.0, 95% CI 1.0–15.2) (Table 2).

Drug resistance mutations in previously treated patients Overall, 84.9% of patients had ≥1 resistance mutation, 80% had ≥1 NRTI mutation, 71.4% had ≥1 NNRTI mutation and 31.7% had ≥1 PI mutation. The most frequent NRTI mutations were M184V/I (75.9%), T215Y/F (37.3%), and M41L (25.7%). The most frequent NNRTI mutations were K103N/S (28.6%), Y181C/I/V (26.4%) and G190S/A (21.7%). The most common PI mutations were L90M (16.3%), M46I/L (15.9%) and V82A/T/F/S (10.3%) (Table 3).

Frequency of drug resistance mutations to any drug class was significantly higher in patients who had undergone ≥3 therapy regimens ($p = 0.0149$, OR 2.1, 95% CI 1.1–3.8) compared to those with fewer regimens. Mutations associated with NRTIs, NNRTIs and PIs were observed in 74.4%, 69.9% and 9.7% of first-line failures, respectively. In patients failing second-line therapy, the respective frequencies were 79.2%, 72.2% and 31.9%. In patients exposed to ≥3 ART regimens, these values increased to 84.1%, 72% and

Table 1: Characteristics of patients with HIV-1

Characteristic	Total	Treatment naïve	Previously treated
Patients [n (%)]	926 (100)	342 (36.9)	584 (63.1)
Age [median years (IQR)]	37.8 (30.0–45.0)	32.4 (24.6–41.3)	40.5 (33.6–46.6)
Male [n (%)]	771 (83.3)	290 (82.6)	481 (82.4)
Transmission route MSM [n (%)]	711 (76.8)	270 (76.9)	441 (75.5)
CD4 [median cell count/mm^3 (IQR)]	241 (138–382)	349(201–479)	208 (111–305)
Viral load median RNA copies/mL (IQR)	20,000 (3966–80,458)	18,966 (3794–84,768)	21,264 (4052–80,458)
HIV status [n (%)]			
Recent diagnosis[a]	199 (21.5)	178 (52.0)	21 (3.6)
Chronic infection[b]	727 (78.5)	164 (48.0)	563 (96.4)
Therapy history			
Years since therapy initiation [median years (IQR)]	3.0 (1.1–5.6)	—	3.0 (1.1–5.6)
Previous therapy exposure [n (%)]			
Mono or dual	75 (12.8)	—	75 (12.8)
NRTI	584 (100.0)	—	584 (100.0)
NNRTI	526 (90.1)	—	526 (90.1)
PI	366 (62.7)	—	366 (62.7)
ART at time of resistance testing [n (%)]			
NRTI			
3TC	540 (92.5)	—	540 (92.5)
ABC	93 (15.9)	—	93 (15.9)
AZT	261 (44.7)	—	261 (44.7)
D4T	129 (22.1)	—	129 (22.1)
DDI	6 (1.0)	—	6 (1.0)
FTC	17 (2.9)	—	17 (2.9)
TDF	93 (15.9)	—	93 (15.9)
NNRTI			
EFV	54 (9.2)	—	54 (9.2)
NVP	258 (44.2)	—	258 (44.2)
PI			
ATV/r	5 (0.9)	—	5 (0.9)
FPV/r	71 (12.2)	—	71 (12.2)
IDV/r	37 (6.3)	—	37 (6.3)
LPV/r	63 (10.8)	—	63 (10.8)
NFV	40 (6.8)	—	40 (6.8)
SQV/r	52 (8.9)	—	52 (8.9)
TPV/r	5 (0.9)	—	5 (0.9)
HIV-1 subtype			
B	286 (30.9)	104 (30.4)	182 (31.2)
C	51 (5.5)	12 (3.5)	39 (6.7)
G	21 (2.3)	2 (0.6)	19 (3.3)
CRF 18_cpx	91 (9.8)	35 (10.2)	56 (9.6)
CRF 19_cpx	169 (18.3)	65 (19.0)	104(17.8)
CRF_BGs (20, 23, 24)	204 (22.0)	91 (26.6)	113 (19.3)
URF	60 (6.5)	17 (5.0)	43 (7.4)
Other	44 (4.7)	16 (5.2)	28 (4.8)

[a]sampling <1 year after HIV-1 diagnosis (recent infections included)
[b]sampling >one year after HIV-1 diagnosis
3TC: lamivudine ABC: abacavir ART: antiretroviral therapy ATV: atazanavir AZT: zidovudine
CRF: circulating recombinant form D4T: stavudine DDI: didanosine EFV: efavirenz FPV: fosamprenavir
FTC: emtricitabine IDV: indinavir IQR: interquartile range LPV: lopinavir
MSM: men who have sex with men NFV: nelfinavir NNRTI: non-nucleoside reverse transcriptase inhibitor
NRTI: nucleoside reverse transcriptase inhibitor NVP: nevirapine PI: protease inhibitor /r: ritonavir
SQV: saquinavir TDF: tenofovir TPV: tipranavir URF unique recombinant form

46.2%, respectively. For each specific NRTI and PI mutation, significant differences were observed between patients exposed to 1 or 2 regimens compared to those exposed to ≥3 regimens ($p < 0.05$). The number of patients harboring viruses with NNRTI mutations did not significantly increase in those exposed to ≥2 regimens, but the frequency of K103N/S and V108I was higher ($p = 0.0402$ and $p = 0.0049$, respectively) in patients exposed to ≥3 than in patients failing the first regimen. Dual-class resistance mutations to NRTI+NNRTI were more frequently observed in patients exposed to 2 therapies ($p = 0.0017$, OR 2.0, 95% CI 1.3–3.2) compared with first therapy failures. The same was observed for dual-class resistance to NRTI+PI ($p < 0.001$, OR 14.2, 95% CI 6.6–30.5) and for triple class resistance ($p = 0.0067$, OR 2.0, 95% CI 1.2–3.4) (Table 3).

Prevalence of resistance mutations among different subtypes in patients on active ART As shown in Table 4, NRTI resistance mutation K65R was significantly more frequent among subtype C isolates from patients treated with 3TC whereas L210W was present in higher proportions among CRF19_cpx isolates from individuals failing AZT or stavudine (D4T) regimens. NNRTI resistance mutation K101E was more frequent in subtype C isolates from patients failing NVP therapy. PI mutation M47V/I was more frequent among recombinant forms CRF_BGs (20, 23, 24) isolates from patients failing LPV/r therapy.

Drug resistance prevalence and trends in previously treated patients The highest drug resistance levels against NRTI were detected for 3TC/FTC (76.9%) and ABC (50.2%); against NNRTIs were for NVP (71.2%) and EFV (70.9%); against PI were NFV (31.8%) and SQV/r (26.4%) (Figure 1a).

The average proportions of patients harboring NRTI, NNRTI and PI resistance were 52.7%, 54.7% and 21.4%, respectively. This average significantly increased in patients failing ≥2 regimens for NRTI ($p < 0.0001$) and PI resistance ($p < 0.0001$). FCR to NRTI, NNRTI and PI was observed in 21.2%, 32.4% and 8%, respectively. FCR to NRTI and PI also significantly increased after two regimens failures ($p = 0.0001$ and $p < 0.0001$, respectively). MDR was present in 4.1% of studied patients and significantly increased after two regimens failures ($p = 0.0017$) (Figure 1b).

From 2009 to 2014, a significant declining trend in MDR prevalence was noticed. In 2009 12.6% of patients harbored an MDR virus, whereas in 2011 prevalence fell to 2.3% (OR = 0.80, 95% CI 0.69–0.93; $p = 0.003$) (Figure 2a). Furthermore, a significant decline

Table 2: HIV-1 drug resistance mutations in treatment-naïve patients

Mutation	Total n (%)	Recent diagnosis n (%)[a]	Chronic infection n (%)[b]	OR (95% CI)
Any	39 (100)	20 (51.3)	19 (48.7)	
NRTI[c]				
Single TDR against NRTI	8 (20.5)	2 (10.0)	6 (31.6)	4.2 (0.7–24.0)
Any	27 (69.2)	12 (60.0)	15 (78.9)	2.5 (0.6–10.3)
M41L	6 (15.4)	3 (15.0)	3 (15.8)	–
D67N/G	7 (17.9)	2 (10.0)	5 (26.3)	3.2 (0.5–19.1)
M184V/I	18 (46.2)	6 (30.0)	12 (63.2)	4.0 (1.0–15.2)
T215Y/I/S/D	10 (25.6)	4 (20.0)	6 (31.6)	1.8 (0.4–8.0)
K219Q/E/N/R	8 (20.5)	5 (25.0)	3 (15.8)	1.8 (0.4–8.8)
NNRTI[c]				
Single TDR against NNRTI	5 (12.8)	3 (15.0)	2 (10.5)	1.5 (0.2–10.1)
Any	18 (46.2)	10 (50.0)	8 (42.1)	1.4 (0.4–4.9)
K103N	9 (23.1)	5 (25.0)	4 (21.1)	1.3 (0.3–5.6)
Y181C/I	11 (28.2)	6 (30.0)	5 (26.3)	1.2 (0.3–4.9)
G190A	7 (17.9)	6 (30.0)	1 (5.3)	7.7 (0.8–71.7)
PI[c]				
Single TDR against PI	7 (17.9)	5 (25.0)	2 (10.5)	2.8 (0.5–16.8)
Any	10 (25.6)	6 (30.0)	4 (21.1)	1.6 (0.4–6.9)
M46I/L	6 (15.4)	4 (20.0)	2 (10.5)	2.1 (0.3–13.2)
Dual or triple TDR				
NRTI+NNRTI	16 (41.0)	9 (45.0)	7 (36.8)	1.4 (0.4–5.1)
NRTI+PI	1 (2.6)	0 (0.0)	1 (5.3)	—
NRTI+NNRTI+PI	2 (5.1)	1 (5.0)	1 (5.3)	–

[a]sampling <1 year after HIV-1 diagnosis (recent infections included)
[b]sampling >one year after HIV-1 diagnosis
[c]NRTI mutations K70R, L210W; NNRTI mutations L100I, K101P/E, Y188L, P225H and PI mutations V32I, I47V/A, I54L/M/V/T/A/S, G73C/S/T/A, L76V, V82A, I85V, N88D/S, L90M were observed <15% of patients.
NNRTI, non-nucleoside reverse transcriptase inhibitor
NRTI: nucleoside reverse transcriptase inhibitor PI: protease inhibitor
TDR: transmitted drug resistance

was observed for FCR NRTI and FCR PI. Statistical analysis demonstrated that FCR NRTI is significantly decreasing over time, from 37.6% in 2009 to 9.5% in 2014 (OR = 0.74, 95% CI 0.70–0.82; p <0.001). For FCR PI, a significant decrease was also observed between 2009 and 2012, from 24.7% to 0.8% (OR = 0.80, 95% CI 0.71–0.89; p <0.001). When this analysis was performed to include any drug in each drug class, PI resistance showed a similar declining trend (p <0.001) (Figure 2b).

DISCUSSION

These results describe circulating subtypes and prevalence of drug resistance for HIV-1 infections in Cuba during 2009–2014. The finding that HIV-1 non-B subtypes were more frequent is consistent with previous studies[6,11–14] and in contrast with the high proportion of subtype B reported in the Caribbean.[15,16] The broad genetic diversity of HIV-1 in Cuba is thought to be due to its originating from contacts in Central Africa.[14,17,18]

Cuba has made great strides in decreasing HIV-related morbidity and mortality by providing universal free access to ART.[11] Because of economic constraints, the most common drug combinations for first-line ART are restricted to nationally manufactured generic drugs.[12] Drug resistance testing was not available until May 2009, so a substantial number of patients may have been treated with failed virological regimens.[6]

The high overall TDR prevalence detected confirms previous reports in Cuba,[11,19] and is higher than reported in other Caribbean countries, Mexico and Central America.[20–26] Particularly alarming is the frequent detection of dual-class resistance to NRTI+NNRTI, since these classes of drugs constitute the backbone of first-line therapy in Cuba.

Table 3: HIV-1 drug resistance mutations in previously treated patients

Mutation[a]	Total n (%)	Previous regimen exposure[b] n (%)						
		1 regimen			2 regimens			≥3 regimens
		Total	NRTI+NNRTI	NRTI+PI	Total	PI/NNRTI	NNRTI/PI	Total
NRTI[c]								
Any	467 (80.0)	131 (74.4)	112 (63.6)	19 (10.8)	114 (79.2)	64 (83.1)	46 (74.2)	222 (84.1)
M41L	150 (25.7)	31 (17.6)	27 (15.3)	4 (2.3)	39 (27.1)	19 (24.7)	18 (29.0)	80 (30.3)
D67N	140 (24.0)	22 (12.5)	17 (9.7)	5 (2.8)	33 (22.9)	19 (24.7)	12 (19.4)	85 (32.2)
K70R/E	124 (21.2)	25 (14.2)	22 (12.5)	3 (1.7)	32 (22.2)	22 (28.6)	7 (11.3)	67 (25.4)
M184V/I	443 (75.9)	126 (71.6)	108 (61.4)	18 (10.2)	107 (74.3)	60 (77.9)	44 (71.0)	210 (79.5)
T215Y/F	218 (37.3)	48 (27.3)	43 (24.4)	5 (2.8)	59 (41.0)	29 (37.7)	29 (46.8)	111 (42.0)
NNRTI[c]								
Any	417 (71.4)	123 (69.9)	110 (62.5)	13 (7.4)	104 (72.2)	68 (88.3)	34 (54.8)	190 (72.0)
K103N/S	167 (28.6)	42 (23.9)	38 (21.6)	4 (2.3)	38 (26.4)	28 (36.4)	10 (16.1)	87 (33.0)
Y181C/I/V	154 (26.4)	44 (25.0)	40 (22.7)	4 (2.3)	39 (27.1)	23 (29.9)	15 (24.2)	71 (26.9)
G190S/A	127 (21.7)	33 (18.8)	28 (15.9)	5 (2.8)	35 (24.3)	28 (36.4)	7 (11.3)	59 (22.3)
PI[c]								
Any	185 (31.7)	17 (9.7)	7 (4.0)	10 (5.7)	46 (31.9)	14 (18.2)	30 (48.4)	122 (46.2)
M46I/L	93 (15.9)	9 (5.1)	4 (2.3)	5 (2.8)	14 (9.7)	3 (3.9)	10 (16.1)	70 (26.5)
L90M	95 (16.3)	9 (5.1)	3 (1.7)	6 (3.4)	20 (13.9)	7 (9.1)	12 (19.4)	66 (25.0)
Any	496 (84.9)	138 (78.4)	117 (66.5)	21 (11.9)	124 (86.1)	70 (90.9)	50 (80.6)	234 (88.6)
Any NRTI+NNRTI	388 (66.4)	116 (65.9)	105 (59.7)	11 (6.3)	94 (65.3)	50 (64.9)	26 (41.9)	178 (67.4)
Any NRTI+PI	182 (31.2)	17 (9.7)	7 (4.0)	10 (5.7)	46 (31.9)	0 (0.0)	0 (0.0)	119 (45.1)
Any NRTI+NNRTI+PI	139 (23.8)	10 (5.7)	5 (2.8)	5 (2.8)	32 (22.2)	0 (0.0)	0 (0.0)	97 (36.7)

[a]amino acid changes at positions included in HIV genotypic drug resistance interpretation algorithm Rega v9.1.0
[b]NRT+NNRTI, NNRTI-based first-line regimen; NRTI+PI, PI-based first-line regimen; NNRTI/PI, NNRTI-based first-line regimen, followed by PI-based second-line regimen; PI/NNRTI, PI-based first-line regimen followed by NNRTI-based second-line regimen
[c]NRTI mutations K65R/E/N, V75I, F77L, Y115F, F116Y, Q151M; NNRTI mutations L100I, V179L, Y188C/L/H, P225H, F227C, M230I/L; and PI mutations D30N, V32I, I47V/A, G48V, I50L/V, Q58E, T74P, L76V, V82A/T/F/S/L, N83D, N88S were observed in <15% of patients.
NNRTI: non-nucleoside reverse transcriptase inhibitor NRTI: nucleoside reverse transcriptase inhibitor PI: protease inhibitor

The most frequent mutations found for NRTI and NNRTI in previously treated patients were expected because, for over a decade, AZT+3TC+NVP has been the most common combination used in Cuba for first-line therapy.[12] Worrisome is the high prevalence of V82A mutation which is selected by ritonavir and produces treatment failure with most PI.[27]

In Cuba, HIV-1 patients can only receive ART if it is prescribed by authorized HIV specialists; thus, our observation of NNRTI and PI resistance mutations in patients never exposed to these drug classes (Table 2) supports previous reports that drug-resistant strains are in circulation.[11,19] Subtype B viruses played a major role in the earliest ARV resistance studies, most of which reported that existing ARVs are equally effective at treating subtype B and non-B viruses. However, protease and reverse transcriptase sequence data from non-B subtypes isolated from previously treated patients have shown several drug resistance mutations that preferentially occur in certain HIV-1 subtypes. Most of these subtype-specific differences in drug resistance mutation distribution are attributed to differences in codon usage.[28]

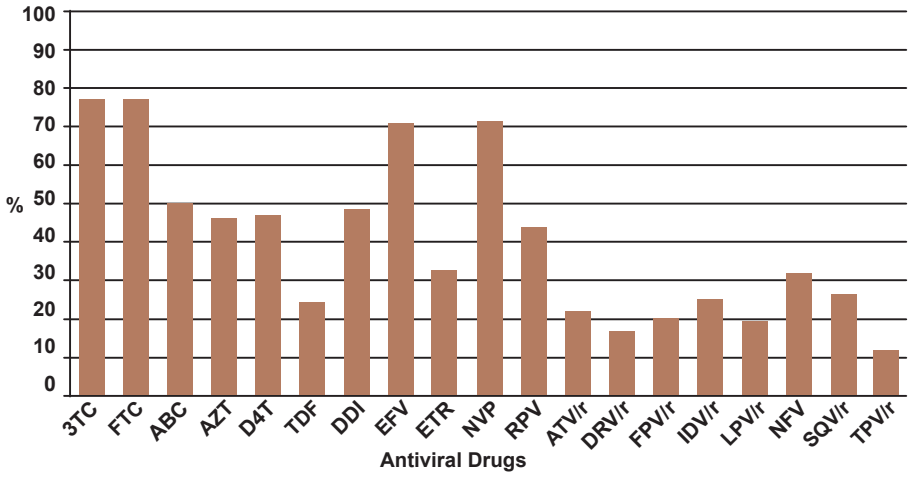
Table 4: Resistance mutations with HIV-1 viral variant in previously treated patients on active ART at time of testing

Subtype (n)	ART	Mutation (n)	p Value*	OR (95% CI)
		NRTI		
C (34)	3TC	K65R (5)	0.001	10.733 (3.303–34.873)
CRF 19_cpx (68)	AZT o d4T	L210W (15)	0.007	2.479 (1.260–4.878)
		NNRTI		
C (6)	NVP	K101E (3)	0.037	6.636 (1.285–34.267)
		PI		
CRF_BG (10)	LPV/r	M47V/I (4)	0.029	6.4 (1.338–30.606)

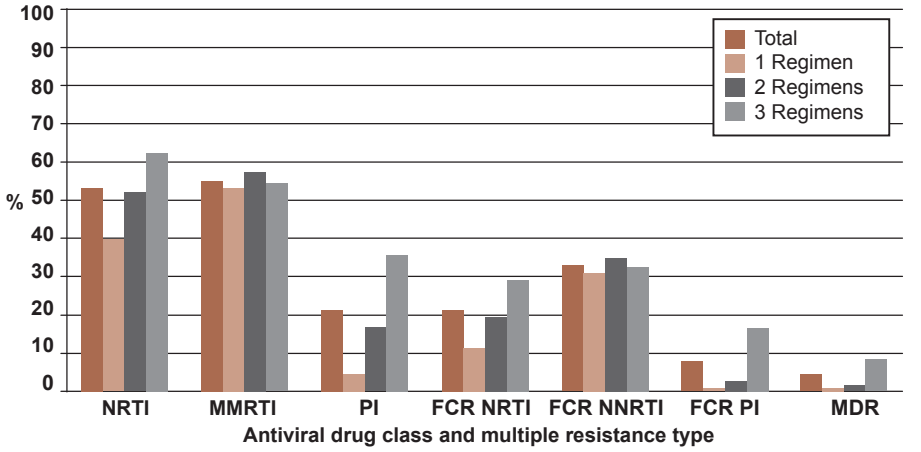
*chi square with Yate's continuity correction
ART: antiretroviral therapy NNRTI: non-nucleoside reverse transcriptase inhibitor
NRTI: nucleoside reverse transcriptase inhibitor OR: odds ratio PI: protease inhibitor

Figure 1: Antiviral resistance in previously treated patients, Cuba, 2009–2014

a. By drug



b. By drug class

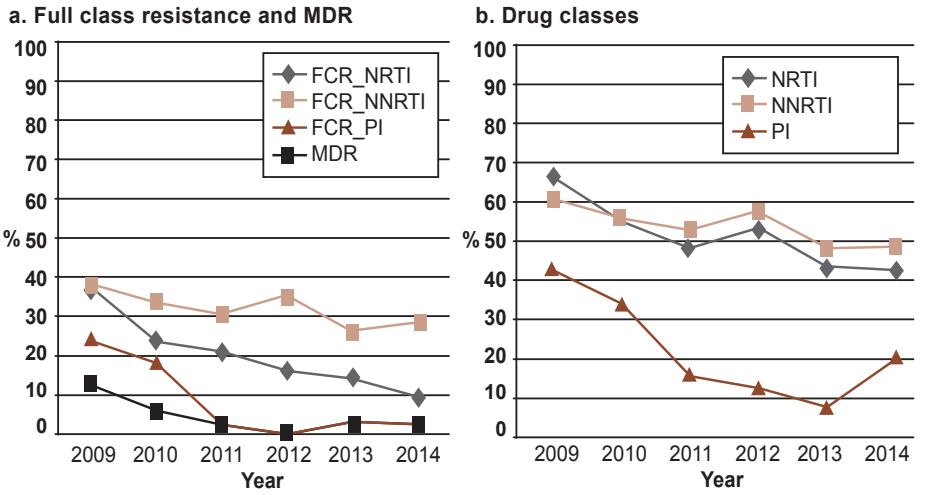


3TC: lamivudine ABC: abacavir ATV: atazanavir AZT: zidovudine D4T: stavudine DDI: didanosine
DLV: delavirdine DRV: darunavir EFV: efavirenz ETR: etravirine FCR: full class resistance
FPV: fosamprenavir FTC: emtricitabine IDV: indinavir LPV: lopinavir MDR: multidrug resistance
NFV: nelfinavir NNRTI, non-nucleoside reverse transcriptase inhibitor
NRTI: nucleoside reverse transcriptase inhibitor NVP: nevirapine PI: protease inhibitor RPV: rilpivirine
/r: ritonavir SQV: saquinavir TDF: tenofovir TPV: tipranavir

ART susceptibility of different HIV-1 subtypes is currently the subject of much attention and hence, further research on this topic is encouraged. Our finding that K65R resistance mutation was more likely detected in subtype C is consistent with previous reports.[29–32] The higher prevalence of NRTI mutation L210W in the viral strain CRF19_cpx, has important implications for NRTI-based ART regimens in Cuba, because CRF19_cpx is the third most frequent strain in the Cuban HIV-1 epidemic,[11–13] and has recently been associated with rapid progression to AIDS.[33] Moreover, the higher prevalence of PI mutation M47V/I among Cuban recombinants represents a hazard for PI-based ART.[34] CRF19_cpx and CRFs BGs circulate almost exclusively in Cuba,[11–13] so there are no previous prevalence studies of resistance mutations among these CRFs. Further studies are required to confirm our findings.

Overall, drug resistance to NRTI, NNRTI and PI in the sample studied is high, probably due to the combination's lack of potency, acquisition of resistant virus[12,35,36] and lower frequency of viral load testing. Despite overall high resistance, our analysis showed a significantly declining trend over time for FCR NRTI, FCR PI and MDR. This might be due to changes in patient selection for resistance testing. In the first years after implementation of the test, samples were selected mainly from patients failing multiple therapy regimens; after 2011, all patients failing first therapy regimen were tested. It might also reflect better clinical management of HIV ART, greater experience of clinicians, and virologists' assistance in interpreting genotypic resistance assays, resulting in increasing ART effectiveness.[8] The declining resistance observed in Cuba is in line with a trend observed in recent years in high-income countries in Western Europe and North America.[35,37,38]

Figure 2: Drug resistance trends, Cuba, 2009–2014



FCR: full class resistance MDR: multidrug resistance
NNRTI: non-nucleoside reverse transcriptase inhibitor
NRTI: nucleoside reverse transcriptase inhibitor PI: protease inhibitor

The study's main limitation is that it does not meet WHO standards for a national surveillance study, which require a nationally representative sample.[39]

CONCLUSIONS

TDR levels observed reinforce the need for a national surveillance study of Cuban treatment-naïve patients. Despite the high prevalence of resistance in patients failing ART, its frequency seems to be decreasing over time. The frequency of specific drug resistance mutations in recombinant forms of HIV in Cuba needs further attention.

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