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Genotypic resistance profiles of HIV-2 infected patients from Cape Verde failing first-line antiretroviral therapy

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The *pol* gene from HIV-2 infected patients from Cape Verde experiencing virologic failure was sequenced and drug resistance mutations were determined. Most patients were taking a first line regimen of AZT, 3TC and LPV/r. Resistance mutations were found in most patients (11/17, 64.7%) especially I82F (4/7, 57.1%) and M184V (10/17, 58.8%). Resistance to all reverse transcriptase and protease inhibitors was found in 58.8% (10/17) of the patients. Integrase inhibitors are warranted to treat these patients.

Keywords

HIV-2 infection in Cape Verde; antiretroviral therapy; drug resistance mutations; resistance profiles.

HIV-2 infection is endemic in some West African countries including Cape Verde. Patients living with HIV in Cape Verde at the end of 2018 were estimated to be 2,400 (2,100-2,900) and the prevalence rate among adults was 0.6% (0.5%-0.7%) [1]. In 2015, HIV-2 represented 12% of the new HIV infections in Cape Verde (39 cases in 327 new HIV infections) [2]. As for HIV-1, without effective antiretroviral treatment HIV-2-infected individuals have a high probability of developing and dying from AIDS [3]. As it suppresses virus replication, effective antiretroviral therapy (ART) is also crucial to prevent virus transmission. However, many current antiretroviral drugs and combination regimens fail to suppress HIV-2 replication [4]. Preferred regimens combine two nucleoside reverse transcriptase inhibitors (NRTI) (TDF with 3TC, TDF with FTC and AZT with 3TC) with protease inhibitors (PIs) or integrase strand transfer inhibitors (INSTIs) [5]. K65R, Q151M and M184V are the most prevalent resistance mutations in HIV-2 infected patients failing therapy with NRTIs [6-8]. Likewise, V71I, V47A and L90M are often selected on the protease of patients failing therapy with protease inhibitors [9, 10].

In Cape Verde about 2,200 HIV-infected patients were receiving antiretroviral therapy in 2017, representing a coverage of 89% (75- 95%) [1]. As of 2018, ART is freely available for all HIV-infected patients. Until 2017 the preferential first line regimen adopted for HIV-2 was AZT+3TC+LPV/r; in 2018 it changed to TDF + 3TC + DTG. We have recently identified in Cape Verde several HIV-2 infected patients with virologic failure to first-line ART [11]. The aim of this study was to characterize the genotypic resistance profiles in these patients. The study was approved by the Cape Verde National Ethics Committee in health research and all participants in the study signed informed consent. Blood samples were draw from 23 (17 failing ART and 6 drug-naïve as controls) HIV-2 infected patients living in Santiago and São Vicente

islands in Cape Verde in 2014 and 2015 (Table 1 and SDC1 http://links.lww.com/QAD/B583). Duration of treatment was highly heterogeneous ranging from 10 to 112 months (median 55 months). The median age was 52 (IQR=47.5-58.0) for patients on ART and 55 (IQR=54.0-61.8) for untreated patients. 47.1% of the patients on ART were female. Median number of $CD4^+$ T cells/mm^3 in patients on ART was 166 (IQR=95.0-221.5) and median viral load was 43,352 HIV-2 RNA copies/ml (IQR=13,874-98,307) which were not significantly different from drug-naïve patients [median number of $CD4^+$ T cells/mm^3= 205 (IQR= 127.3-432.0); median viral load = 27,887 HIV-2 RNA copies/ml (IQR=3,630-220,923].

HIV-2 viral RNA extraction was done using QIAamp Viral RNA miniKit (QIAGEN) and quantified by ExaVir™ Load assay version 3, according to manufacturer instructions [12]. The PR/RT gene was amplified using an in-house method developed by Brandin et al., with modification of the primer JA220 [(–3178] 5′-GTCTTT AT(T/C)CCTGGGTAGATTTGTG-3′] to JA220MOD [(–3178] 5′-GTCTTTATICCTGGGTAGAI(T/G)TGTG-3′] [13]. DNA sequences were obtained using Sanger sequencing. Sequences were given the following GenBank accession numbers: MK493081-493103. Phylogenetic analysis of HIV-2 sequences was done by maximum likelihood. HIV-GRADE Algorithm [8] was used to identify drug resistance mutations and resistance profiles.

Phylogenetic analysis confirmed clustering in a patient-specific manner and excluded the possibility of contamination (SDC2 http://links.lww.com/QAD/B584). All sequences belong to group A, which is the prevailing group in Cape Verde [2, 14]. Drug resistance mutations were only present in treated patients (Table 1). Seven NRTI resistance mutations were identified in eleven patients: K65R (n=4), N69T (n=1), K70N (n=1), K70R (n=2), V111I (n=6), Q151M (n=4), M184V (n=10). In line with previous studies, including one performed in Cape Verde, M184V was the most prevalent DRM and was selected in most patients on FTC or 3TC-based regimens (59%, 10/17) [2, 4, 15-18]. Q151M was associated with other mutations such as K65R (n=3) and M184V (n=4), as in previous studies [7, 19]. In patients 20, 22 and 23 these associations lead to class-wide NRTI resistance (Table 1). Like in previous studies these three patients, which were on FTC or 3TC-based regimens, developed the V111I mutation in association with K65R and Q151M [20]. V111I increases the fitness of NRTI-resistant K65R and Q151M viruses [6]. V111I was present in patient 17 without any other DRM suggesting poor adherence to treatment [18, 21].

The following PI resistance mutations were present in 7 (41%) treated patients: V47A (n=3), I54M (n=3), I82F (n=4), I84V (n=2), L90M (n=1). I54M and L90M are associated with treatment with DRV/r, IDV/r, LPV/r or SQV/r and consequently with virologic failure to these drugs [9, 10, 22]. As in previous studies, I82F was often associated with I54M (3/4 patients) [4, 8, 9, 22]. V47A mutation was present in three patients on LPV/r-based regimens further

confirming its role in resistance to this drug [15, 17, 18, 22, 23]. A potential second line treatment for these patients could include SQV because of the hypersusceptibility conferred by V47A to this drug [15]. Notably, 4 (24%) treated patients (1, 4, 11 and 18) did not exhibit DRMs despite detectable viral load (Table 1)..Poor adherence to treatment may also explain virologic failure in these patients [18, 21]. Finally, 3 patients were treated with efavirenz despite ample evidence for its lack of activity on HIV-2 [24]. Not surprisingly, these patients harbored 3 of the 4 isolates with mutations conferring resistance to all NRTIs. There was no significant correlation or discernible trend between the duration of treatment and viral load (Spearman r=-0.3029; P= 0.2373) or the number of DRMs (Spearman r=0.2839; P= 0.2695) at the time of failure.

In conclusion, most isolates from HIV-2 infected patients from Cape Verde showing virologic failure to first-line ART harbor multiple DRMs that confer resistance to all available RT and PR inhibitors. Dolutegravir have been recently introduced in the country and will be crucial to treat these patients. Low adherence and incorrect choice of drug regimens seem to be the main causes of current treatment failures. This supports the need for improved education of clinicians and patients on the best therapeutic practices for HIV-2 infection.

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Table legend

Table 1 - Viral load, ART regimens, drug resistance mutations and resistance profiles of HIV-2 infected patients

Patient	ART Initiation (duration, (months)	ART Regimen	Viral Load (HIV-2 RNA copies/ml)	PR		RT	
				DRMs	Susceptibility profile	DRMs	Susceptibility profile
1	2012 (34.5)	AZT+3TC+LPV/r	15,237	None	Sensitive to all drugs	None	Sensitive to all drugs
2	2013 (16)	AZT+3TC+LPV/r	152,222	L90L/M	Intermediate resistance to: DRV/r and LPV/r Resistance to: IDV/r, SQV/r	None	Sensitive to all drugs
3	2009 (63)	AZT+3TC+LPV/r	43,352	I54M, I82F	Resistance to: ATV/r, DRV/r, IDV/r, LPV/r	Q151M, M184V	Resistance to: 3TC, ABC, AZT, D4T, DDI, FTC
4	2012 (30)	AZT+3TC+LPV/r	101,183	None	Sensitive to all drugs	None	Sensitive to all drugs
5	Untreated	-	4,156	None	Sensitive to all drugs	None	Sensitive to all drugs
6	Untreated	-	7,133	None	Sensitive to all drugs	None	Sensitive to all drugs
7	Untreate	-	48,641	None	Sensitive to	None	Sensitive to

	d				all drugs		all drugs
8	2010 (49)	FTC/TDF+ LPV/r	12,786	V47A, I82F	Resistance to: ATV/r, IDV/r, LPV/r	K70N, V111I, M184V	Resistance to: 3TC, FTC,
9	2013 (20)	AZT+3TC+LPV/r	87,859	I54M, I82F	Resistance to: ATV/r, DRV/r, IDV/r, LPV/r	M184V	Resistance to: 3TC, FTC,
10	2009 (58)	AZT+3TC+LPV/r	52,860	I54M, I82F	Resistance to: ATV/r, DRV/r, IDV/r, LPV/r	M184V	R- NRTI: 3TC, FTC
11	2009 (60)	AZT+3TC+LPV/r	5,522	None	Sensitive to all drugs	None	Sensitive to all drugs
12	2010 (55)	AZT+3TC+LPV/r	74,391	V47A, I84V	Resistance to: LPV/r	None	Sensitive to all drugs
13	2013 (14)	AZT+3TC+LPV/r	288,433	None	Sensitive to all drugs	M184M/V	Resistance to: 3TC, FTC
14	2013 (10)	AZT+3TC+LPV/r	18,052	None	Sensitive to all drugs	M184V	Resistance to: 3TC, FTC
15	Untreated	-	2,051	None	Sensitive to all drugs	None	Sensitive to all drugs
16	Untreated	-	89,362	None	Sensitive to all drugs	None	Sensitive to all drugs
17	2010 (58)	AZT+3TC+LPV/r	120,546	None	Sensitive to all drugs	V111I	Sensitive to all drugs
18	2005	AZT+3TC+LP	1,284	None	Sensitive to	None	Sensitive to

	(112)	V/r			all drugs		all drugs
19	Untreated	-	615,604	None	Sensitive to all drugs	None	Sensitive to all drugs
20	2009 (62)	AZT+3TC+EFV	27,641	None	Sensitive to all drugs	K65R, N69T, V111I, Q151M, M184V	Resistance to all NRTIs
21	2013 (13)	FTC+TDF+EFV	8,558	None	Sensitive to all drugs	K65R, V111I/V, M184V	Intermediate resistance to: D4T Resistance to: 3TC, ABC, DDI, FTC, TDF/TAF
22	2005 (104)	FTC+TDF+EFV	14,962	None	Sensitive to all drugs	K65R, K70R, V111I, Q151M, M184V	Resistance to all NRTIs
23	2007 (84)	AZT+3TC+LPV/r	95,431	V47A, I84V	Intermediate resistance to: DRV/r, Resistance to: SQV/r; IDV/r, LPV/r	K65R, K70R, V111I, Q151M, M184V	Resistance to All NRTIs

ART- Antiretroviral therapy; DRMs- Drug resistance mutations; NRTIs- Nucleoside reverse transcriptase inhibitors; PIs Protease inhibitors; 3TC- lamivudine, ABC- abacavir, AZT- zidovudine, D4T- stavudine, DDI- didanosine, FTC- emtricitabine, TDF- Tenofovir disoproxil fumarate, ATV/r- Atazanavir/ritonavir, DRV/r- Darunavir/ritonavir, IDV/r- Indinavir/ritonavir, LPV/r - Lopinavir/ritonavir, SQV/r- Saquinavir/ritonavir.