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Molecular Characterization of Human Immunodeficiency Virus Type 1 (HIV-1) and HIV-2 in Yaoundé, Cameroon: Evidence of Major Drug Resistance Mutations in Newly Diagnosed Patients Infected with Subtypes Other than Subtype B[▽]

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Prior to current studies on the emergence of drug resistance with the introduction of antiretroviral therapy (ART) in Cameroon, we performed genotypic analysis on samples from drug-naïve, human immunodeficiency virus (HIV)-infected individuals in this country. Of the 79 HIV type 1 (HIV-1) *pol* sequences analyzed from Cameroonian samples, 3 (3.8%) were identified as HIV-1 group O, 1 (1.2%) was identified as an HIV-2 intergroup B/A recombinant, and the remaining 75 (95.0%) were identified as HIV-1 group M. Group M isolates were further classified as subtypes A1 ($n = 4$), D ($n = 4$), F2 ($n = 6$), G ($n = 12$), H ($n = 2$), and K ($n = 1$) and as circulating recombinant forms CRF02_AG ($n = 41$), CRF11_cpx ($n = 1$), and CRF13_cpx ($n = 2$). Two *pol* sequences were identified as unique recombinant forms of CRF02_AG/F2 ($n = 2$). M46L ($n = 2$), a major resistance mutation associated with resistance to protease inhibitors, was observed in 2/75 (2.6%) group M samples. Single mutations associated with resistance to nucleoside reverse transcriptase inhibitors (T215Y/F [$n = 3$]) and nonnucleoside reverse transcriptase inhibitors (V108I [$n = 1$], L100I [$n = 1$], and Y181C [$n = 2$]) were observed in 7 of 75 (9.3%) group M samples. None of the patients had any history of ART exposure. Population surveillance of transmitted HIV drug resistance is required and should be included to aid in the development of appropriate guidelines.

The current standard for antiretroviral drug therapy (ART) in developed countries is the combination of two nucleoside reverse transcriptase (RT) inhibitors (NRTIs) plus a nonnucleoside RT inhibitor (NNRTI) or a protease inhibitor (PI). Since the successful trials in the late 1990s, combination ART has benefited and continues to aid many human immunodeficiency virus type 1 (HIV-1)-infected patients in developed countries, and it is becoming increasingly available in resource-constrained countries (17, 20, 24, 29, 30).

In countries with multiple antiretrovirals (ARVs) readily available, the prevalence of drug-resistant variants has ranged from 10 to 20% among drug-naïve patients (33), while in resource-constrained areas, resistance in the untreated HIV-infected population is rarely reported (23, 31). Recent interventions through such programs as the World Health Organization (WHO)'s 3 by 5 plan to treat 3 million people by the end of 2005 (33a) and the President's Emergency Plan for AIDS Relief have promoted significant access to ART in low- and middle-income countries. As of June 2005, about 500,000 people in sub-Saharan Africa were receiving ART, although the regional coverage rate was still 11% of the estimated number of patients with CD4 cell counts of ≤300/ml (2% of all HIV-infected patients in this region) (33a). Developing countries, including Cameroon, are moving towards universal access to HIV prevention, care, and treatment for those in need and at high risk of infection. This has led to the widespread use of antiretroviral drugs through structured national ART scale-up plans. Because of the complexity and open-ended duration of HIV treatments and the need to begin programs to treat many patients quickly, fears have been raised that emergence of ARV resistance may become a serious public health concern and render anti-HIV drugs useless. To assist ART programs and to minimize the emergence and transmission of HIV drug resistance strains and their public health consequences, WHO has developed a minimum-resource strategy for the surveillance and monitoring of HIV drug resistance in resource-limited countries. In Kenya, for example, where ART has been provided for 12 to 17% of the estimated need, the prevalence of resistant strains among drug-naïve patients has recently risen from 1% (2002) to more than 5% (2003) (WHO, personal communication). In Botswana, where treatment is available to all patients with <300 CD4 cells/ml, the prevalence of major mutations conferring PI resistance was estimated to be 4% among drug-naïve patients (4).

Unlike the case in southern and eastern African countries, where one or two HIV-1 subtypes dominate (22), all major groups and subtypes of HIV-1 cocirculate in Cameroon (1, 6, 14–19, 21, 22, 24, 28, 34–38). According to WHO/UNAIDS, as of the end of 2004, the prevalence of HIV-1 infection was estimated to be 4.8% overall and 9.8% for adults. To date,

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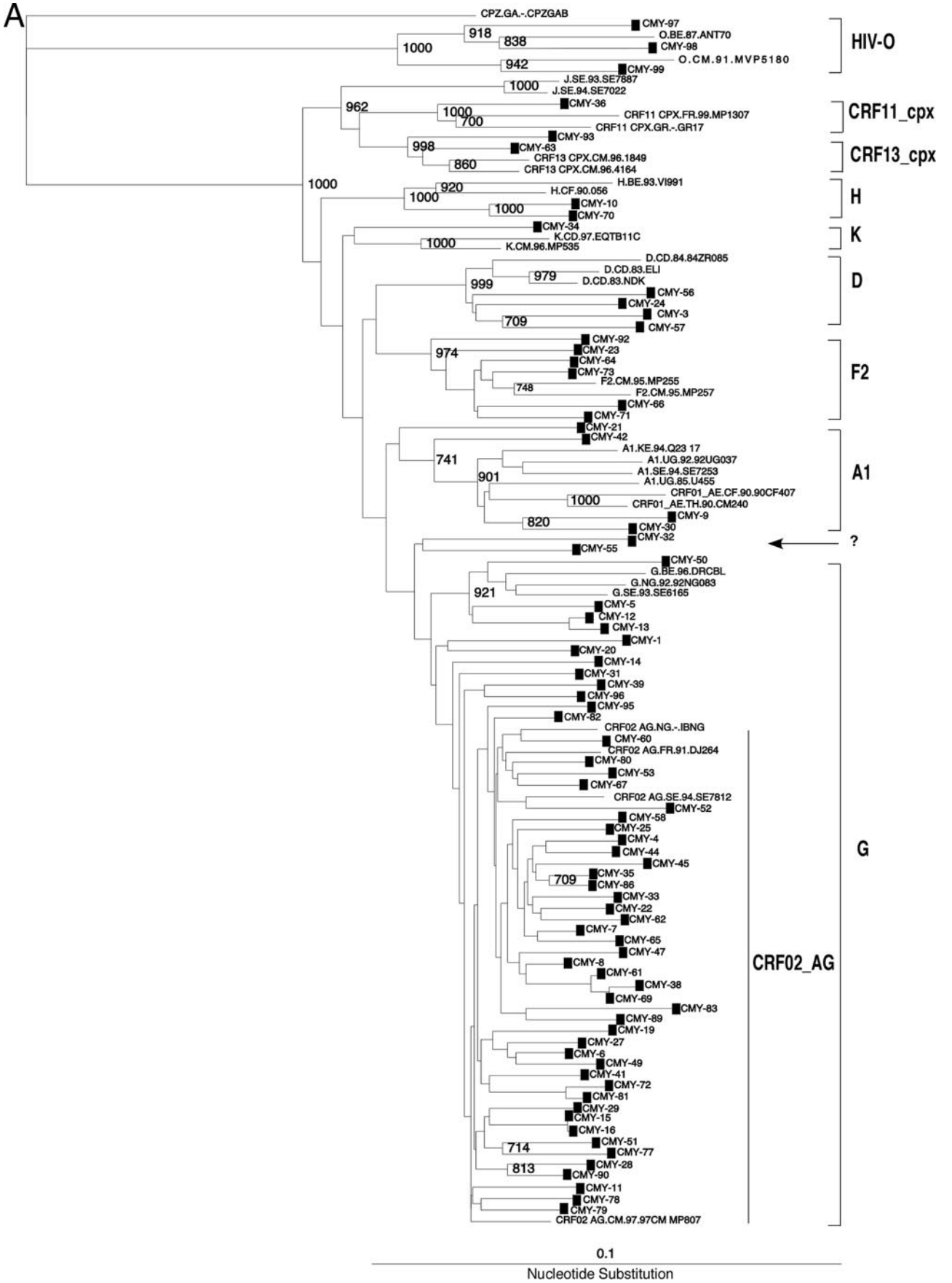
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O.CM.91.MVP5180
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J.SE.94.SE7022
CMY-36
CRF11 CPX.FR.99.MP1307
CRF11 CPX.GR-.GR17
CMY-93
CMY-63
CRF13 CPX.CM.96.1849
CRF13 CPX.CM.96.4164
H.BE.93.VI991
H.CF.90.056
CMY-10
CMY-70
CMY-34
K.CD.97.EQTB11C
K.CM.96.MP535
D.CD.84.84ZR085
D.CD.83.ELI
D.CD.83.NDK
CMY-56
CMY-24
CMY-3
CMY-57
CMY-92
CMY-23
CMY-64
CMY-73
F2.CM.95.MP255
F2.CM.95.MP257
CMY-66
CMY-71
CMY-21
CMY-42
A1.KE.94.Q23 17
A1.UG.92.92UG037
A1.SE.94.SE7253
A1.UG.85.U455
CRF01_AE.CF.90.90CF407
CRF01_AE.TH.90.CM240
CMY-9
CMY-30
CMY-32
CMY-55
CMY-50
G.BE.96.DRCBL
G.NG.92.92NG083
G.SE.93.SE6165
CMY-5
CMY-12
CMY-13
CMY-1
CMY-20
CMY-14
CMY-31
CMY-39
CMY-96
CMY-95
CMY-82
CRF02 AG.NG.-.IBNG
CMY-60
CRF02 AG.FR.91.DJ264
CMY-80
CMY-53
CMY-67
CRF02 AG.SE.94.SE7812
CMY-52
CMY-58
CMY-25
CMY-4
CMY-44
CMY-45
CMY-35
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CMY-19
CMY-27
CMY-6
CMY-49
CMY-41
CMY-72
CMY-81
CMY-29
CMY-15
CMY-16
CMY-51
CMY-77
CMY-28
CMY-90
CMY-11
CMY-78
CMY-79
CRF02 AG.CM.97.97CM MP807
1000
918
838
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942
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962
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700
998
860
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920
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999
979
709
974
748
741
901
1000
820
921
714
813
709
HIV-O
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H
K
D
F2
A1
?
G
CRF02_AG
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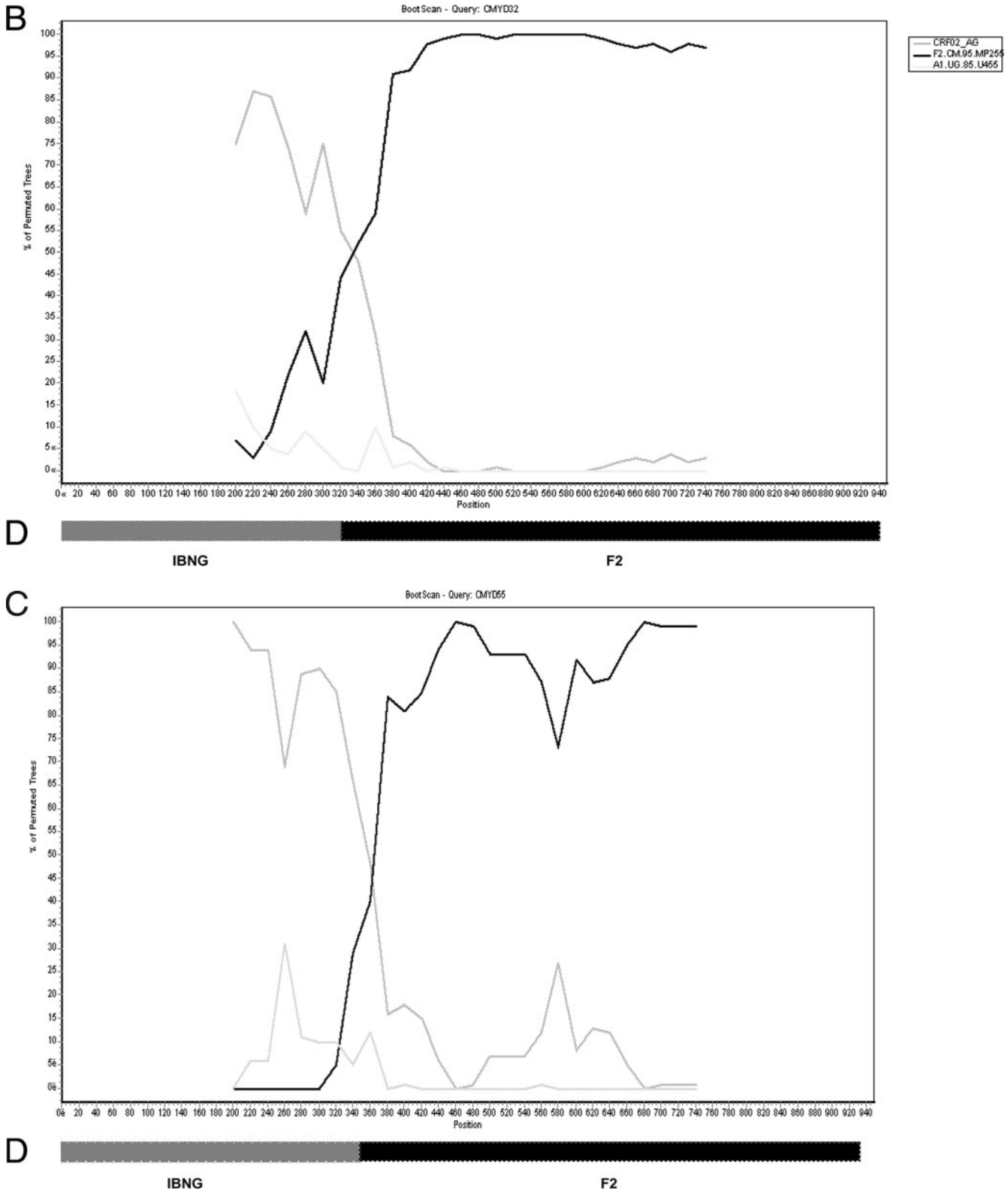
Nucleotide Substitution



there have been several reports on the prevalence of ARV resistance mutations in the drug-naïve HIV-1-infected population of Cameroon (1, 6, 14–16, 19, 31). Baseline information on the frequency and types of ARV resistance mutations in Cameroon will help to inform optimal ART and enable the government to monitor the success of the national AIDS treatment program.

ART in Cameroon is based on the WHO guidelines, i.e., the

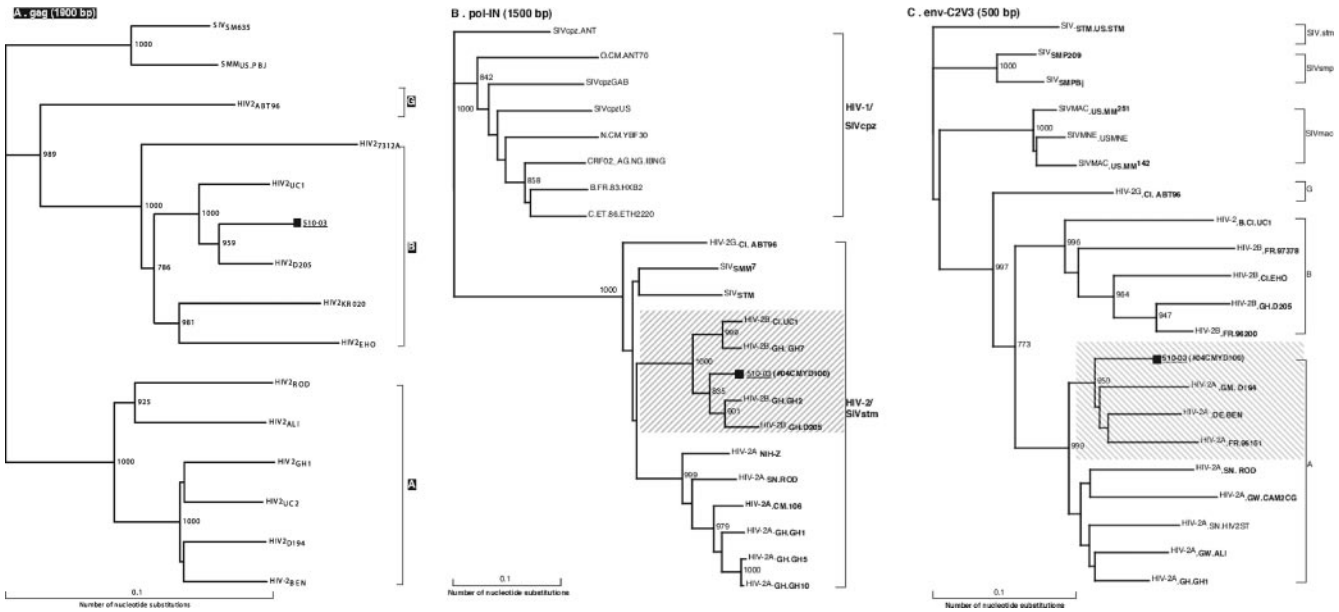


FIG. 2. Phylogenetic trees based on *gag-p17/p24* gene (1,900 bp) of HIV-2 (510-03/04CMYD-100) subtype B/A recombinant strain (A) and the *pol-IN* (1,500 bp) gene (B) and *env-C2V3* (500 bp) gene (C) from the Cameroonian HIV-2 strain. The bootstrap value at each node represents the number among 1,000 replicates that supported the branching order. Bootstrap values of >70% are shown. The brackets on the right represent the major HIV-2 subtypes. The newly analyzed sequence (510-03) is marked with a filled square.

combination of two NRTIs and one NNRTI. With the rapid introduction of ART and with limited health care infrastructure for care and monitoring, this country may face similar emergence rates of ARV resistance to those described for other developing countries (29, 30). With a higher prevalence of ARV resistance in the drug-naïve population (18, 32), resistance may emerge at an even higher rate.

In this study, we evaluated the prevalence of drug-resistant HIV-1 strains in treatment-naïve HIV-1-infected individuals in a resource-limited country where ART is being scaled up rapidly to determine whether standard first-line regimens will continue to be effective. Samples were obtained prior to the rollout of significant ART programs in Yaoundé, the capital city of Cameroon. We examined the prevalence of ARV resistance mutations in 79 patient samples and found a low rate of major drug resistance mutations to RTIs and PIs.

MATERIALS AND METHODS

Study population. Blood specimens were drawn in 2004 from newly diagnosed HIV-1 patients attending a clinic in Yaoundé, Cameroon. All participants provided written informed consent and were likely to be recently infected. Sera found to be reactive for HIV by enzyme-linked immunosorbent assay confirmed with Western blotting were included in this study to explore the prevalence of intrinsic resistance to ARV drugs from treatment-naïve patients. This study received ethical clearance from the National Ethics Committee of Cameroon. Exclusion criteria included any previous form of ARV treatment, including that given to women for prevention of mother-to-child transmission.

PCR and sequencing. Peripheral blood mononuclear cells (PBMCs) from HIV-seroreactive blood donors were obtained by Ficoll-Hypaque density gradient centrifugation. Proviral DNA was extracted from uncultured PBMCs with a DNA extraction kit (Qiagen, Hilden, Germany). Nested PCR amplification was performed using AmpliTaq DNA polymerase (Roche Molecular Systems, Branchburg, NJ). A segment of the PR-RT region of the *pol* gene was first PCR amplified using the universal external primers univ-PS1 (TTTTTTAGGGAAA ATTTGGCCTTC) and univ-RTA4 (CTGTATATCATTGACAGTCCAGCT), resulting in a 1.2-kbp product. Nested PCR was then performed with the uni-

FIG. 1. (A) Phylogenetic tree of HIV-1 PR-RT sequences from 78 HIV-1 group M and O isolates. "CMY" refers to PR-RT sequences from the cross-sectional analysis and indicates the country (Cameroon) and location (Yaoundé) of sample collection. The bootstrap value at each node represents the number among 1,000 bootstrap replicates that supported the branching order. Bootstrap resampling values of 70% or higher are shown. Brackets on the right represent the major group M subtypes. Newly derived sequences are marked with filled squares, and the novel unique recombinant form CRF02_AG/F2 is shown by an arrow. A 950-nt segment of the PR-RT coding region was used to construct this tree by the neighbor-joining method. PR-RT genetic subtypes A, D, F, G, H, and K and recombinants CRF02_AG, CRF11.cpx, and CRF13.cpx, as well as HIV-1 group O, are indicated. GenBank accession numbers for the reference sequences are as follows: A1.KE.93.Q23-17, AF004885; A1.UG.85.U455, M62320; A1.UG.92.92UG037, U51190; D.CD.83.ELI, K03454; D.CD.83.NDK, M27323; DCD.84.84ZR085, U88822; F2.CM.95.MP257, AJ249237; G.NG.92.92NG083, U88826; G.SE.93.SE6165, AF061642; G.BE.96.DRCBL, AF084936; H.BE.93.VI991, AF190127; J.SE.93SE7887, AF082394; J.SE.94.SE7022, AF082395; K.CM.96.MP535, AJ249239; K.CD.97.EQTB11C, AJ249234; 01_AE.TH. 90.90CM240, U54771; 01_AE.CF.90.90CF4071, AF197341; 02_AG.NG.-.IBNG, L39106; 02_AG.FR91.DJ264, AF063224; 02_AG.SE.94.SE7812, AF107770; 02_AG.CM.97.97CM.MP807, AJ251056; 11_CPX.CM.97.MP818, AJ291718; 13_CPX.CM.96.1849, AF460972; 13_CPX.CM.96.4164, AF460974; O.CM.-.ANT70, L20587; O.CM.91.MVP5180, L20571; and CPZ.GA.-.CPZGAB, X52154. (B and C) SimPlot analyses of unclassifiable Cameroonian PR-RT (approximately 1,000 nt) sequences 04CMY-32 (B) and 04CMY-55 (C), showing the recombination between subtype F2 and CRF02_AG (A). The bootscan analysis was performed against reference strains from clades A (strain A1.UG.85.U455), B (strain B.US.83.RF), D (strain D,CD.84.84ZR085), F1 (strain F1.FI.93.FIN9363), F2 (strain F2.CM.95,MP255), G (strain G.SE.93.SE6155), and 02_AG (strain AG.NG.-.IBNG). (D) Segments derived from an IBNG-like strain and subtype F2 are shown.

versal primers univ-PS2 (5′-TCCCTCAAATCACTCTTTGGCAAC-3′) and univ-RTA3 (5′-TTCATAACCCATCCAAAGAAATGG-3′) to generate a fragment of 1.0 kbp. The PCR products were then purified with a QIAquick PCR purification kit (Qiagen, Valencia, CA) and sequenced in the sense and antisense directions with a set of nested primers (25). All sequencing reactions were performed using an ABI Prism Big Dye Terminator cycle sequencing ready reaction kit (Applied Biosystems, Foster City, CA) and an ABI 3730 DNA sequencer by Davis Sequencing, Inc. The chromatogram files were read using the Chromas 1.6 program (Helensvale, Australia). All sequences were edited with the BioEdit program.

Phylogenetic analysis and subtyping. Neighbor-joining phylogenetic trees including reference *pol* sequences were constructed using Clustal W and then drawn using Treeview PPC, version 1.6.6 (Institute of Biochemical and Life Sciences, Scotland, United Kingdom). Bootstrap resampling (1,000 data sets) of multiple alignments was performed to test the statistical robustness of the trees. Kimura-2 parameters were calculated with the DNADIST program in the PHYLIP package (13, 27).

Genotypic resistance analysis. Genotypic resistance was defined as the presence of one or more resistance-related mutations, as specified by the consensus mutation figures of the International AIDS Society—USA (11). The emergence of amino acid substitutions associated with resistance to RTIs and PIs has been characterized extensively, and these substitutions can be classified into major and accessory/minor (modifying) mutations. Major mutations lead to severalfold decreases in sensitivity to one or more ART drug. Accessory mutations may not result in a significant decrease in sensitivity but are associated with an increase in viral fitness (replication capacity) (9). Although resistance testing was performed retrospectively, for ethical reasons these results were fed back to the clinicians at the study site regarding the relative merits of change in therapy.

Nucleotide sequence accession numbers. The DNA sequences of HIV-1 *pol* PR-RT regions determined as part of this study were submitted to GenBank under the following accession numbers: DQ990400 to DQ990455.

RESULTS

HIV-1 subtype distribution. Seventy-nine HIV-infected samples from drug-naïve patients were obtained in 2004. A 1.0-kbp fragment encompassing amino acids 1 to 99 of PR and 1 to 234 of RT was PCR amplified and sequenced as described above. Sequences were then aligned and phylogenetic trees constructed to classify the different HIV sequences into groups, subtypes, and recombinant forms (Fig. 1A). Three sequences (3.8%; 95% confidence interval [CI], 1.6 to 5.9%) belonged to HIV-1 group O, and 75 sequences (94.9%; CI, 94.8 to 95.0%) were identified as HIV-1 group M. Group M isolates were further classified into the following six subtypes and three circulating recombinant forms (CRFs): subtypes A1 ($n = 4$), D ($n = 4$), F2 ($n = 6$), G ($n = 12$), H ($n = 2$), and K ($n = 1$) and CRF02_AG ($n = 41$), CRF11_cpx ($n = 1$), and CRF13_cpx ($n = 2$), with an intersubtype recombinant, CRF02_AG/F2 ($n = 2$). The two CRF02_AG/F2 isolates were identified using SimPlot for bootscanning analysis (Fig. 1B and C), with a 400-nucleotide (nt) rolling window and a significance threshold of 95% over the 1,000-bp PR-RT gene. Figure 1D shows the SimPlot output and a schematic representative plot and indicates that samples CMYD-32 and CMYD-55 have different breakpoints in the PR-RT gene, at 350 nt and 425 nt, respectively.

HIV-2 intersubtype B/A recombinant. One sample was seropositive for HIV infection but could not be PCR amplified by our set of primers. PCR amplification and subsequent DNA sequencing with a set of HIV-2-specific primers confirmed the identity of this isolate as not only HIV-2 but also the first documented case of an HIV-2 intersubtype B/A recombinant, based on *gag-p17/pol-IN/env-C2V3* sequence analyses, with three breakpoints in the *env-nef* gene (Fig. 2). The HIV-2 isolate (510-03) was subtype B based on *gag* and *pol* sequences, while the *env-nef* region is an intersubtype recombinant of subtypes A and B, with three recombination breakpoints identified. Further data analyses are in progress (N. Ndembi and C. Brennan, unpublished data).

PI resistance-associated mutations. The amino acid sequence of each strain was compared to the subtype B consensus amino acid sequence, using the published HIV drug resistance algorithm from the International AIDS Society (10, 11) for mutations associated with resistance to PIs and RTIs. Based on subtype B sequences, drug resistance mutations in the protease region at positions 10, 13, 16, 20, 24, 30, 32, 33, 34, 36, 43, 46, 47, 48, 50, 53, 54, 58, 60, 62, 63, 64, 71, 73, 76, 77, 82, 84, 85, 88, 89, 90, and 93 (11), i.e., 33 mutations in total, have been shown to be associated with resistance to PIs.

Primary PI resistance-associated mutations were found in 2 of 75 cases (2.6%). These two patients harbored a CRF02_AG or CRF13_cpx HIV-1 isolate with an M46L amino acid substitution in the protease coding region. The M46L mutation in subtype B is associated with resistance to amprenavir, atazanavir (ATV), indinavir, and nelfinavir. The CRF02_AG-infected patient CMY-72 also contained a G48R mutation linked to the M46L mutation in the protease gene. The G48V mutation in subtype B is responsible for saquinavir, ritonavir, and ATV resistance (9). A V82I mutation was detected in the protease sequences of three patients, but the V82I mutation is a minor/accessory mutation and confers only minimal resistance to ATV and ritonavir (10). An alanine, threonine, phenylalanine, or serine at this position, however, is responsible for resistance to all PIs. Isoleucine at position 82 is also a naturally occurring polymorphism in subtype strains (9, 23) and was observed in 3 of 12 (25%; CI, 5.5 to 57.2%) of our G isolates. Minor or accessory PI resistance mutations were also found as wild-type sequences in Cameroonian isolates at the following positions, in order of decreasing frequency: M36I (74/75 isolates; 98.7%), K20I/M/R (67/75 isolates; 89.3%), L10V (5/75 isolates; 6.7%), L63P (4/75 isolates; 5.3%), and D60E (4/75 isolates; 5.3%).

RTI resistance-associated mutations. Based on subtype B consensus sequences, mutations leading to resistance to NRTIs and NNRTIs are well defined and differ between the two classes of inhibitors. The most common major RT mutations leading to NRTI resistance occur at positions 41, 62, 65, 67, 69, 70, 74, 75, 77, 115, 116, 151, 184, 210, 215, and 219 (16 in total), and major mutations leading to NNRTI resistance are known to occur at positions 100, 103, 106, 108, 181, 188, 190, 225, (11), and 236 (9 in total).

Of the 79 cases analyzed, 7 (9.3%) showed major mutations associated with resistance to RTIs (zidovudine [ZDV], nevirapine [NVP], delavirdine [DLV], and efavirenz [EFV]). A V108I mutation was found in a CRF02_AG-infected patient, a Y181C mutation was found in a CRF13_cpx-infected patient, and V118C and V179E mutations were found in subtype G isolates. The subtype B mutations V118C and V179E result in moderate NNRTI resistance, whereas Y181C and V108I mutations are responsible for high-level NNRTI resistance (DLV, EFV, and NVP resistance and EFV and NVP resistance, respectively). The L210W mutation in subtype B (ZDV resistance) and the Y181C mutation (in subtype B [NNRTI resistance]) are found as the wild-type sequences in most HIV-1 group O isolates, including the three group O samples from

TABLE 1. Overview of epidemiologic and genetic information for acutely HIV-1-infected subjects in central Cameroon

Patient no.	Age (yr)	Sex[d]	Genetic subtype[a]				Drug resistance-associated mutation(s)[c]			
			GenBank accession no.	Pol-PR	Pol-RT	Unique recombinant form	PR		RT	
							Primary	Secondary	Primary	Secondary
04CMYD1	50	F	DQ990377	G	G			K20I, M36I		
04CMYD3	21	M	DQ990378	D	D			M36I		
04CMYD4	29	F	DQ990379	CRF02_AG	CRF02_AG			K20R, M36I		
04CMYD5	28	F	DQ990380	G	G			K20I, M36I		
04CMYD6	25	F	DQ990381	CRF02_AG	CRF02_AG			K20I, M36I		
04CMYD7	45	M	DQ990382	CRF02_AG	CRF02_AG			K20I, M36I		
04CMYD8	27	F	DQ990383	CRF02_AG	CRF02_AG			K20I, M36I		
04CMYD9	23	F	DQ990384	A1	A1			M36I, D60E, V77I		
04CMYD10	33	M	DQ990385	H	H			K20R, M36I, D60E		
04CMYD11	23	F	DQ990386	CRF02_AG	CRF02_AG			L10I, K20I, M36I		
04CMYD12	21	F	DQ990387	G	G			K20I, M36I, (V82I)		
04CMYD13	23	F	DQ990388	G	G			K20I, M36I		
04CMYD14	40	F	DQ990389	G	G			K20I, M36I		
04CMYD15	34	M	DQ990390	CRF02_AG	CRF02_AG			K20I, M36I		
04CMYD16	25	F	DQ990391	CRF02_AG	CRF02_AG			K20I, M36I	**V100I**	
04CMYD19	29	F	DQ990392	CRF02_AG	CRF02_AG			K20I, M36I		
04CMYD20	33	F	DQ990393	G	G			K20I, M36I		
04CMYD21	47	M	DQ990394	A1	A1			M36I		
04CMYD22	43	M	DQ990395	CRF02_AG	CRF02_AG			K20I, M36I		
04CMYD23	54	M	DQ990396	F2	F2			M36I		
04CMYD24	28	M	DQ990397	D	D			K20I, M36I		
04CMYD25	14	M	DQ990398	CRF02_AG	CRF02_AG			K20I, M36I		
04CMYD27	35	F	DQ990455	CRF02_AG	CRF02_AG			K20I, M36I		
04CMYD28	56	M	DQ990399	CRF02_AG	CRF02_AG			K20I, M36I		
04CMYD29	45	F	DQ990400	CRF02_AG	CRF02_AG			K20I, M36I		
04CMYD30	26	M	DQ990401	A1	A1			M36I		
04CMYD31	46	M	DQ990402	G	G			K20I, M36I, (V82I)		
04CMYD32	30	F	DQ990403	CRF02_AG	F2	CRF02_AG)/F2[b]		K20I, M36I, V77I		
04CMYD33	40	M	DQ990404	CRF02_AG	CRF02_AG			K20I, M36I		
04CMYD34	35	F	DQ990405	K	K			K20R, M36I		
04CMYD35	35	M	DQ990406	CRF02_AG	CRF02_AG			K20I, M36I	**Y188C**	
04CMYD36	31	F	DQ990407	CRF11_cpx	CRF11_cpx			D60E, V77I		
04CMYD38	49	M	DQ990408	CRF02_AG	CRF02_AG			K20I, M36I		
04CMYD39	34	F	DQ990409	G	G			K20I, M36I		
04CMYD41	32	M	DQ990410	CRF02_AG	CRF02_AG			K20I, M36I		
04CMYD42	43	F	DQ990411	A1	A1			K20I, M36I, L63P		
04CMYD44	33	M	DQ990412	CRF02_AG	CRF02_AG			K20I, M36I		
04CMYD45	36	F	DQ990413	CRF02_AG	CRF02_AG			K20I, M36I		
04CMYD47	29	F	DQ990414	CRF02_AG	CRF02_AG			K20I, M36I		
04CMYD49	24	M	DQ990415	CRF02_AG	CRF02_AG			K20I, M36I		
04CMYD50	35	M	DQ990416	G	G			K20I, M36I		
04CMYD51	28	F	DQ990417	CRF02_AG	CRF02_AG			K20I, M36I		
04CMYD52	26	M	DQ990418	CRF02_AG	CRF02_AG			K20I, M36I		
04CMYD53	50	M	DQ990419	CRF02_AG	CRF02_AG			K20I, M36I, L63P		
04CMYD55	20	M	DQ990420	CRF02_AG	F2	CRF02_AG/F2[b]		K20I, M36I		
04CMYD56	26	M	DQ990421	D	D			L10V, K20R, M36I		
04CMYD57	35	F	DQ990422	D	D			M36I		
04CMYD58	60	F	DQ990423	CRF02_AG	CRF02_AG			K20I, M36I	**V108I**	
04CMYD60	43	M	DQ990424	CRF02_AG	CRF02_AG			L10V, K20R, M36I		
04CMYD61	21	F	DQ990425	CRF02_AG	CRF02_AG			K20I, M36I		
04CMYD62	32	M	DQ990426	CRF02_AG	CRF02_AG			K20I, M36I		
04CMYD63	30	F	DQ990427	CRF13_cpx	CRF13_cpx			K20I, M36I, V77I		
04CMYD64	36	M	DQ990428	F2	F2			L10V, K20R, M36I		
04CMYD65	42	F	DQ990429	CRF02_AG	CRF02_AG			K20I, M36I		
04CMYD66	28	M	DQ990430	F2	F2			L10V, K20R, M36I		
04CMYD67	35	M	DQ990431	G	G			K20I, M36I, (V82I)		
04CMYD69	43	M	DQ990432	CRF02_AG	CRF02_AG			K20I, M36I		
04CMYD70	38	F	DQ990433	H	H			K20R, M36I, D60E		
04CMYD71	44	F	DQ990434	F2	F2			K20R, M36I, D60E		
04CMYD72	48	M	DQ990435	CRF02_AG	CRF02_AG		**M46L**	K20I, M36I		
04CMYD73	39	F	DQ990436	F2	F2			K20R, M36I		
04CMYD77	22	F	DQ990437	CRF02_AG	CRF02_AG			K20I, M36I	**T215Y**	
04CMYD78	36	F	DQ990438	CRF02_AG	CRF02_AG			K20I, M36I		
04CMYD79	33	F	DQ990439	CRF02_AG	CRF02_AG			K20I, M36I		
04CMYD80	55	M	DQ990440	CRF02_AG	CRF02_AG			K20I, M36I, L63P	**T215F**	
04CMYD81	33	F	DQ990441	CRF02_AG	CRF02_AG			K20I, M36I	**T215Y**	
04CMYD82	37	M	DQ990442	CRF02_AG	CRF02_AG			K20I, M36I		
04CMYD83	47	M	DQ990443	CRF02_AG	CRF02_AG			K20I, M36I		
04CMYD86	45	F	DQ990444	CRF02_AG	CRF02_AG			K20I, M36I		
04CMYD89	40	F	DQ990445	CRF02_AG	CRF02_AG			K20I, M36I		
04CMYD90	24	M	DQ990446	CRF02_AG	CRF02_AG			K20I, M36I		
04CMYD92	32	M	DQ990447	F2	F2			M36I, L63P		
04CMYD93	42	M	DQ990448	CRF13_cpx	CRF13_cpx		**M46L**	K20I, M36I	**Y181C**	
04CMYD95	31	M	DQ990449	CRF02_AG	CRF02_AG			K20I, M36I		
04CMYD96	37	F	DQ990450	G	G			K20I, M36I, L63P		
04CMYD97	23	M	DQ990451	HIV-1 group O	HIV-1 group O			M36L, I93L	**Y181C, L210W**	
04CMYD98	38	M	DQ990452	HIV-1 group O	HIV-1 group O			M36L, I93L	**Y181C, L210W**	
04CMYD99	25	F	DQ990453	HIV-1 group O	HIV-1 group O			M36L, I93L	**Y181C, L210W**	
04CMYD100	28	F	DQ990454	HIV-2 group A	HIV-2 group A	HIV2.B/A		NA	NA	

[a] Typing of the *pol* gene (approximately 1,000 bp), encoding the Pol protease (Pol-PR) and Pol reverse transcriptase (Pol-RT) regions.

[b] Possible recombination between subtype F and CRF_02 within the region.

[c] Amino acid changes denote International AIDS Society (30) recognized mutations, while amino acid changes in parentheses stand for the presence of resistance mutations as minor mutations and subtype G naturally occurring polymorphisms. Primary drug resistance-associated mutations, shown in boldface type, lead to severalfold decreases in sensitivity to one or more ARTs. NA, not analyzed. All HIV group O samples contained Y181C as a natural occurring polymorphism. HIV-2B/A is a new recombinant strain, based on its *gag-p17/24* (1,500 bp), *pol-IN* (1,500 bp), and *env-C2V3* (500 bp) sequences.

[d] F, female; M, male.

this cohort, i.e., CMYD-97, -98, and -99 (5, 19, 26). Possible accessory amino acid mutations R211K and G333E in subtype B isolates were also observed in the RT genes of viruses from 54 patients (Table 1).

Dual-class resistance-associated mutations. In one of the CRF13_cpx isolates (1.2%; CI, 0.93 to 1.46%), we identified primary amino acid sites associated with resistance to PIs (M46L mutation [resistance to amprenavir, indinavir, ATV, and nelfinavir]) and NRTIs (Y181C mutation [resistance to DLV, EFV, and NVP). Further phenotypic resistance would be needed to confirm these genotypic analyses.

DISCUSSION

In the current study, we found 2.6% PI resistance and 9.3% major RTI resistance mutations in HIV-1-infected drug-naïve individuals in Yaoundé, Cameroon. Unlike the case in developed countries, where antiretroviral regimens containing PIs are readily available, the first line of ART in Cameroon is the combination of two NRTIs plus one NNRTI. Very few patients in Cameroon are currently being or have been treated with PIs (1, 16–18). Konings et al. (16) reported that only the minor mutations associated with PI resistance were detected among HIV-1-infected drug-naïve patients in Cameroon during the period of 2000 to 2002. Our study confirmed previous reports and describes a high frequency of minor mutations (isoleucine or valine at position 10 in CRF02_AG; K20I and M36I mutations), which were found in all sequences except one, i.e., the CMY-36 isolate classified as CRF11_cpx. Of greater concern is the appearance of the major PI resistance mutation M46L in two infected patients (one with CRF02_AG and another with CRF13_cpx). The identification of this amino acid mutation in the protease warrants a more thorough screen of CRF02_AG and CRF13_cpx protease sequences, which is currently under way.

Three major NRTI resistance mutations were observed as wild-type sequences in three CRF02_AG (T215Y/F) and one CRF13_cpx (Y118C) virus. The T215Y/F mutation confers resistance to ZDV in nearly all HIV-1 isolates, whereas Y118C is a mutation related to native versus nucleoside analog discrimination but confers only low-level resistance (10, 11, 30). Limited studies on ART drug resistance in Africa, especially for non-B subtypes in Europe, have shown a strong correlation between the presence of major mutations and phenotypic resistance, similar to the case for mutations seen in subtype B infections with similar treatment regimens (31, 33). However, studies have also documented some salient differences among patients infected with non-B subtypes. A study of single-dose NVP to prevent mother-to-child transmission of HIV-1, conducted in Uganda, showed that selection of genotypic mutations associated with resistance to NVP occurred more frequently in women infected with subtype D than in women infected with subtype A viruses (23, 24). In addition, there has been identification of new mutational patterns conferring high-level drug resistance, previously not characterized for subtype B isolates (3, 23, 25, 26). For example, the V106M mutation in subtype C, as opposed to the V106A mutation of subtype B, is generally selected and confers resistance to EFV (3, 4). In addition, a combination of three mutations (I135L, T139V, and V245T) found as "wild-type" sequences in a subtype D HIV-1 isolate in Uganda conferred over 1,000-fold resistance to NVP and DLV and some cross-resistance to EFV (8). We are currently examining the phenotypic resistance of the PR-RT coding regions of Cameroonian HIV-1 isolates with or without any ARV resistance sequences. Although resistance testing was performed on PBMCs, this is a more sensitive method for detection of archived resistant mutants in persons lacking evidence of resistance by conventional assays.

This study provides the most recent data on molecular characterization of HIV-1 isolates in treatment-naïve individuals in Yaoundé, Cameroon. Overall, there is clear documentation of cocirculating HIV-1 group M and O strains as well as evidence for HIV-2 B/A recombinants, which are the subject of further investigation. At least six genetic subtypes (A, D, F2, G, H, and K) and three CRFs (CRF02_AG, CRF11_cpx, and CRF13_cpx) have been identified in HIV-1-infected patients in Yaoundé. Subtype CRF02_AG was responsible for 51.89% of the infections and was previously identified as predominant in west and west-central Africa (1, 6, 14–19, 21, 22, 24, 28). HIV-2 has been observed with a very low prevalence (0.06% of total HIV infections) in Douala but at a higher frequency in Yaoundé (0.2% to 1.2% of total HIV infections), based on independent epidemiological surveys (28, 36). A higher prevalence of HIV-2 infections was observed in commercial sex workers and tuberculosis patients, with no apparent link to other West African countries (36). However, the origin of the HIV-2 infection in our study was not available (7).

An obvious challenge in resource-limited settings such as Yaoundé, Cameroon, is maintaining a balance between rapid introduction of ART and continual surveillance of drug resistance to prevent treatment failures and to avoid a public health crisis. Expansion of molecular characterization on a nationwide basis would be useful to scientists developing prevention strategies based on vaccines and microbicides. Although there may be a cost factor involved, ART should be accompanied by testing for resistance before the choice of a particular ART regimen is made. This will reduce the selection pressure of resistance types, thus making first-line therapy more effective.

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