Original Study Article
https://doi.org/10.36233/0372-9311-616

Analysis of HIV-1 genetic variants and drug resistance among men with high-risk sexual behavior, Cuban citizens, living in Moscow in 2022–2024

Ilya A. Lapovok[1]✉, Alina A. Kirichenko[1], Anastasia V. Shlykova[1], Dmitry E. Kireev[1], Anna A. Popova[1, 2], Anastasia V. Pokrovskaya[1, 3]

[1]Central Research Institute of Epidemiology, Moscow, Russia;
[2]Russian Medical Academy of Continuous Professional Education, Moscow, Russia;
[3]Peoples' Friendship University of Russia, Moscow, Russia

Abstract

Introduction. The characteristics of the HIV epidemic in Cuba are comparable to those of the HIV epidemic in Russia. Migration between Cuba and Russia can affect HIV-1 genetic diversity and drug resistance in Russia. The vulnerable group of men with risk sexual behavior including men who have sex with men (MSM) and commercial sex workers (CSW) deserves special attention. The **aim** of our study was the analysis of HIV-1 genetic variants and HIV-1 drug resistance in blood plasma samples obtained from MSM migrants from Cuba living in Moscow.

Materials and methods. A collection of blood plasma samples, epidemiological and clinical information was collected from MSM patients — migrants from Cuba, nucleotide sequences of the HIV-1 genome were obtained. HIV-1 genotyping, cluster analysis and analysis of drug resistance (DR) were carried out.

Results. Samples and epidemiological data obtained in 2022–2024 from 27 patients were analyzed. 24/27 patients (including 12/15 suspected of infection in Moscow and all 10 — in Cuba) harbored HIV-1 variants typical for Cuba, not Russia. This indicates that 88.89% of patients were infected by their fellow citizen. DR was detected in 9 patients (33.33%; 95% CI 15.55–51.11). The most common resistance was DR to efavirenz (EFV) and nevirapine (NVP), which was associated with *K103N, Y181C* and *P225H* mutations.

Conclusion. The migration factor should be taken into account in HIV-1 prevention and control of HIV-spreading programs in Russia, and the genetic characteristics of HIV-1 in migrants should be taken into account in effective therapy selecting.

Keywords: *HIV-1, MSM, migrants, viral variant, CRF, BG-recombinants, drug resistance, cluster*

Ethics approval. The study was conducted with the informed consent of the patients. The research protocol was approved by the Ethics Committee of the Central Research Institute of Epidemiology (protocol No. 142, April 25, 2024).

Funding source. This study was not supported by any external sources of funding.

Conflict of interest. The authors declare no apparent or potential conflicts of interest related to the publication of this article.

For citation: Lapovok I.A., Kirichenko A.A., Shlykova A.V., Kireev D.E., Popova A.A., Pokrovskaya A.V. Analysis of HIV-1 genetic variants and drug resistance among men with high-risk sexual behavior, Cuban citizens, living in Moscow in 2022–2024. *Journal of microbiology, epidemiology and immunobiology.* 2025;ONLINE FIRST
DOI: https://doi.org/10.36233/0372-9311-616
EDN: https://www.elibrary.ru/zoyqjd

DOI: https://doi.org/10.36233/0372-9311-616

Оригинальное исследование
https://doi.org/10.36233/0372-9311-616

Анализ генетических вариантов и лекарственной устойчивости ВИЧ-1 среди мужчин с рискованным сексуальным поведением, граждан Кубы, проживающих в Москве в 2022–2024 годах

Лаповок И.А.[1]✉, Кириченко А.А.[1], Шлыкова А.В.[1], Киреев Д.Е.[1], Попова А.А.[1, 2], Покровская А.В.[1, 3]

[1]Центральный научно-исследовательский институт эпидемиологии Роспотребнадзора, Москва, Россия;
[2]Российская медицинская академия непрерывного профессионального образования, Москва, Россия;
[3]Российский университет дружбы народов имени Патриса Лумумбы, Москва, Россия

Аннотация

Введение. Эпидемия ВИЧ-инфекции на Кубе имеет свои особенности по сравнению с эпидемией в России. Миграционные потоки между Кубой и Россией способны оказать влияние на генетическое разнообразие и лекарственную устойчивость ВИЧ-1 в России. Отдельного внимания заслуживает уязвимая группа мужчин с рискованным сексуальным поведением, в частности — мужчины, практикующие секс с мужчинами (МСМ), и работники коммерческого секса.

Целью нашего исследования был анализ генетических вариантов ВИЧ-1 и лекарственной устойчивости ВИЧ-1 в образцах плазмы крови, полученных от мигрантов — МСМ с Кубы, проживающих в Москве.

Материалы и методы. Была собрана коллекция образцов плазмы крови, сопутствующая эпидемиологическая и клиническая информация от пациентов — МСМ с Кубы, получены нуклеотидные последовательности генома ВИЧ-1. Были проведены генотипический, кластерный анализ и анализ лекарственной устойчивости (ЛУ) ВИЧ-1.

Результаты. Были проанализированы образцы и эпидданные, полученные от 27 пациентов в 2022–2024 гг. Было выявлено, что 24/27 пациентов (включая 12/15, предполагающих факт инфицирования в Москве, и 10, предполагающих инфицирование на Кубе), были инфицированы вариантами ВИЧ-1, типичными для Кубы, а не для России. Это говорит об инфицировании 88,89% пациентов их согражданином. ЛУ была выявлена у 9 (33,33%; 95% ДИ 15,55–51,11) пациентов. Наиболее часто отмечалась устойчивость к эфавирензу и невирапину, что было связано с мутациями *K103N*, *Y181C* и *P225H* гена обратной транскриптазы.

Заключение. Фактор миграции должен учитываться в программах профилактики и противодействия распространению ВИЧ-инфекции в России, а генетические особенности ВИЧ-1 необходимо принимать во внимание при подборе эффективной терапии у мигрантов.

Ключевые слова: *ВИЧ-1, МСМ, мигранты, вирусный вариант, CRF, BG-рекомбинанты, лекарственная устойчивость, кластер*

Этическое утверждение. Исследование проводилось при добровольном информированном согласии пациентов. Протокол исследования одобрен Этическим комитетом ЦНИИ Эпидемиологии (протокол № 142 от 25.04.2024).

Источник финансирования. Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Для цитирования: Лаповок И.А., Кириченко А.А., Шлыкова А.В., Киреев Д.Е., Попова А.А., Покровская А.В. Анализ генетических вариантов и лекарственной устойчивости ВИЧ-1 среди мужчин с рискованным сексуальным поведением, граждан Кубы, проживающих в Москве в 2022–2024 годах. *Журнал микробиологии, эпидемиологии и иммунобиологии.* 2025;ONLINE FIRST
DOI: https://doi.org/10.36233/0372-9311-616
EDN: https://www.elibrary.ru/zoyqjd

Introduction

The relationships between Cuba and Russia have been significantly strengthened in recent years. In recent years, cooperation between the two countries has been based on the Declaration on Principles of Relations between the Russian Federation and the Republic of Cuba[1], the Memorandum on Principles of Strategic Cooperation[2] and the Joint Statement of the Presidents of the two countries[3], adopted in 1996, 2009 and 2018, respectively.

The close relationships between Russia and Cuba lead to increased migration processes between the countries. Since the spread of HIV infection is a problem in both countries, there is always a risk that HIV-1 variants circulating in the one country could be brought into the other country. The spread of these variants within certain vulnerable groups can then lead to an increase in infections, which can affect the genetic landscape of HIV infection and the drug resistance (DR) profile of a country. One such vulnerable group is men with high-risk sexual behaviors, particularly men who have sex with men (MSM) as well as commercial sex workers (CSW). Sexual contacts with citizens of other countries are typical for members of this group, which can affect the genetic diversity of HIV-1 and lead to the generation of new recombinant forms of the virus.

Cuba has seen an increase in the number of HIV infections in recent years. According to the United Nations Joint Program on HIV/AIDS (UNAIDS), the total number of people living with HIV-1 in Cuba has continued to increase in the last decade, rising from 3,100 in 2000 to 14,000 in 2011 [1] and by 44,000 in 2023[4]. Meanwhile, in recent years, Cuba has shown success diagnostic and treatment of HIV infection. A total of 33,000 (75%) people knew their HIV status in 2023. Of these, 28,000 (84.84%) were on antiretroviral therapy (ART). Finally, 24,000 (85.72%) patients on ART had virological success. Thus, Cuba is well on its way to meeting the WHO's 90–90–90 HIV strategy and has the prospect of reaching the main targets of the 95–95–95 strategy by 2030 [2].

The genetic diversity of HIV-1 in Cuba is very different from that of the Caribbean. While virus of subtype B is dominated in most countries of the region (which was associated with more than 90% of infections in the early 2000s) a large number of other HIV-1 genetic variants are actively circulating in Cuba, including recombinant forms that are spreading only on the island [3]. Thus, while back in the mid-1990s the dominant virus variant in Cuba was subtype B [4], a 2002 study showed that only 48% of patients were infected with this genetic variant [5]. In 2017, the proportion of subtype B in samples from patients over 18 years of age collected during the 1st half of 2017 was only 26.9%, and infection with various recombinant forms accounted for 59.5% of HIV infections [6].

With regard to subtype B prevalent in Cuba, it should be mentioned that this variant is genetically similar to the virus prevalent in the United States and most Western European countries, but differs from viruses prevalent in other Caribbean countries [1, 7, 8]. Some researchers attribute this to the repeated unrelated importations of subtype B from the United States, Canada and the European continent in the late 1970s [7]. Others suggest that it arrived in Cuba in the early 1990s from the United States, when, against the background of the economic crisis caused by the collapse of the USSR, the tourist business in Cuba began to develop and migration flows shifted to the United States [1].

In addition to subtype B, a recombinant form CRF19_cpx, whose genome is represented by fragments of the HIV-1 genome of subtypes D, A1, and G, is circulating in Cuba [3, 9]. CRF19_cpx was first described in Cuba in 1999. [10]. A detailed analysis revealed that CRF19_cpx is a recombinant of two viral variants: AG recombinant from Cameroon, later described as CRF37_cpx recombinant, and a subtype D virus from Gabon [9-11]. CRF19_cpx appears to have emerged in the Cuban community in the Democratic Republic of Congo (DRC) in 1966–1970, from where it entered the province of Villa Clara, Cuba in the late 1970s, i.e., before the spread of HIV-1 into Western European countries (in the late 1970s). Then there was its spreading in Havana and other provinces of Cuba [8, 10, 12]. By 2017, this genetic variant accounted for 24.1% of new HIV infections among patients over 18 years of age [6].

Also another recombinant CRF18_cpx with a complex, mosaic genome structure is widely distributed in Cuba. Apparently, it is also of African origin and could have come from either the DRC or the Central African Republic or the Republic of Cameroon or the Republic of Angola [1, 12, 13]. The genome structure of CRF18_cpx is more mosaic than that of CRF19_cpx and is represented by regions identical to viruses of subtypes A, F, G, H, K and U. In describing this genetic variant, 40 genetically related virus samples were identified, including CRF04_cpx and CRF13_cpx viruses [13].

---

[1] Electronic fund of legal and normative-technical documents. Declaration on the principles of relations between the Russian Federation and the Republic of Cuba. 1996. URL: https://docs.cntd.ru/document/1902532?section=text

[2] Electronic fund of legal and normative-technical documents. Memorandum on the principles of strategic cooperation between the Russian Federation and the Republic of Cuba. 2009. URL: https://docs.cntd.ru/document/902161646?section=text

[3] Official website of the President of the Russian Federation. Joint statement of the President of the Russian Federation V.V. Putin and the Chairman of the State Council and the Council of Ministers of the Republic of Cuba M. Diaz-Canel Bermudez on common approaches to international affairs. 2018. URL: http://www.kremlin.ru/supplement/5354

[4] UNAIDS Country Fact Sheet. Cuba, 2023. URL: https://www.unaids.org/en/regionscountries/countries/cuba

DOI: https://doi.org/10.36233/0372-9311-616

The period from the mid-1980s to the mid-1990s was marked in Cuba by the appearance of other recombinant forms, including BG recombinants, among HIV-infected people [1]. The BG recombinants circulating in Cuba have Cuban origin [3], having resulted from the recombination of HIV-1 variants of subtypes B and G that had previously circulated on the island [1]. All Cuban BG recombinants (CRF20, CRF23, CRF24) have a common origin from subtype G from Central Africa, circulating among heterosexuals, and subtype B (close to the one prevalent in the USA), circulating among MSM in Havana in the early 2000s. It is logical that BG recombinants were initially detected among MSM in Havana, and by 2003 were responsible for more than 30% of HIV infections in the Cuban capital [1, 8, 12].

This fact clearly illustrates the role of recombination as one of the drivers of HIV-1 genetic variability and makes Cuba one of the HIV-1 recombination hotspots along with Myanmar, South China, East Africa, Argentina and Brazil [8]. Moreover, recombinants generated in Cuba subsequently began to spread around the world. Thus, Cuban CRF20_BG is found in Spain and Greece [1].

Except for the recombinant forms mentioned above, other HIV-1 variants circulate in Cuba: at least 2 subtype C lineages from East and South Africa [1]. HIV-1 subtype G, the progenitor of BG recombinants, is likely has the Central African origin [1]. In addition, active circulation of HIV-1 subtype H has been noted in the province of Santiago de Cuba [12, 14]. Finally, some cases of HIV infection caused by CRF05_DF, which was previously reported in Belgium, DRC, Spain, and Costa Rica, have been identified [14].

The role of the vulnerable MSM group in the HIV epidemic in Cuba is crucial. While in 2002 just over 81% of HIV-infected men were MSM [5], in the first half of 2017 this index reached to 94.31% [6]. This vulnerable group is currently the dominant group in Cuba. The invasion of HIV-1 subtype B into the MSM group at the end of the twentieth century led to the active spreading and domination of this variant in Cuba [7]. The same factor became the basis for the increase in genetic diversity in Cuba: already in the early 2000s, Cuban BG-recombinant forms of HIV-1 began to spread among MSM [1, 8, 12].

Full-scale ART implementation began in Cuba in 2001 through the use of primarily generic nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) [6], achieving virologic success in 83% of patients in 2017 and nearly 86% in 2023. [6]. However, the use of ART has also led to an increase in DR in the country. In 2017, the prevalence of DR in ART-naïve patients to at least one drug was 29.8%, which is higher than this index in 2007–2011 (12.5%) and 2009–2012 (20.2%) [6].

In 2017, the primary DR to NRTIs was 10.6% and to NNRTIs was more than 23%. The prevalence of high-level DR to NNRTIs was associated with the frequency of K103N, G190A, and Y181C mutations [6]. Moreover, DR was significantly more frequently detected in people 18–25 years old compared to groups of older people.

There are significant differences between the genetic diversity of HIV-1 in Cuba and Russia. In Russia, since the late 1990s, HIV-1 sub-subtype A6 spreading in Russia and the former Soviet Union through injecting drug users has been dominant [15, 16]. The second most common HIV-1 variant is recombinant CRF63_02A1, which in recent years has been actively spreading in Central Asian countries and the Siberian region of Russia [15, 17].

In Russia, circulation of two variants of HIV-1 subtype B has been detected: Bwest, characteristic of Western Europe and Cuba, and Bfsu (IDU-B), which circulation was noted in the Russian Far East and which, together with sub-subtype A6, became the progenitor of CRF03_AB [15, 16, 18, 19]. The circulating of HIV-1 subtype C, CRF03_AB, and subtype G have also been observed in Russia [15].

In recent years, unique and rare AG-recombinant forms, probably imported from Central Asian countries, have been frequently detected in Russia [15].

The problem of HIV infection among MSM in Russia also has its own peculiarities, primarily due to the insufficient study of this vulnerable group. MSM were the main vulnerable group until the mid-1990s But after HIV-1 invasion in community of injecting drug users, these people became the main vulnerable group, and the contribution of MSM to the HIV epidemic in Russia became vanishingly low [15, 16, 18, 20]. This can be largely explained by the stigmatization of MSM and the lack of work on monitoring this group in Russia [18, 20]. Meanwhile, there are estimates of the probable prevalence of HIV-1 among MSM in different regions of the country — 5–25%, and UNAIDS in 2016 indicated the value of this index in Russia at 6% [18].

In recent years, the share of MSM among new HIV infections in Russia has been gradually increasing. Thus, by the end of 2020, only 2.8% of HIV cases in the country were associated with MSM. By the end of 2021, this figure increased to 3%, and by the end of 2023 — to 4.1%[5].

Bwest has historically dominated among Russian MSM, but in the recent past there has been a trend towards an increase in the proportion of HIV-1 sub-subtype A6 in this vulnerable group [15, 18], which suggests that the boundaries of this vulnerable group are blurring. In addition, the circulation in Russian MSM of BG recombinants genetically similar to viruses circulating in Spain and Portugal, but not to

[5] Federal Scientific and Methodological Center for the Prevention and Control of AIDS. URL: http://www.hivrussia.info

CRF20/23/24 viruses prevalent in Cuba, has been detected among Russian MSM [21].

Regarding the problem of HIV-1 DR in Russia, the most frequently detected resistance mutations were *K103N/S*, *G190A/S* in the same positions as for the Cuban samples. Meanwhile, instead of *Y181C*, the *K101E* substitution causing resistance to all NNRTIs is more often detected in Russian samples [22].

Taking into account the above-mentioned features of HIV infection in Cuba and Russia, as well as the role of MSM in the HIV epidemic in both countries, **the aim** of our study was to analyze HIV-1 genetic variants and viral drug resistance in blood plasma samples obtained from MSM migrants from Cuba living in Moscow, one of the most economically developed centers of Russia, where historically there is an extensive community of MSM and migrants.

Materials and methods

The collection of 27 blood plasma samples obtained from MSM Cuban citizens in period since March 2022 till June 2024 was studied. At the same time, clinical and epidemiologic data were collected and processed: age, dates of the last negative and first positive HIV test, information on the likely place and time of infection, number of sexual partners, experience with ART, as well as the stage of HIV infection. Patients were recruited for inclusion in the study in collaboration with non-profit organizations: Steps Foundation[6] and LaSky Center[7].

HIV-1 RNA concentration (viral load, VL) and CD4-lymphocyte count were determined in blood plasma samples.

The nucleotide sequences of the *pol* region (positions 2253–3353 of the reference strain HXB-2, GenBank number K03455) encoding HIV-1 protease and reverse transcriptase fragment in the studied samples were obtained. Sequencing was performed using the AmpliSens HIV-Resist-Seq reagent kit (Central Research Institute of Epidemiology) and an Applied Biosystems genetic analyzer (Life Technologies).

A sequence analysis was performed, including preliminary determination of the genetic variant using the HIVBlast online application[8], phylogenetic analysis in the MEGA 6.0 program [23], and cluster analysis of nucleotide sequences using the ClusterPicker 1.2.3 program (genetic distance threshold of 4.5% with bootstrap support of more than 90%)[9].

For phylogenetic and cluster analyses, the obtained sample was supplemented with nucleotide sequences of Cuban patients (n = 430) from the international HIV-1 database of the Los Alamos Institute (USA)[10], described in publications devoted to the analysis of HIV-1 samples isolated in Cuba in 2007–2017 [3, 6, 10]. For the phylogenetic analysis of genomes genetically close to HIV-1 variants circulating in Russia, we used a collection of reference sequences used earlier in the analysis of HIV-1 variants circulating in Eastern Europe and Central Asia in 2010–2019 [15].

HIV-1 DR was analyzed using the web service HIVdb of the Stanford University database[11] with the determination of both resistance mutations and DR level based on the Stanford Penalty Score calculation [22, 24].

The nucleotide sequences obtained in this study were uploaded to the Russian database of HIV resistance to antiretroviral drugs, RuHIV (https://ruhiv.ru/) under accession numbers RHD10698, RHD10712, RHD10720, RHD10721, RHD10725, RHD10727, RHD10733-RHD10736, RHD10739, RHD16068, RHD16123, RHD16132, RHD17497, RHD17505, RHD17513, RHD17514, RHD20769, RHD20773, RHD20782, RHD20783, RHD20796-RHD20798, RHD20806, RHD20813.

Results

The mean VL level was 5.33 (95% CI 5.19–5.44) log copies/mL and the mean CD4-lymphocyte count was 405 (95% CI 296.34–513.34) cells/μL. For 23 (85.19%) patients, stage 2a HIV infection was determined. Another 4 patients were at stage 3a. No significant associations were found between HIV infection stage and VL value or CD4-lymphocyte count.

We analyzed the genetic variants of HIV-1 in the samples based on a search for maximally genetically similar reference sequences from the GenBank database using the HIVBlast online application. The results of the analysis are presented in the **Table**. 21/27 (77.78%) samples were genetically close to HIV-1 reference sequences from Cuba. At the same time, 12 (57.14%) of the 21 patients infected with these HIV-1 variants suggested that their infection occurred in Moscow and not in Cuba.

Two patients were infected with HIV-1 subtype C, typical of Botswana. Another 2 patients had HIV-1 subtype B, which is close to the viruses isolated in Germany: patient M151, infected with HIV-1 subtype B, presumed to have been infected in Cuba, and patient M80 — in Moscow.

[6] Foundation for the Prevention of Socially Significant Diseases "Steps". URL: http://stepsfund.ru

[7] Low-threshold center for the prevention of HIV infection and support for people living with HIV in Moscow and the Moscow region. URL: https://lasky.ru

[8] Los Alamos National Laboratory. HIV BLAST. URL: https://www.hiv.lanl.gov/content/sequence/BASIC_BLAST/basic_blast.html

[9] Leigh Brown HIV Research Group. Picking and Describing HIV Clusters in Phylogenetic Trees. URL: https://hiv.bio.ed.ac.uk/software.html

[10] Los Alamos National Laboratory. HIV databases. URL: https://www.hiv.lanl.gov

[11] Stanford HIV Drug Resistance Database. URL: https://hivdb.stanford.edu

DOI: https://doi.org/10.36233/0372-9311-616

Results of preliminary genotyping of HIV-1 nucleotide sequences in the HIVBlast online application

Sample	Presumed place of infection	Reference sequence in HIVBlast			
		GenBank number	genetic variant	country	genetic similarity, %
M52	Moscow	MZ004274	CRF19_cpx	Cuba	98
M67	Moscow	MZ004382	CRF19_cpx	Cuba	95
M75	Moscow	MK817409	CRF20_BG	Cuba	98
M76	Moscow	MK817388	CRF20_BG	Cuba	96
M80	Moscow	MH471360	Subtype B	Germany	96
M82	Moscow	MH667011	Subtype B	Russia	98
M88	Moscow	DQ113271	CRF19_cpx	Cuba	95
M89	Moscow	MZ004339	CRF19_cpx	Cuba	96
M90	Unknown	DQ113301	Subtype B	Cuba	96
M91	Moscow	JQ585469	Subtype B	Cuba	97
M94	Moscow	DQ113060	CRF19_cpx	Cuba	96
M96	Moscow	OL792340	Sub-subtype A6	Russia	97
M151	Cuba	KJ770458	Subtype B	Germany	96
M160	Moscow	MZ004178	CRF19_cpx	Cuba	97
M169	Moscow	MK817435	CRF18_cpx	Cuba	96
M177	Moscow	AY900579	CRF24_BG	Cuba	97
M185	Cuba	KR860993	Subtype C	Botswana	95
M186	Cuba	KR860993	Subtype C	Botswana	94
M195	Moscow	JN000054	CRF20_BG	Cuba	97
M199	Cuba	JN000009	Subtype B	Cuba	97
M208	Cuba	MK817498	CRF18_cpx	Cuba	96
M209	Cuba	MK817361	Subtype B	Cuba	97
M222	Cuba	JN000021	CRF24_BG	Cuba	96
M223	Cuba	MK817465	CRF18_cpx	Cuba	96
M224	Unknown	DQ020274	CRF20_BG	Cuba	90
M232	Cuba	MK817454	CRF18_cpx	Cuba	98
M240	Cuba	DQ113256	Subtype B	Cuba	96

Only 2 patients yielded HIV-1 samples typical for Russia: one patient was infected with a sub-subtype A6 virus and another with a virus genetically similar to subtype B references from the Czech Republic and Russia, and the Russian A6 and B reference viruses were isolated from male patients with homosexual and heterosexual transmission of HIV-1 in 2019 and 2015, respectively.

The preliminary genotyping results obtained were mostly confirmed by phylogenetic analysis with HIV-1 reference nucleotide sequences isolated from Cuban patients between 2007 and 2017 (**Fig. 1**). The same set of reference sequences and genomes under study (n = 457) were subjected to cluster analysis.

We identified 3 clusters formed by the samples studied:

1) a cluster formed by 2 HIV-1 subtype C samples from epidemiologically related patients M185 and M186;

2) a cluster formed by a sample from patient M222 and reference MK817363;

3) cluster formed by sample M160 and reference sequences MZ004165 and MZ004178.

Patient M222 was CSW practicing sex under chemical drugs. He was diagnosed with HIV infection in 2013, and his presumed site of infection in the same 2013 was Cuba. Therefore, the formation of a cluster

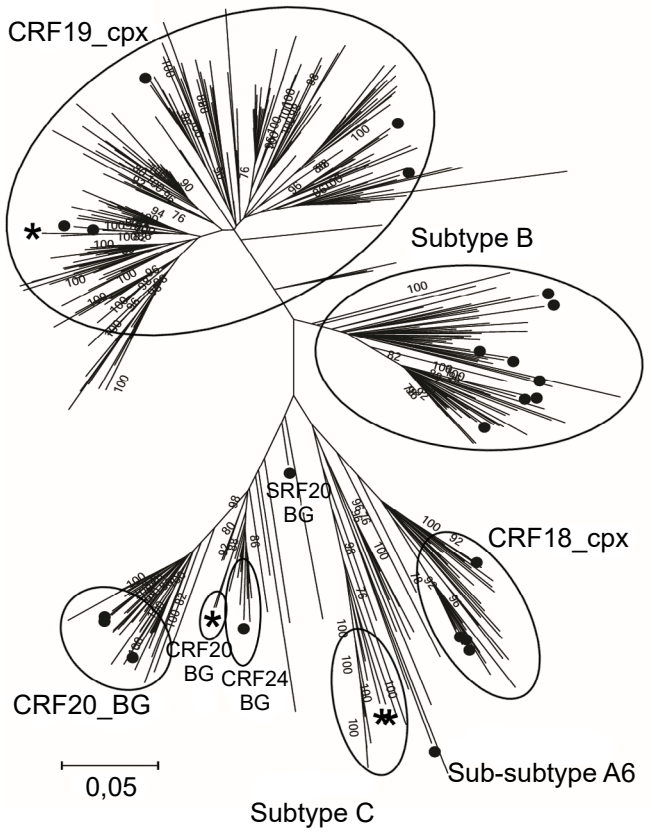

Fig. 1. Results of phylogenetic and cluster analysis of the pol gene fragment (positions 2253–3353) nucleotide sequences of the studied HIV-1 samples' collection (*n* = 27) and the comparison group (*n* = 430) of Cuban HIV-1 samples.

Phylogenetic analysis was performed by the maximum likelihood method using the GTR+G+I model of nucleotide substitutions in 500 independent constructions. Cluster analysis was performed with a genetic distance threshold of 4.5% and bootstrap support of more than 90%. Sequences of the studied collection that formed clusters are indicated by asterisks, those that did not are marked with black circles.

between M222 and MK817363, a CRF20_BG virus isolated in Cuba in 2017, is not unexpected. Interestingly, this sample was initially identified in the HIVBlast program as CRF24_BG (Table), since CRF20 and CRF24 are very close in terms of the genetic fragment studied. Cluster analysis corrected these data by assigning the investigated sample to CRF20.

At the same time, patient M160, whose sample shared a common cluster with CRF19_cpx samples MZ004165 and MZ004178 isolated in Havana in 2013 from a male and a female, respectively, was also a CSW and a transgender person. The patient was diagnosed with HIV infection in June 2023, and the estimated site and date of infection was Moscow, January 2022.

Samples from patients M82 and M96, genetically similar to Russian subtype B and sub-subtype A6 viruses, respectively, were subjected to additional phylogenetic analysis with reference sequences of HIV-1 genetic variants circulating in Russia (**Fig. 2**). Sample M96, in 82% of possible constructs, formed a common branch with the sub-subtype A6 AF413987 and AY500393 reference sequences. Sample M82, in turn, with 78% reliability formed a common branch with the Russian reference Bwest AY819715 and the main world reference of the same lineage HXB-2 K03455.

No DR to protease inhibitors was detected in any HIV-1 sample (**Fig. 3**). DR to at least one reverse transcriptase inhibitor was detected in 9 (33.33%; 95% CI 15.55–51.11) samples, and 3 (11.11%; 95% CI 0–22.97) samples (one CRF19_cpx and two CRF20_BG) were resistant to both NRTIs and NNRTIs. Typical Russian viruses from the above-mentioned samples from patients M82 and M96 did not contain DR, as well as subtype C viruses from patients M185 and M186 and HIV-1 subtype B from patient M80. The most frequently we detected DR (predominantly high level) to the NNRTIs efavirenz and nevirapine (in 29.63% of samples; 95% CI 12.41–46.85), associated with *K103N*, *Y181C* and *P225H* mutations (Fig. 3). Resistance to rilpivirine was also associated with the presence of *Y181C*, *K101E* and *E138A* mutations. Finally, high-level DR to the NNRTIs emtricitabine and lamivudine in the virus from 2 (7.41%; 95% CI 0–17.29) samples was associated with the presence of the *M184V* mutation.

Six of 9 patients infected with DR HIV-1 had no experience with therapy and 3 had past experience. Patient M222 with experience of taking efavirenz + tenofovir disoproxil + lamivudine had a virus with high level DR to efavirenz (and cross-DR to nevirapine) and lamivudine due to a combination of *M184V*, *K103N* and *P225H* mutations. Patient M208, with a history of taking Truvada 2 years prior to the study, had HIV-1 with only the *K103N* mutation causing high-level DR to efavirenz and nevirapine. The same substitution was detected in patient sample M223.

Discussion

The results of HIV-1 genetic analysis indicate that the absolute majority of 21 patients (77.78%) were infected with the virus variant typical of the patient's country of origin — Cuba. For 12 of the 15 patients who indicated Moscow as the probable place of infection, there was either infection from a Cuban citizen or a common sexual partner; or an incorrect assessment of the place of probable infection had a place. The 2 cases of infection with a typical Botswana subtype C virus most likely occurred in Cuba, as the patients assumed, bacause African variants of HIV-1 were detected in Cuba in 2013 [1]. The same is real for patient M151, who was found to have HIV infection with a subtype B virus genetically similar to the virus circulating in Germany, reflecting the link between the HIV epidemic in Cuba and Western Europe [1, 7].

Two samples, M222 (CRF20_BG) and M160 (CRF19_cpx), formed two active (expanding) clusters that also included samples isolated from Cubans in a

DOI: https://doi.org/10.36233/0372-9311-616

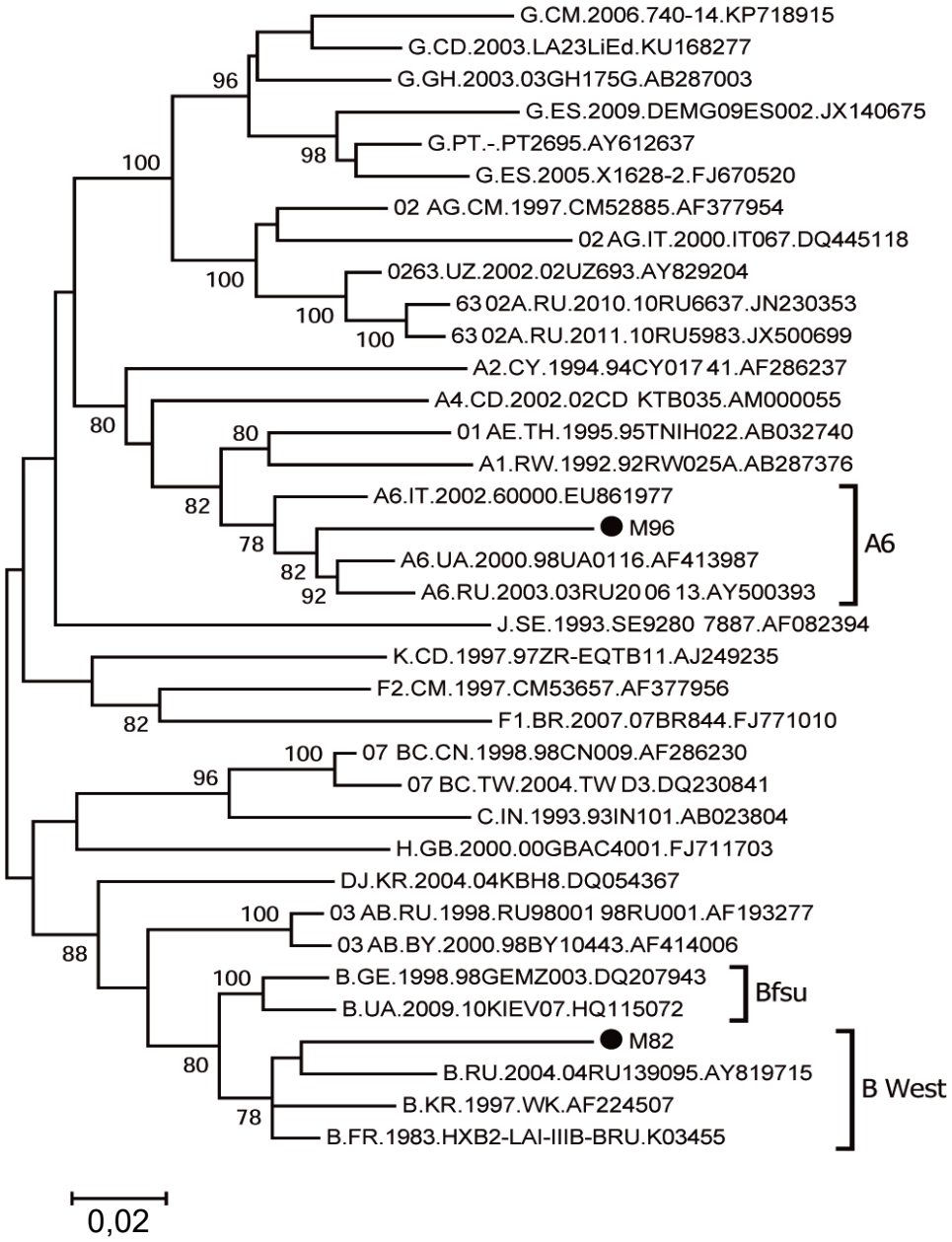

Fig. 2. Results of phylogenetic analysis of the *pol* gene fragment (positions 2253–3353) nucleotide sequences of HIV-1 samples from patients M82 and M96, presumably belonging to genetic variants circulating in Russia.

Phylogenetic analysis was performed using the maximum likelihood method with the HKY+G model of nucleotide substitutions and bootstrap support of 500. Sequences of M82 and M96 are indicated by black circles. Sub-branches formed by viruses of sub-subtype A6 and genetic variants Bwest and Bfsu are highlighted by frames.

different time period (a difference of 4 and 10 years, respectively). This suggests continued circulation and further spread of these viral genetic lineages worldwide.

Thus, a total of 24 (88.88%) of the Cuban nationals MSM we studied living in Moscow were probably infected either in their home country or from a fellow citizen. Only 3 patients could have been infected in Russia:

- M82 and M96 patients infected with HIV-1 typical for the territory of Russia;
- patient M80 infected with a subtype B virus genetically close to the strain circulating in Germany. Meanwhile, the circulation of Western European variants of HIV-1 is typical for the vulnerable MSM group in Russia [18, 20, 21].

Our data on the incidence of DR have low statistical reliability due to the small size of sample collection (n = 27), which does not allow reliable comparison of our results with published data on HIV-1 DR in Cuba. However, we detected DR in 9 virus samples, 3 of

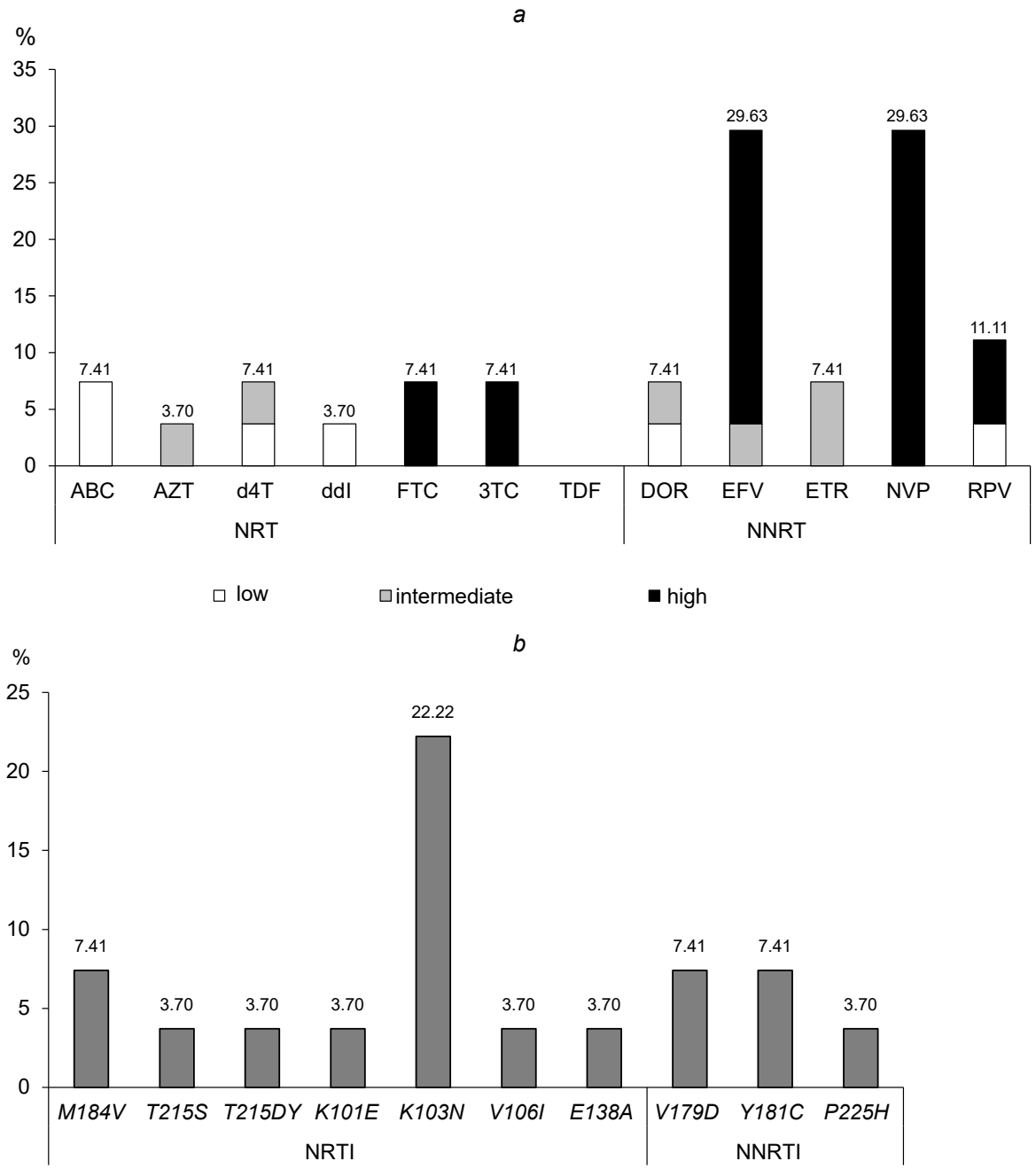

Fig. 3. Results of the analysis of occurrence frequency for drug resistance (*a*) and drug resistance mutations to HIV-1 inhibitors of the NRTI and NNRTI classes (*b*).

ABC — abacavir; AZT — zidovudine; d4T — stavudine; ddI — didanosine; FTC — emtricitabine; 3TC — lamivudine; TDF — tenofovir disoproxil; DOR — doravirine; EFV — efavirenz; ETR — etravirine; NVP — nevirapine; RPV — rilpivirine. The analysis was carried out using the HIVdb online application, the degree of resistance was determined based on the calculation of the Stanford Penalty Score.

which were obtained from patients with previous therapy experience. The most frequently detected high-level DR to efavirenz and nevirapine is a trend in recent years in Russia and low- and middle-income countries [22, 24]. The presence of HIV-1 with DR in 6 (25%; 95% CI 8.67–41.33) of 24 patients, who probably received HIV-1 from Cuban citizens, is a consequence of the problem of DR spread in Cuba [6]. Moreover, the use of effective therapeutic regimens may be the key to virologic success even in the face of HIV-1 resistance to efavirenz and nevirapine.

Conclusion

Our results suggest a contribution of migration from Cuba to HIV-1 genetic diversity among MSM in Moscow in recent years and its impact on the spread of HIV-1 DR in this vulnerable group. However, reliable data on the extent of such influence can only be

JOURNAL OF MICROBIOLOGY, EPIDEMIOLOGY AND IMMUNOBIOLOGY. 2025: ONLINE FIRST
DOI: https://doi.org/10.36233/0372-9311-616

obtained by studying a wider collection of MSM group patients. The HIV-1 DR profile in the studied collection was close to published data on DR in Cuba as a whole. Thus, the migration factor should be taken into account in programs aimong to prevent and counteract the spread of HIV infection in Russia, and effective treatment of patients with HIV-1 DR variants requires the selection of an effective therapy regimen.

СПИСОК ИСТОЧНИКОВ | REFERENCES

1. Delatorre E., Bello G. Phylodynamics of the HIV-1 epidemic in Cuba. *PLoS One*. 2013;8(9):e72448. DOI: https://doi.org/10.1371/journal.pone.0072448
2. Frescura L., Godfrey-Faussett P., Feizzadeh A.A., et al. Achieving the 95 95 95 targets for all: A pathway to ending AIDS. *PLoS One.* 2022;17(8):e0272405. DOI: https://doi.org/10.1371/journal.pone.0272405
3. Kouri V., Khouri R., Alemán Y., et al. CRF19_cpx is an evolutionary fit HIV-1 variant strongly associated with rapid progression to AIDS in Cuba. *EBioMedicine*. 2015;2(3):244–54. DOI: https://doi.org/10.1016/j.ebiom.2015.01.015
4. Rolo F., Miranda L., Wainberg M., et al. Envelope V3 region sequences of Cuban HIV-1 isolates. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol*. 1995;9(2):123–5.
5. Cuevas M.T., Ruibal I., Luisa M., et al. Villahermosa high HIV-1 genetic diversity in Cuba. *AIDS.* 2002;16(12):1643–53. DOI: https://doi.org/10.1097/00002030-200208160-00010
6. Machado L.Y., Blanco M., López L.S., et al. National survey of pre-treatment HIV drug resistance in Cuban patients. *PLoS One*. 2019;14(9):e0221879. DOI: https://doi.org/10.1371/journal.pone.0221879
7. Machado-Zaldívar L.Y., Díaz-Torres H.M., Blanco-de Armas M., et al. Origin and evolutionary history of HIV-1 subtype B in Cuba. *MEDICC Rev*. 2017;19(2-3):40. DOI: https://doi.org/10.37757/MR2017.V19.N2-3.7
8. Pérez L., Thomson M.M., Bleda M.J. HIV type 1 molecular epidemiology in Cuba: high genetic diversity, frequent mosaicism, and recent expansion of BG intersubtype recombinant forms. *AIDS Res. Hum. Retroviruses*. 2006;22(8):724–33. DOI: https://doi.org/10.1089/aid.2006.22.724
9. Casado G., Thomson M.M., Sierra M., et al. Identification of a novel HIV-1 circulating ADG intersubtype recombinant form (CRF19_cpx) in Cuba. *J. Acquir. Immune Defic. Syndr.* 2005;40(5):532–7. DOI: https://doi.org/10.1097/01.qai.0000186363.27587.c0
10. Zhukova A., Voznica J., Dávila F.M., et al. Cuban history of CRF19 recombinant subtype of HIV-1. *PLoS Pathog*. 2021;17(8):e1009786. DOI: https://doi.org/10.1371/journal.ppat.1009786
11. Powell R.L.R., Zhao J., Konings F.A.J., et al. Circulating recombinant form (CRF) 37_cpx: an old strain in Cameroon composed of diverse, genetically distant lineages of subtypes A and G. *AIDS Res. Hum. Retroviruses*. 2007;23(7):923–33. DOI: https://doi.org/10.1089/aid.2007.0040..
12. Sierra M., Thomson M.M., Posada D. Identification of 3 phylogenetically related HIV-1 BG intersubtype circulating recombinant forms in Cuba. *J. Acquir. Immune Defic. Syndr*. 2007;45(2):151–60. DOI: https://doi.org/10.1097/QAI.0b013e318046ea47
13. Thomson M.M., Casado G., Posada D. Identification of a novel HIV-1 complex circulating recombinant form (CRF18_cpx) of Central African origin in Cuba. *AIDS.* 2005;19(11):1155–63. DOI: https://doi.org/10.1097/01.aids.0000176215.95119.1d
14. Machado L.Y., Blanco M., Dubed M., et al. HIV type 1 genetic diversity in newly diagnosed Cuban patients. *AIDS Res. Hum. Retroviruses*. 2012;28(8):956–60. DOI: https://doi.org/10.1089/aid.2011.0295
15. Лаповок И.А., Кириченко А.А., Шлыкова А.В. и др. Молекулярно-эпидемиологический анализ генетических вариантов ВИЧ-1, циркулировавших в странах Восточной Европы и Центральной Азии в 2010–2019 гг. *Эпидемиология и инфекционные болезни. Актуальные вопросы*. 2022;12(3):31–40. Lapovok I.A., Kirichenko A.A., Shlykova A.V., et al. Molecular epidemiological analysis of HIV-1 genetic variants circulating in the countries of Eastern European and Central Asia in 2010–2019. *Epidemiology and Infectious Diseases. Current Items.* 2022;12(3):31–40. DOI: https://doi.org/10.18565/epidem.2022.12.3.31-40 EDN: https://elibrary.ru/poaufi
16. Bobkova M. Current status of HIV-1 diversity and drug resistance monitoring in the former USSR. *AIDS Rev.* 2013;15(4):204–12.
17. Kostaki E.G., Karamitros T., Bobkova M., et al. Spatiotemporal characteristics of the HIV-1 CRF02_AG/CRF63_02A1 epidemic in Russia and Central Asia. *AIDS Res. Hum. Retroviruses.* 2018;34(5):415–20. DOI: https://doi.org/10.1089/aid.2017.0233
18. Kazennova E., Laga V., Gromov K., et al. Genetic variants of HIV type 1 in men who have sex with men in Russia. *AIDS Res. Hum. Retroviruses*. 2017;33(10):1061–4. DOI: https://doi.org/10.1089/aid.2017.0078
19. Лаповок И.А., Лопатухин А.Э., Киреев Д.Е. и др. Молекулярно-эпидемиологический анализ вариантов ВИЧ-1, циркулировавших в России в 1987–2015 гг. *Терапевтический архив.* 2017;89(11):44–9. Lapovok I.A., Lopatukhin A.E., Kireev D.E., et al. Molecular epidemiological analysis of HIV-1 variants circulating in Russia in 1987–2015. *Terapevticheskiy arkhiv.* DOI: https://doi.org/10.17116/terarkh2017891144-49 EDN: https://elibrary.ru/zwosol
20. Лаповок И.А., Кириченко А.А., Шлыкова А.В. и др. Анализ генетических вариантов ВИЧ-1 среди мужчин-мигрантов гомосексуальной ориентации, проживающих в Москве. *Журнал инфектологии*. 2023;15(2 S2):71–2. Lapovok I.A., Kirichenko A.A., Shlykova A.V., et al. Analysis of HIV-1 genetic variants among homosexual male migrants living in Moscow. *Journal Infectology*. 2023;15(2 S2):71–2. EDN: https://elibrary.ru/qtszlb
21. Murzakova A., Kireev D., Baryshev P., et al. Molecular epidemiology of HIV-1 subtype G in the Russian Federation. *Viruses*. 2019;11(4):348. DOI: https://doi.org/10.3390/v11040348
22. Kirichenko A., Kireev D., Lapovok I., et al. HIV-1 drug resistance among treatment-naïve patients in Russia: analysis of the national database, 2006–2022. *Viruses.* 2023;15(4):991. DOI: https://doi.org/10.3390/v15040991
23. Tamura K., Stecher G., Peterson D., et al. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol. Biol. Evol*. 2013;30(12):2725–9. DOI: https://doi.org/10.1093/molbev/mst197
24. Kirichenko A., Kireev D., Lopatukhin A., et al. Prevalence of HIV-1 drug resistance in Eastern European and Central Asian countries. *PLoS One*. 2022;17(1):e0257731. DOI: https://doi.org/10.1371/journal.pone.0257731

DOI: https://doi.org/10.36233/0372-9311-616

Information about the authors

Ilya A. Lapovok✉ — Cand. Sci. (Biol.), senior researcher, HIV diagnostic and molecular epidemiology laboratory, Central Research Institute of Epidemiology, Moscow, Russia, i_lapovok@mail.ru, https://orcid.org/0000-0002-6328-1415

Alina A. Kirichenko — Cand. Sci. (Med.), senior researcher, HIV diagnostic and molecular epidemiology laboratory, Central Research Institute of Epidemiology, Moscow, Russia, kirichenko@cmd.su, https://orcid.org/0000-0002-7116-0138

Anastasia V. Shlykova — researcher, HIV diagnostic and molecular epidemiology laboratory, Central Research Institute of Epidemiology, Moscow, Russia, murzakova_a.v@mail.ru, https://orcid.org/0000-0002-1390-8021

Dmitry E. Kireev — Cand. Sci. (Biol.), Head, HIV diagnostic and molecular epidemiology laboratory, Central Research Institute of Epidemiology, Moscow, Russia, dmitkireev@yandex.ru, https://orcid.org/0000-0002-7896-2379

Anna A. Popova — Cand. Sci. (Med.), senior researcher, Department of specialized research laboratory for AIDS epidemiology and prevention, Central Research Institute of Epidemiology, Moscow, Russia; Associate Professor, Department of infectious diseases, Russian Medical Academy of Continuous Professional Education, Moscow, Russia, asya-med@mail.ru, https://orcid.org/0000-0001-9484-5917

Anastasia V. Pokrovskaya — D. Sci. (Med.), senior researcher, Central Research Institute of Epidemiology, Moscow, Russia; Professor, Department of infectious diseases with courses of epidemiology and phthisiology, Medical Institute, Peoples' Friendship University, Moscow, Russia, pokrovskaya_av@mail.ru, https://orcid.org/0000-0002-2677-0404

Author contribution: *Lapovok I.A., Kirichenko A.A., Shlykova A.V.* — literature selection and analysis, data analysis, statistical processing, writing the text, editing; *Kireev D.E., Popova A.A., Pokrovskaya A.V.* — study concept and design, organization of biomaterial collection, editing. All authors made a significant contribution to the search and analytical work and preparation of the article, read and approved the final version before publication.

The article was submitted 14.10.2024;
accepted for publication 28.12.2024;
published 28.02.2025

Информация об авторах

Лаповок Илья Андреевич✉ — канд. биол. наук, с. н. с. лаб. диагностики и молекулярной эпидемиологии ВИЧ-инфекции ЦНИИ Эпидемиологии, Москва, Россия, i_lapovok@mail.ru, https://orcid.org/0000-0002-6328-1415

Кириченко Алина Алексеевна — канд. мед. наук, с. н. с. лаб. диагностики и молекулярной эпидемиологии ВИЧ-инфекции ЦНИИ Эпидемиологии, Москва, Россия, kirichenko@cmd.su, https://orcid.org/0000-0002-7116-0138

Шлыкова Анастасия Вениаминовна — н. с. лаб. диагностики и молекулярной эпидемиологии ВИЧ-инфекции ЦНИИ Эпидемиологии, Москва, Россия, murzakova_a.v@mail.ru, https://orcid.org/0000-0002-1390-8021

Киреев Дмитрий Евгеньевич — канд. биол. наук, зав. лаб. диагностики и молекулярной эпидемиологии ВИЧ-инфекции ЦНИИ Эпидемиологии, Москва, Россия, dmitkireev@yandex.ru, https://orcid.org/0000-0002-7896-2379

Попова Анна Анатольевна — канд. мед. наук, с. н. с. Специализированного научно-исследовательского отдела по профилактике и борьбе со СПИДом ЦНИИ Эпидемиологии, Москва, Россия; доцент каф. инфекционных болезней РМАНПО, Москва, Россия, asya-med@mail.ru, https://orcid.org/0000-0001-9484-5917;

Покровская Анастасия Вадимовна — д-р мед. наук, с. н. с. ЦНИИ Эпидемиологии, Москва, Россия; профессор каф. инфекционных болезней с курсами эпидемиологии и фтизиатрии Медицинского института РУДН им. Патриса Лумумбы, Москва, Россия, pokrovskaya@cmd.su, https://orcid.org/0000-0002-2677-0404.

Участие авторов: *Лаповок И.А., Кириченко А.А., Шлыкова А.В.* — подбор и анализ литературы, анализ данных, статистическая обработка, написание текста, редактирование; *Киреев Д.Е., Попова А.А., Покровская А.В.* — концепция и дизайн исследования, организация сбора материала, редактирование. Все авторы внесли существенный вклад в проведение поисково-аналитической работы и подготовку статьи, прочли и одобрили финальную версию до публикации.

Статья поступила в редакцию 14.10.2024;
принята к публикации 28.12.2024;
опубликована 28.02.2025