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The pilot study of the features of HIV-1 resistant variants spread using molecular clusters

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Abstract

Introduction. As a result of routine testing of HIV-1 drug resistance (DR), a significant amount of viral nucleotide sequences and epidemiological data of HIV-infected individuals have been collected. Combined with the increasing use of bioinformatics methods in practice, it has become possible to study the features of HIV-1 resistant variants spread using molecular clustering analysis.

The **aim** of the study was to validate the molecular clustering analysis in a pilot region of Russia using a significant number of nucleotide sequences to study the features of the spread of HIV-1 resistant variants.

Materials and methods. HIV-1 nucleotide sequences were obtained from 899 HIV-infected patients who were registered at the Oryol AIDS Center in 2016–2021. HIV-1 genetic variants were determined using the Stanford University database, REGA and HIV BLAST. Resistance mutations and prognostic HIV-1 DR were determined using the Stanford University database. Phylogenetic analysis was carried out using the MEGA program. HIV-1 molecular clusters were identified using Cluster Picker software.

Results. In the pilot region, sub-subtype A6 dominated (85.7%); an increase in the share of CRF63_02A6 was noted. HIV-1 resistance was found in 13.6% of patients without antiretroviral therapy (ART) experience and in 52.0% with ART experience. Molecular clusters were more often formed by HIV-1 nucleotide sequences from ART-naïve patients. HIV-1 DR variants were less likely to fall into molecular clusters. The sources of transmitted mutations were more often patients with ART experience. The most actively and efficiently transmitted mutations were *K103N*, *V179E/T*, *Y181C* and *G190S*, associated with virus resistance to efavirenz and nevirapine.

Keywords: *HIV-1, drug resistance, resistance mutations, antiretroviral therapy, molecular clusters, transmission clusters, surveillance, genomic surveillance*

Ethics approval. The study was conducted with the informed consent of the patients. The local Ethics Committee of the Central Research Institute of Epidemiology (Protocol No. 93, June 18, 2019) approved the study.

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Оригинальное исследование
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Пилотное исследование по изучению особенностей распространения резистентных вариантов ВИЧ-1 с помощью молекулярных кластеров

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Аннотация

Введение. Благодаря рутинному тестированию лекарственной устойчивости (ЛУ) ВИЧ-1 накапливается значимое количество нуклеотидных последовательностей (НП) вируса и эпидемиологических данных о ВИЧ-инфицированных лицах, что вместе с активным внедрением в практику биоинформатических методов позволяет использовать их для изучения особенностей распространения резистентных вариантов ВИЧ-1 с помощью анализа молекулярных кластеров.

Цель исследования — апробация анализа молекулярных кластеров на территории пилотного региона России с использованием значимого количества НП для изучения особенностей распространения резистентных вариантов ВИЧ-1.

Материалы и методы. Получены НП ВИЧ-1 от 899 ВИЧ-инфицированных пациентов, состоявших на диспансерном учёте в Орловском центре СПИД в 2016–2021 гг. Определены генетические варианты ВИЧ-1 с помощью базы данных Стенфордского университета, REGA и HIV BLAST. Выявлены мутации резистентности и определена прогностическая ЛУ ВИЧ-1 с использованием базы данных Стенфордского университета. Проведён филогенетический анализ в программе «MEGA». Выявлены молекулярные кластеры ВИЧ-1 с помощью программного обеспечения «Cluster Picker».

Результаты. На территории пилотного региона доминировал суб-субтип A6 (85,7%), отмечено увеличение доли CRF63_02A6. Резистентность ВИЧ-1 была обнаружена у 13,6% пациентов без опыта антиретровирусной терапии (АРТ) и у 52,0% с опытом АРТ. Молекулярные кластеры чаще образовывали НП ВИЧ-1 от пациентов без опыта АРТ. ЛУ-варианты ВИЧ-1 реже попадали в молекулярные кластеры. Источниками передаваемых мутаций чаще являлись пациенты с опытом АРТ. Наиболее активно и эффективно передавались мутации *K103N*, *V179E/T*, *Y181C*, *G190S*, ассоциированные с устойчивостью вируса к эфавирензу и невирапину.

Заключение. Применение анализа молекулярных кластеров, предоставляющего информацию об особенностях распространения резистентных вариантов ВИЧ-1, может быть рекомендовано к использованию в России с целью разработки стратегий профилактики предотвращения передачи ЛУ-вариантов вируса и повышения эффективности лечения.

Ключевые слова: *ВИЧ-1, лекарственная устойчивость, мутации резистентности, антиретровирусная терапия, молекулярные кластеры, кластеры передачи, эпидемиологический надзор, геномный эпидемиологический надзор*

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Introduction

Widespread use of antiretroviral therapy (ART) significantly reduces morbidity and mortality of people living with HIV (PLHIV) [1, 2], as well as the risk of HIV transmission [3, 4]. However, the expansion of ART coverage in HIV-infected patients inevitably leads to the emergence and spread of drug resistance (DR) of the virus, which jeopardizes the efficacy of ART [5, 6]. At least 20% of HIV-infected patients[1] in Russia annually experience virologic failure of ART, the main cause of which is HIV-1 DR.

Lack of measures to counteract the emergence and spread of HIV-1 DR variants will lead to reduced ART efficacy, increased morbidity and mortality, poorer health of PLHIV, reduced therapeutic options available to patients, which will result in increased economic costs of counteracting the HIV epidemic [7, 8]. Thus, HIV-1 DR poses clinical, epidemiological and economic threats to achieving control of the HIV epidemic.

In this regard, recommendations were developed in Russia to identify HIV-1 DR in clinical practice to improve the effectiveness of treatment at the individual level[2] and as one of the components of epidemiological surveillance of HIV infection to improve the effective-

[1] Federal Scientific and Methodological Center for AIDS Prevention and Control of the Central Research Institute of Epidemiology of Rospotrebnadzor. Reference. HIV infection in the Russian Federation as of December 31, 2022. URL: http://www.hivrussia.info/wp-content/uploads/2023/09/Spravka-VICH-v-Rossii-na-31.12.2022.pdf (date of access: July 8, 2024).

[2] Clinical Guidelines “HIV Drug Resistance Analysis”. Moscow; 2017. URL: https://fedlab.ru/upload/medialibrary/f38/_-_10_04_2017_.pdf (date of access: July 8,.2024).

ness of ART and reduce the spread of HIV-1 DR at the population level among HIV-infected individuals[3].

In Russia, HIV-1 DR surveillance studies are rarely and unsystematically conducted; however, HIV-1 DR testing at the individual level is one of the routine types of analysis of ART efficacy, is included in the standards of primary health care for HIV infection[4] and is performed annually on at least 7,000 HIV-infected patients[5]. Thanks to routine HIV-1 DR testing, a significant amount of HIV-1 nucleotide sequences and related epidemiological data on HIV-infected individuals has been accumulated, which, together with the active introduction of bioinformatic methods into practice, has allowed the development of a new area called genomic epidemiological surveillance [9]. In 2021, during a pandemic caused by a new coronavirus infection, the World Health Organization (WHO) called on countries to strengthen the role of genomic epidemiological surveillance to better understand the transmission of infectious agents with pandemic and epidemic potential in order to develop vaccines, drugs, diagnostic test systems, as well as to take measures aimed at preventing the spread of infections[6].

One of the most important tools for genomic epidemiological surveillance of HIV infection is the identification of molecular clusters, i.e., HIV-1 nucleotide sequences that have high genetic similarity[7] and suggest an epidemiological link between the HIV-infected individuals from whom they are derived [10]. Clustering of HIV-1 nucleotide sequences indicates a more active transmission of the virus [11] and makes it possible to identify foci of increased morbidity, as well as to characterize the cohort with the most active HIV-1 transmission for effective anti-epidemic measures. Currently, the US Centers for Disease Control and Prevention (CDC) emphasize the identification of molecular clusters and rapid response to them as one of the main principles necessary to end the HIV epidemic, along with early diagnosis, rapid and effective treatment and prevention in at-risk groups [12].

For such studies, the key factor is the "sampling density" of HIV-infected persons, i.e. the proportion of persons for whom the nucleotide sequence of HIV-1 is known among all identified PLHIV in the region under study. If the "sample density" is less than 10%, the reliability and accuracy of the results obtained are significantly reduced [13].

Currently in Russia, bioinformatic methods in epidemiological surveillance are used only for the purpose of investigating cases of HIV infection, presumably related to the provision of medical care[8]. In turn, there have been no studies devoted to the analysis of molecular clusters in epidemiological surveillance of DR variants of HIV in Russia.

Therefore, the **aim** of this study was to validate molecular cluster analysis in a pilot region of Russia using a significant number of nucleotide sequences to study the features of the spread of resistant HIV-1 variants.

Materials and methods

Study samples

The study included 899 HIV-infected patients who were registered at the Oryol Region AIDS Center from 2016 to 2021.

The criteria for inclusion in the study were the diagnosis of HIV infection confirmed in accordance with national clinical protocols, as well as the availability of patient data: gender, date of the first positive immune blot result, and experience of taking antiretroviral drugs.

Inclusion criteria were a viral load of less than 500 copies/mL of HIV-1 RNA.

Informed consent was obtained for all patients or their legal representatives (if the patient was less than 18 years of age at the time of the study) before performing procedures related to this study. The study was approved by the local ethics committee of the Central Research Institute of Epidemiology of Rospotrebnadzor (protocol No. 93 of 18.06.2019).

RNA extraction and HIV-1 sequencing

Extraction of HIV-1 RNA from blood plasma and sequencing of amplified fragments of the pol gene encoding protease and part of reverse transcriptase (2253-3368 bp. relative to the reference strain HXB2, GenBank #K03455) were performed using the AmpliSense HIV-Resist-Seq reagent kit (Central Research Institute of Epidemiology of Rospotrebnadzor) with an Applied Biosystems 3500 Genetic Analyzer (Life Technologies) or with the in-house method using the next-generation sequencer MiSeq (Illumina).

Sequencing data were processed and consensus sequences were obtained using DEONA software (ver-

[3] Methodological Guidelines MG 3.1.5.0075/1-13 "Surveillance of the spread of HIV strains resistant to antiretroviral drugs" Moscow; 2013.

[4] Order of the Ministry of Health of the Russian Federation № 438n from June 23, 2022 "On approval of the standard of primary medical and sanitary care for adults with HIV infection (diagnosis, treatment and dispensary monitoring)".

[5] ITPC, Eastern Europe and Central Asia. Analysis of procurement of diagnostics for HIV treatment in Russia in 2020-2021. (2022) URL: https://itpc-eeca.org/wp-content/uploads/2022/07/monitoring-testov-vich-2020-21-gg-1.pdf

[6] World Health Organization. Global genomic surveillance strategy for pathogens with pandemic and epidemic potential, 2022–2032. 2022. 32 p. URL: https://www.who.int/publications/i/item/9789240046979 (date of access: 08.07.2024).

[7] CDC. A guide for health departments: detecting and responding to HIV Transmission Clusters, 2018. 2019. 131 p. URL: https://www.cdc.gov/hiv/programresources/guidance/cluster-outbreak/index.html (date of access: 08.07.2024).

[8] Methodological guidelines of Rospotrebnadzor MG 3.1.3342-16 "Epidemiological surveillance of HIV infection". Moscow; 2016.

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sions 1.2.3, 1.7.0) (RMBit) for classical sequencing data and with the help of the Trimmomatic [14] and VirGenA programs [15] for next-generation sequencing data with a 20% sensitivity threshold for HIV-1 minor variants.

All nucleotide sequences were subjected to quality control using the WHO BCCfE HIVDR QC instrument[9].

The obtained HIV-1 nucleotide sequences as well as related epidemiological and laboratory data on patients were uploaded to the Russian Database of HIV resistance to antiretroviral drugs[10].

Identification of HIV-1 genetic variants

HIV-1 genetic variants were identified using the Stanford University HIVdb (v. 9.1)[11] and REGA HIV-1 Subtyping Tool (v. 3.0)[12].

In case of discordant results between the two tools, nucleotide sequences were analyzed using the HIV BLAST (Basic Local Alignment Search Tool) tool of the Los Alamos Institute international database[13].

Determination of HIV-1 drug resistance

Resistance mutations were identified using the Stanford University HIVdb database (v. 9.1), according to which each mutation and combination of mutations are assigned penalty scores characterizing the level of HIV-1 prognostic DR: potential-low (10–14 points), low (15–29 points), intermediate (30–59 points) and high (more than 60 points). The DR variants of the virus were considered to be those for which 15 or more penalty scores were obtained.

HIV-1 prognostic DR was assessed to:

- protease inhibitors (PIs): atazanavir (ATV), darunavir (DRV), fosamprenavir (FPV), indinavir (IDV), lopinavir (LPV), nelfinavir (NFV), ritonavir (RTV), saquinavir (SQV), tipranavir (TPV);
- nucleoside reverse transcriptase inhibitors (NRTIs): abacavir (ABC), zidovudine (ZDV), stavudine (d4T), didanosine (ddI), emtricitabine (FTC), lamivudine (3TC), tenofovir (TDF);
- non-nucleoside reverse transcriptase inhibitors (NNRTIs): doravirine (DOR), efavirenz (EFV), etravirine (ETR), nevirapine (NVP), rilpivirine (RPV).

The 2009 Surveillance Drug Resistance Mutation (SDRM) list, significant for surveillance of transmitted DR HIV-1, was used to assess mutations in ART-naïve patients [16].

Phylogenetic analysis

HIV-1 nucleotide sequences were aligned using the online software of the Los Alamos Institute International Database using the HMMER method.

Editing and trimming of the aligned nucleotide sequences was performed using the BioEdit 7.0.9.0 program.

Phylogenetic analysis was performed by maximum likelihood method with bootstrap 100 and general reversion model with invariant sites and gamma distribution (G+I) in MEGA6 program.

Identification of HIV-1 molecular clusters

HIV-1 molecular clusters were identified using the Cluster Picker 1.2.3 software [17] with a bootstrap threshold of 0.9 and a genetic distance threshold of 0.045 nucleotide substitutions per position (4.5%).

Molecular clusters were visualized using the MicrobeTrace online software[14].

Clusters were classified as large if they consisted of 4 or more nucleotide sequences and active if they contained at least 1 nucleotide sequence from a patient diagnosed with HIV infection between 2019 and 2021.

Statistical analysis

The data obtained in the study were statistically processed using Microsoft Excel and GraphPad Prism online software. The statistical significance of differences between quantitative indicators was assessed using Fisher's two-sided exact test. Differences were considered significant at $p < 0.05$.

Results

Patient characteristics

The study included 899 HIV-infected patients, representing 34.1% of identified PLHIV in the study region as of the end of 2021[15].

At the time of blood collection, 354 (39.4%) patients were ART-naïve and 545 (60.6%) patients were ART-naïve.

The median age of patients at the time of blood collection for the study was 37 (32–42) years. Among ART-experienced patients, there were 6 (1.7%) HIV-infected patients less than 18 years of age.

The route of HIV-1 transmission was known for 874 (97.2%) study patients. The main routes of HIV-1 transmission were sexual (507; 65.0%) and parenteral through intravenous drug administration (283; 31.5%).

[9] URL: http://pssm.cfenet.ubc.ca/who_qc/
[10] URL: https://ruhiv.ru
[11] URL: https://hivdb.stanford.edu/
[12] URL: http://dbpartners.stanford.edu:8080/RegaSubtyping/stanford-hiv/typingtool
[13] URL: https://www.hiv.lanl.gov
[14] URL: https://microbetrace.cdc.gov
[15] Федеральный научно-методический центр по профилактике и борьбе со СПИДом ФБУН ЦНИИ Эпидемиологии Роспотребнадзора. Информационный бюллетень № 46 «ВИЧ-инфекция». 2021. URL: http://www.hivrussia.info/wp-content/uploads/2022/05/Byulleten-46-VICH-infektsiya-za-2020-g.-.pdf (дата обращения: 08.07.2024).

Male patients made up the majority (512; 57.0%).

Table 1 presents the clinical and epidemiological characteristics of all patients included in the study.

HIV-1 genetic variants

The dominant genetic variant of HIV-1 was sub-subtype A6, which was detected in 85.7% of HIV-infected patients. The circulating recombinant form (CRF) 63_02A6 was detected with high frequency (10.6%), the prevalence of which increased among the study patients diagnosed with HIV infection in 2015-2021 (**Fig. 1**). The other HIV-1 genetic variants were much less common: subtype B, 2.4%; CRF02_AG, 1.0%; CRF03_A6B, 0.2%; and subtype F, 0.1%.

Drug resistance and HIV-1 resistance mutations

Among 545 ART-naïve patients, HIV-1 DR to at least one antiretroviral drug was detected in 74 (13.6%) HIV-infected individuals: most often to NNRTIs (11.4%), significantly less often to PIs (2.8%) and NRTIs (0.7%).

Among the NNRTI class, HIV-1 DR was detected most frequently to RPV (7.3%), NVP (6.4%), and EFV (6.1%), with predominantly high-level DR to 1st generation drugs (**Figure 2**). Among PIs, HIV-1 DR was most frequently detected to NFV (2.6%). Among drugs of the NRTI class, HIV-1 resistance was identified most frequently to ABC (0.7%), FTC (0.7%) and 3TC (0.7%).

HIV-1 DR among ART-naïve patients was predominantly detected to only one class of drugs — NNRTIs (10.1%). HIV-1 resistance only to PIs was detected in 12 (2.2%) patients, HIV-1 DR only to NNRTIs was not detected. Multidrug resistance was detected rarely and only simultaneously to two classes of antiretroviral drugs: PIs + NRTIs (0.6%) and NRTIs + NNRTIs (0.7%).

Analysis of HIV-1 DR patterns revealed that at least one resistance mutation, including polymorphic mutations for sub-subtype A6 — A62V and E138A, was detected in 273 (50.1%) HIV-1-infected individuals. The most common mutations were *K103N* (4.6%), *E138A* (4.2%), *G190S* (1.5%), *V179E* (1.3%), and *K101E* (1.1%), and the most common mutation for the NNRTI class was A62V (39.4%). The remaining mutations, including those to the PI class, occurred at a frequency of less than 1%.

Table 1. Clinical and epidemiological characteristics of patients

Characteristic	ART-experienced patients	ART-naïve patients	Total
Number of patients	354	545	899
Age, years, median (IQR)	37 (33–42)	36 (31–42)	37 (32–42)
Sex, *n* (%)			
male	196 (55.4)	316 (58.0)	512 (57.0)
female	158 (44.6)	229 (42.0)	387 (43.0)
Route of transmission, *n* (%)			
sexual (heterosexual)	177 (50.0)	330 (60.6)	507 (56.4)
sexual (homosexual)	1 (0.3)	6 (1.1)	7 (0.8)
sexual (unspecified)	46 (13.0)	24 (4.4)	70 (7.8)
parenteral (narcotic)	116 (32.8)	167 (30.6)	283 (31.5)
mother-to-child	7 (2.0)	0	7 (0.8)
unknown	7 (2.0)	18 (3.3)	25 (2.8)
Viral load, $\log_{10}$ copies/mL, median (IQR)	4.2 (3.7–4.9)	4.6 (4.0–5.2)	4.5 (3.9–5.1)
Sampling year, *n* (%)			
2016	22 (6.2)	0	22 (2.4)
2017	35 (9.9)	0	35 (3.9)
2018	139 (39.3)	341 (62.6)	480 (53.4)
2019	110 (31.1)	113 (20.7)	223 (24.8)
2020	6 (1.7)	0	6 (0.7)
2021	42 (11.9)	91 (16.7)	133 (14.8)

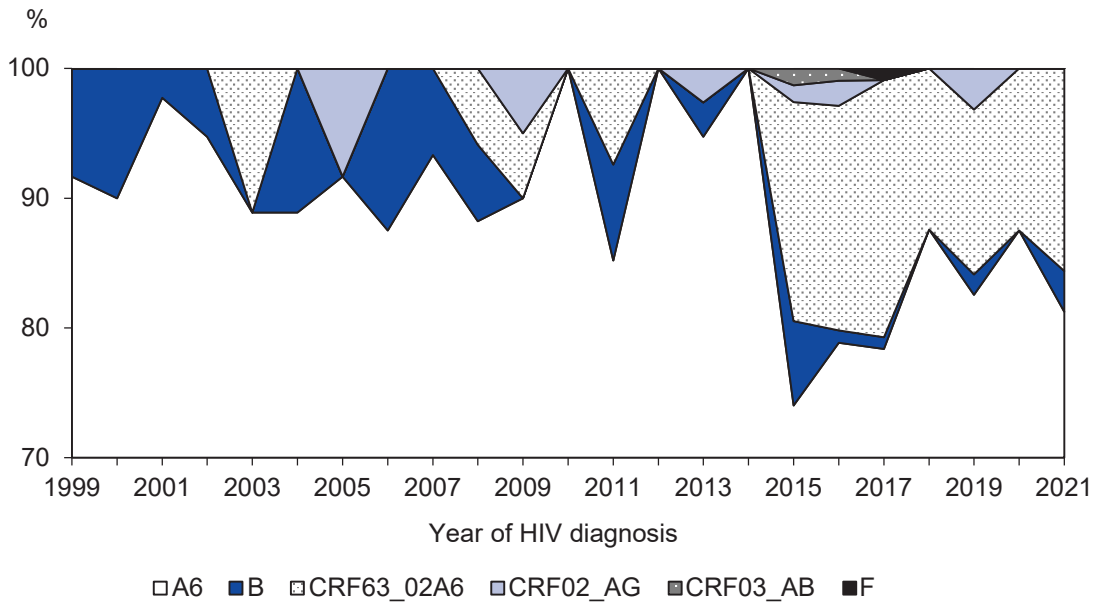

Fig. 1. Distribution of HIV-1 genetic variants by year of diagnosis of HIV infection.

SDRMs were detected in 6.8% of ART-naïve patients, and a tendency to increase their prevalence was noted. For example, in patients with a blood collection date in 2018, 2019, and 2021, at least one surveillance mutation was detected in 5.9% (95% CI 3.8–8.9%), 6.2% (95% CI 2.8–12.4%), and 8.8% (95% CI 4.3–16.6%) of cases, respectively.

The complete list of detected mutations among ART-naïve patients is presented in **Table 2**.

Among 354 ART-experienced patients, HIV-1 DR to at least one antiretroviral drug was detected in 52.0% of cases, most frequently to NNRTIs (44.6%) and NRTIs (36.2%). HIV-1 DR to PIs was detected rarely, in 5.4% of patients.

Among the NNRTI class, HIV-1 DR was detected most frequently to NVP (40.4%), EFV (40.4%), and RPV (32.8%) (**Figure 3**). Meanwhile, resistance was predominantly high to 1st generation NNRTIs (EFV

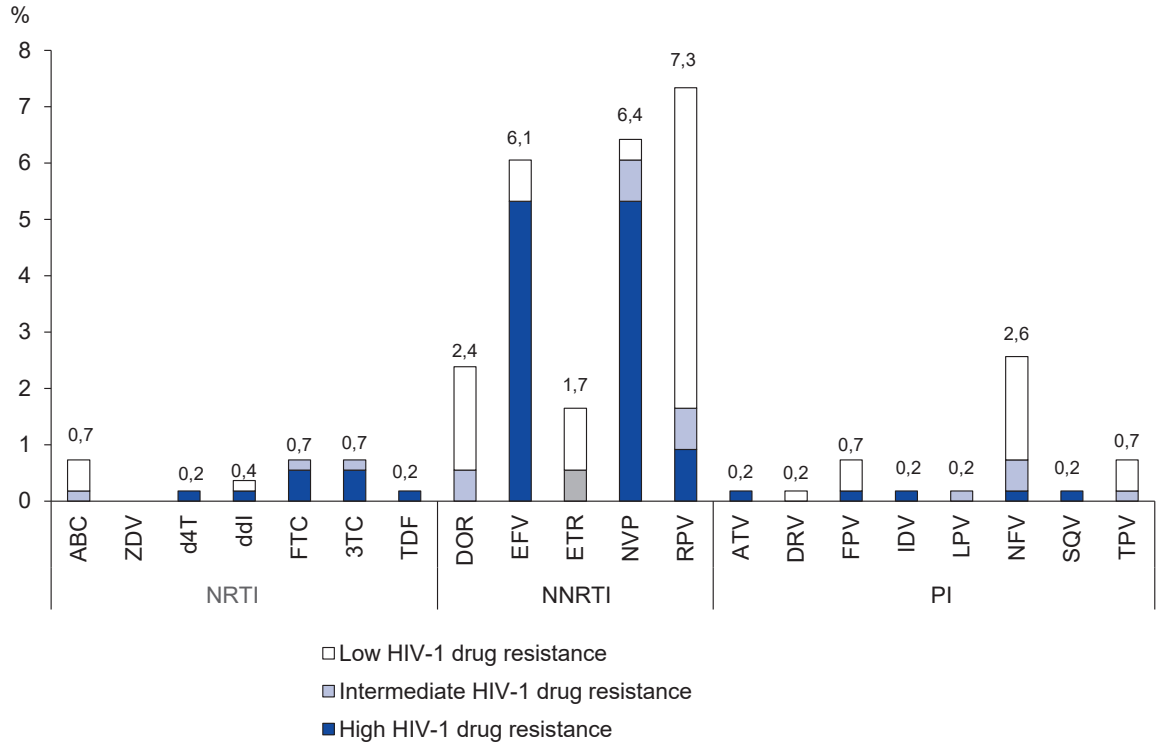

Fig. 2. Prevalence and level of HIV-1 drug resistance among ART-naïve patients.

and NVP). Among the NRTIs, HIV-1 DR was most frequently detected to ABC (35.3%), FTC (34.7%), and 3TC (34.7%), with predominantly high levels to the first 2 drugs. PIs to all drugs of the PI class did not exceed 5% and were most frequently detected to NFV (4.0%).

HIV-1 DR in ART-experienced patients was detected most often, in 27.1% of cases, to two drug classes (NRTI + NNRTI), and to NNRTI class drugs alone in 14.1% of cases. Multidrug resistance to 3 classes of drugs (PI + NRTI + NNRTI) was detected rarely, in 3.1% of patients.

In an analysis of HIV-1 DR patterns in ART-experienced patients, resistance mutations were found in 255 (72.0%) HIV-1-infected patients, including polymorphic mutations. The most frequent mutations to NNRTIs were *G190S* (20.1%), *K103N* (12.7%), *K101E* (10.2%), *Y181C* (6.8%), *E138A* (6.2%); to NRTIs, *A62V* (38.4%), *M184V/I* (26.8%), *K65R* (10.5%); and to PIs, *M46I* (2.5%). **Table 3** presents the list of identified resistance mutations among ART-experienced patients.

HIV-1 molecular clusters

Molecular cluster analysis revealed that 243 out of 899 HIV-1 nucleotide sequences (27.1%) formed 91 clusters. HIV-1 nucleotide sequences from ART-naïve patients were more frequently detected within clusters (69.5% vs 57.3%; $p = 0.0009$).

The prevalence of HIV-1 DR within clusters was 20.2% (49/243) vs 42.7% (209/656) outside clusters

Table 2. Prevalence of resistance mutations among ART-naïve patients

ARV class	Mutations	Mutation detection rate, *n* (%)
NRTI	*E44D*	3 (0.6)
	A62V	215 (39.4)
	*K65R**	1 (0.2)
	*M184I**	1 (0.2)
	*M184V**	2 (0.4)
NNRTI	*A98G*	1 (0.2)
	*K101E**	6 (1.1)
	*K103N**	25 (4.6)
	V106I	1 (0.2)
	V108I	3 (0.6)
	E138A	23 (4.2)
	E138G	5 (0.9)
	E138K	1 (0.2)
	V179D	3 (0.6)
	V179E	7 (1.3)
	V179T	3 (0.6)
	*G190S**	8 (1.5)
PI	*M46I**	3 (0.6)
	M46V	2 (0.4)
	*M46L**	1 (0.2)
	*I84V**	1 (0.2)

Note. *Mutations from the SDRM list.

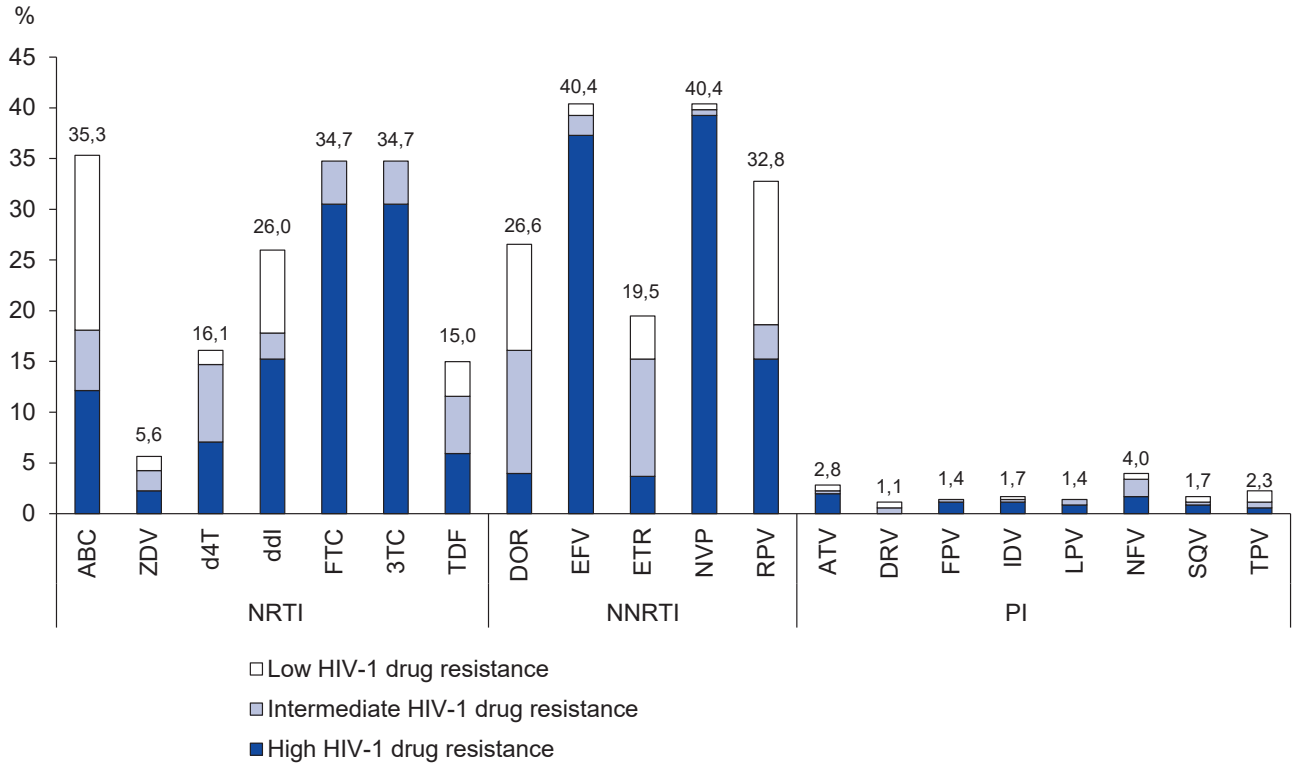

Fig. 3. Prevalence and level of HIV-1 drug resistance among ART-experienced patients.

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Table 3. Prevalence of resistance mutations* among ART-experienced patients

ARV class	Mutations*	Mutation detection rate, *n* (%)
NRTI	*A62V*	136 (38.4)
	K65R	37 (10.5)
	D67N	13 (3.7)
	K70R	10 (2.8)
	K70E	4 (1.1)
	L74I	6 (1.7)
	L74V	13 (3.7)
	M184I	19 (5.4)
	M184V	76 (21.5)
	T215Y	4 (1.1)
	K219E	4 (1.1)
	K219Q	7 (2.0)
NNRTI	*L100I*	4 (1.1)
	K101E	36 (10.2)
	K103N	45 (12.7)
	V106I	8 (2.3)
	V108I	7 (2.0)
	E138A	22 (6.2)
	E138G	8 (2.3)
	V179D	6 (1.7)
	V179E	7 (2.0)
	V179T	4 (1.1)
	Y181C	24 (6.8)
	G190S	71 (20.1)
	H221Y	5 (1.4)
	P225H	9 (2.5)
PI	*M46I*	9 (2.5)
	I50L	4 (1.1)

Note. *Mutations with a prevalence of at least 1% are represented.

($p = 0.0005$). However, there was no correlation of DR HIV-1 nucleotide sequence clustering with ART experience or any other clinical and epidemiological characteristics of patients.

Resistance mutations (excluding polymorphic mutations for sub-subtype A6 – *A62V*, *E138A*) were found in 54 HIV-1 nucleotide sequences in 33 clusters.

The clustering features of HIV-1 nucleotide sequences with mutations occurring with a frequency of more than 1.0% in the study sample were analyzed (**Table 4**). The most common mutations in the detected clusters were *K103N* (6.2%), *V179E* (4.1%), *G190S* (4.9%), and *M184V* (4.9%). At the same time, the *V179E* mutation was significantly more frequent in the clusters, and in contrast, the *M184V*, *K101E*, and *G190S* mutations were less frequent than among all the patients studied.

Transmitted mutations, i.e., those that occurred in at least 2 HIV-1 nucleotide sequences in a cluster, were found in 9 clusters in 27 nucleotide sequences (**Figure 4**). The profile of transmitted mutations was limited and included mutations associated with DR to NNRTIs (*K103N*, *V179E/T*, *G190S*, *Y181C*), NRTIs (*M184I/V*, *K65R*), and PIs (*L33F*).

Within clusters, the transmission efficiency of resistance mutations was determined as the ratio of the number of transmitted mutations in clusters to all mutations in clusters. The highest transmission efficiency (50% or higher) was found for *K103N* (10/15; 66.7%), *V179E/T* (11/12; 91.7%), *Y181C* (2/4; 50.0%) and *G190S* (6/12; 50.0%) mutations.

Based on the date of HIV diagnosis, putative sources of transmitted HIV-1 mutations were identified, which in 6/9 clusters (66.7%) were patients with a history of taking antiretroviral drugs.

In all clusters, patients with an earlier date of diagnosis transmitted the full mutation profile, except for one cluster in which only *K103N* was transmitted from the *M184V* + *K103N* profile.

Most clusters (7/9) with transmitted HIV-1 mutations were small and inactive. Only *K103N* (1 cluster) and *V179E* (1 cluster) mutations were identified in large active clusters.

Discussion

Against the background of a long history of taking antiretroviral drugs and an increasing number of patients on ART, the prevalence of DR HIV variants in Russia is increasing every year [18], which is the reason for increasing the coverage of HIV-1 DR testing among HIV-infected individuals. Thanks to the improvement of sequencing technologies and the strengthening of the national database of HIV resistance to antiretroviral drugs [19], a significant amount of genetic and epidemiological data is being accumulated, which, together with the development of bioinformatic research methods, makes it possible to use them for a more in-depth study of the features of HIV-1 spread.

In this study, a pilot region of Russia was used as an example to demonstrate for the first time in the country the potential of a new direction, genomic epidemiological surveillance, in terms of the spread of resistant HIV-1 variants. For the first time, a high coverage of HIV-1 sequencing of PLHIV was obtained for one region of Russia, which amounted to 34.1% at the end of

Table 4. Prevalence of HIV-1 DR mutations within clusters

ARV class	Mutations	Prevalence among all study patients (*n* = 899), %	Prevalence within clusters (*n* = 243), %	*p**
NRTI	*K65R*	4.2	2.1	0.0604
	D67N	1.4	0.4	0.2039
	K70R	1.1	0	0.0701
	M184I	2.2	2.1	1
	M184V	8.7	4.9	**0.0159**
NNRTI	*K101E*	4.7	2.1	**0.0211**
	K103N	7.8	6.2	0.3270
	V106I	1.0	1.2	0.7091
	V108I	1.1	1.2	0.7349
	E138G	1.4	1.6	0.7568
	V179D	1.0	1.6	0.2625
	V179E	1.6	4.1	**0.0006**
	Y181C	2.7	1.6	0.3515
	G190S	8.8	4.9	**0.0117**
	P225H	1.0	0	0.1233
PI	*M46I*	1.3	0.4	0.1975

Note. *Statistically significant differences are highlighted in bold ($p < 0.05$).

2021[16]. This makes it possible to obtain reliable results of the study.

The assessment of HIV-1 genetic diversity in the study region revealed 5 genetic variants (sub-subtype A6, subtype B, CRF63_02A6, CRF02_AG, CRF03_A6B) characteristic of the Russian genetic landscape [20], as well as subtype F atypical for the Russian epidemic, probably resulting from an imported case of HIV infection. The observed increase in the proportion of CRF63_02A6 among patients diagnosed between 2015 and 2021 reflects the general trend in the country [21, 22].

The analysis of HIV-1 DR revealed that HIV-1 resistance to at least one antiretroviral drug was detected in 13.6% of ART-naïve patients, most often to NNRTIs (11.4%): RPV (7.3%), NVP (6.4%) and EFV (6.1%), which is in line with the data obtained for Russia as a whole [21].

The prevalence of HIV-1 DR in the studied patients with ART experience was 52.0%, most frequently to the same NNRTI class drugs (44.6%): NVP (40.4%), EFV (40.4%) and RPV (32.8%), as well as to NNRTIs (36.2%): ABC (35.3%), FTC (34.7%) and 3TC (34.7%).

The described prevalence of HIV-1 DR in ART-experienced patients in Russia varies considerably from 50% [23] to 82.4% [24], reflecting the correct assignment of genotyping test. In the present study, the relatively low

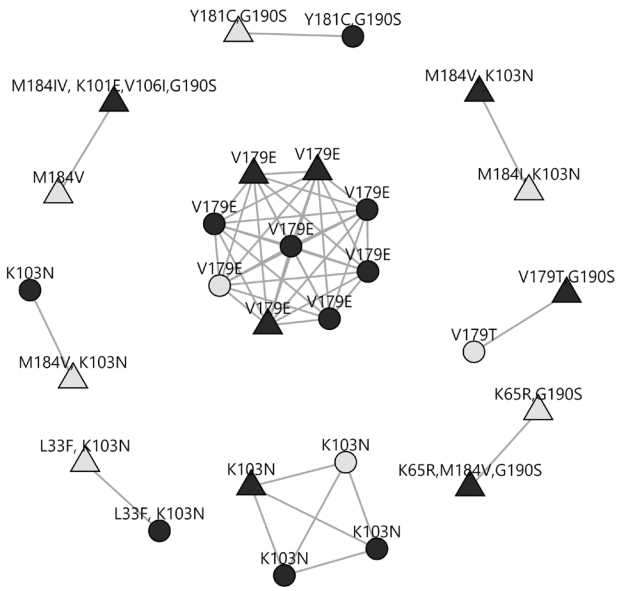

Fig. 4. Clusters with transmitted resistance mutations.
The triangle indicates HIV-1 nucleotide sequences from ART-experienced patients, and the circle indicates HIV-1 nucleotide sequences from ART-naïve patients. HIV-1 nucleotide sequences from patients with the earliest date of diagnosis of HIV infection in the cluster are marked in light grey.

[16] Federal Scientific and Methodological Center for AIDS Prevention and Control FBUN Central Research Institute of Epidemiology of Rospotrebnadzor. Information Bulletin No. 46 "HIV-infection". 2021. URL: http://www.hivrussia.info/wp-content/uploads/2022/05/Byulleten-46-VICH-infektsiya-za-2020-g.-.pdf. (date of access: July 8, 2024).

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HIV-1 DR is associated with the fact that virologic failure of ART wasn't observed in all patients.

It should be noted that high-level HIV-1 DR among all patients, regardless of ART experience, were most often established for NNRTIs (NVP, EFV) and NRTIs (FTC and 3TC), which can be explained by their widespread use and low genetic barrier to the development of HIV-1 DR [25].

During the study period, the most commonly used 1st-line ART regimen in the region included TDF, 3TC and EFV. According to the results of the present study, the primary resistance of HIV-1 to the used nucleoside base did not exceed 1%, and resistance to EFV amounted to 6.1%, which allows recommending replacement of the third component of the regimen in accordance with the national clinical guidelines for the treatment of HIV infection[17].

The most frequent mutations (excluding polymorphic mutations for sub-subtype A6 - *A62V* and *E138A*) among patients without ART experience were *K101E*, *K103N*, *V179E* and *G190S* to NNRTIs, among patients with ART experience — *K101E*, *K103N*, *V179E*, *Y181C*, *G190S* to NNRTIs and *M184V/I*, *K65R* to NRTIs. HIV-1 DR and PI class drug resistance mutations were rare among the study patients.

The prevalence of mutations significant for epidemiological surveillance of transmitted HIV-1 DR in the Orel region was 6.8%, which corresponds to the average level in Russia [18]. Multidrug resistance to three classes of ARVs (PIs + NRTIs + NNRTIs) was detected only in ART-experienced patients in 3.1% of cases. Thus, it can be concluded that the HIV-1 DR level in the study region is moderate, and the HIV-1 DR patterns identified correspond to the ART regimens used and are typical for Russia.

Assessment of HIV-1 DR levels and patterns provides important information about which drugs are effective at the time of the study, but does not allow us to determine the distribution of DR variants or predict which drugs will be effective in the future. For a more in-depth analysis, one of the tools of genomic epidemiological surveillance, the method of molecular cluster analysis, was applied to the spread of HIV-1 DR in this study.

The analysis found that molecular clusters were more likely to be formed by HIV-1 nucleotide sequences from ART-naïve patients, indicating that they were the main sources of HIV infection in the region and suggesting that there was no high risk of transmission of HIV-1 DR variants due to the relatively low prevalence of primary HIV-1 DR.

In addition, it was found that DR variants of the virus from both ART-naïve and ART-experienced patients were less likely to fall into molecular clusters, which is likely due to the fact that most of them have significantly reduced fitness [26].

Despite the fact that HIV transmission in the Orel region was mainly from patients without ART experience, the sources of resistant HIV variants were presumably patients with ART experience. It is interesting to note that foreign studies have described that the source of HIV-1 DR variants was, on the contrary, patients without ART experience [27-29], which is probably due to higher ART efficacy rates in the countries.

An evaluation of transmitted mutations of HIV-1 DR found that the profile is limited to 9 mutations: *K103N*, *V179E/T*, *Y181C*, *G190S* to NNRTIs; *K65R*, *M184I/V* to NRTIs; and *L33F* to PIs, thus predicting which drugs will be ineffective in the future. Thus, the most frequently transmitted mutation was *K103N*, which is associated with the emergence of HIV-1 resistance to NVP and EFV. The results of other studies have also shown that this mutation is transmitted more frequently than others [30, 31], which is due to the fact that viruses with this mutation and wild-type viruses have a similar fitness, as well as the fact that this mutation can persist in the patient's body for a long time [32, 33]. It is also important to note that the presence of this mutation in patients starting treatment with the TDF + 3TC + EFV regimen is associated with increased risks of virologic failure [34].

V179E/T mutations, which also occurred with high frequency in clusters, are associated with decreased response to NNRTI treatment (with the exception of DOR), but generally do not result in the occurrence of virologic failure [35]. However, in the presence of these mutations, it is not recommended to prescribe a regimen containing EFV if the patient has a high viral load at the start of treatment [36].

The next most common transmitted mutation in the clusters, *G190S*, is associated with a 200-fold and 130-fold reduction in HIV-1 susceptibility to NVP and EFV, respectively, and is often found in sub-subtype A6 viruses due to its predisposition to have this mutation [37]. It is noted that the fitness of virus containing this mutation is reduced [38].

The Y181C mutation reduces the susceptibility of the virus to all NNRTIs, especially NVP, and hardly impairs the fitness of the virus [39].

Transmitted mutations to the *M184I/V* NNRTI found in clusters are associated with high levels of HIV-1 resistance to 3TC, FTC and low levels to ABC, while increasing susceptibility to d4T, ZDV and TDF, allowing 3TC and FTC to be retained in regimens when they occur. Foreign studies have described that these mutations are often transmitted from patients with virologic failure of ART [40], and also persist for a long time in HIV reservoirs [41].

The *K65R* mutation is associated with decreased susceptibility to all NRTIs except ZDV, to which, on

[17] Clinical Recommendations "HIV infection in adults" (approved by the Ministry of Health of the Russian Federation). Moscow; 2020.

the contrary, it increases viral sensitivity. *M184V* and *K65R* mutations have been described to significantly reduce virus fitness [42, 43].

The only mutation to PIs, *L33F*, which was found in the clusters, is additional and only slightly affects the susceptibility of the virus to antiretroviral drugs.

The presence of the mutations described above in molecular clusters indicates their active transmission, but the greatest danger is posed by those mutations that have a high efficiency of transmission within these clusters. As a result of the efficiency assessment it was found that such mutations are *K103N*, *V179E/T*, *Y181C*, *G190S*, which cause resistance of the virus to the 1st generation NNRTIs – EFV and NVP.

Thus, based on the data obtained, we can conclude that there is no risk of increased transmission of HIV-1 DR in the Oryol region, as evidenced by the high degree of clustering of ART-naïve patients who have a relatively low level of primary resistance, the low degree of clustering of HIV DR variants, and the fact that transmission of mutations was found mainly in small and inactive clusters. The rapid and efficient transmission of mutations associated with virus resistance to 1st generation NNRTIs established in this study allows us to recommend limiting their use to prevent the spread of DR variants of HIV-1 in the region and to improve the efficacy of ART.

Conclusion

The results of molecular cluster analysis provide information on the peculiarities of HIV-1 DR variants distribution, in particular, on the dynamics of HIV-1 DR transmission, sources of resistant variants of the virus, efficiency of transmission of resistance mutations, which allows us to recommend this method for use within the framework of genomic epidemiological surveillance of HIV infection in Russia to develop prevention strategies to prevent transmission of DR variants of the virus and to improve the effectiveness of treatment.

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