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PRIMARY HIV DRUG RESISTANCE AMONG NEWLY HIV TYPE-1 DIAGNOSED PATIENTS IN ST. PETERSBURG

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There is concern that the widespread use of antiretroviral drugs (ARV) to treat human immunodeficiency virus 1 (HIV-1) infection may result in the emergence of transmission of drug-resistant virus among persons newly infected with HIV-1. Russia is one of a growing number of countries in the world where drug-resistant HIV is becoming a serious health problem because it has the potential to compromise the efficacy of antiretroviral therapy (ART) at the population level.

Materials and methods. We performed a genetic analysis of the HIV-1 plasma derived *pol* gene among the newly diagnosed ART-naïve HIV-1 infected patients during the period from November 2018 to October 2019 in the St. Petersburg Clinical Infectious Diseases Hospital named after S.P. Botkin. We used reverse transcriptase polymerase chain reaction (RT-PCR) followed by direct sequencing of PCR products to determine the prevalence of primary drug resistance (PDR) conferring mutations. HIV-1 genotypes were determined by phylogenetic analysis.

Results. The predominant HIV-1 subtype was A1 (87.2%), followed by B (11.8%) and CRF06_cpx (1%). The overall prevalence of PDR was 11%. Virus with known resistance-conferring mutations to any nucleoside reverse transcriptase inhibitors (NRTIs) was found in 8 individuals, to any non NRTIs in 5 subjects, and to any protease inhibitors in 1 case. Multidrug-resistant virus was identified in 2 individuals (2%).

Conclusion. The distribution of HIV-1 genotypes in St. Petersburg, Russia is diverse. The emerging prevalence of PDR in ART-naïve patients demonstrates the significance of constant monitoring due to the challenges it presents towards treatment.

Key words: HIV-1, drug resistance, mutations, antiretroviral therapy, HIV-1 drug resistance

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ПЕРВИЧНАЯ ЛЕКАРСТВЕННАЯ УСТОЙЧИВОСТЬ СРЕДИ ВПЕРВЫЕ ВЫЯВЛЕННЫХ ПАЦИЕНТОВ С ВИЧ-1 В САНКТ-ПЕТЕРБУРГЕ

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У специалистов есть опасения, что широкое использование антиретровирусных препаратов для лечения инфекции, вызванной вирусом иммунодефицита человека 1 (ВИЧ-1), может привести к возникновению передачи лекарственно-устойчивого вируса среди лиц, впервые инфицированных ВИЧ-1. Россия — одна из растущего числа стран, где лекарственно-устойчивый ВИЧ становится серьезной проблемой для здоровья, поскольку он может снизить эффективность антиретровирусной терапии на уровне населения.

Материалы и методы. Проведен генетический анализ гена *pol*, полученного из плазмы ВИЧ-1, среди впервые выявленных пациентов с ВИЧ-1, не получавших антиретровирусную терапию, в период с ноября 2018 г. по октябрь 2019 г. в СПБ ГБУЗ «Клинической инфекционной больнице им. С. П. Боткина». Использовали полимеразную цепную реакцию с обратной транскриптазой (ОТ-ПЦР) с последующим прямым секвенированием продуктов ПЦР для определения распространенности мутаций, вызывающих первичную лекарственную устойчивость. Генотипы ВИЧ-1 определяли филогенетическим анализом.

Результаты. Преобладающим подтипом ВИЧ-1 был A1 (87,2%), далее за ним следовали B (11,8%) и CRF06_cpx (1%). Общая распространенность первичной лекарственной устойчивости составляла 11%. Вирус с известными мутациями, придающими устойчивость к любым ингибиторам нуклеозидной обратной транскриптазы (НИОТ), был обнаружен у 8 человек, к любым не-НИОТ — у 5 пациентов и к любым ингибиторам протеаз — в одном случае. Вирус с множественной лекарственной устойчивостью выявлен у 2 человек (2%).

Заключение. Распространение генотипов ВИЧ-1 в Санкт-Петербурге (Россия) разнообразно. Растущая распространенность первичной лекарственной устойчивости у пациентов, ранее не получавших антиретровирусную терапию, демонстрирует важность постоянного мониторинга из-за проблем, которые возникают при дальнейшем лечении.

Ключевые слова: ВИЧ-1, лекарственная устойчивость, мутации, антиретровирусная терапия, лекарственная устойчивость ВИЧ-1

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List of abbreviations used: 3TC — Lamivudine, AIDS — Acquired Immune Deficiency Syndrome, ART — Antiretroviral Therapy, ARV — Antiretroviral (drug), CD4 — Cluster of Differentiation 4, cDNA — complementary Deoxyribonucleic Acid, DNA — Deoxyribonucleic Acid, EFV — Efavirenz, EIA — Enzyme Immunoassay, FTC — Emtricitabine, HAART — Highly Active Antiretroviral Therapy, HIV — Human Immunodeficiency Virus, INIs — Integrase Inhibitors, K2EDTA — Dipotassium ethylenediaminetetraacetic Acid, HSX — Heterosexual, MSM — Men who have Sex with Men, NNRTI — Non-Nucleoside Reverse-Transcriptase Inhibitor, NRTIs — Nucleoside Reverse-Transcriptase Inhibitors, PCR — Polymerase Chain Reaction, PDR — Primary Drug Resistance, PMTCT — Prevention of Mother-To-Child Transmission of HIV, PWID — Persons Who Inject Drugs, RAMs — Reference list of Analogue resistance Mutations, RCF — Relative Centrifugal Force, RNA — Ribonucleic Acid, RT — Reverse Transcription, RT-PCR — Reverse Transcription Polymerase Chain Reaction, STIs — Sexually Transmitted Infections, TB-Tuberculosis, TDF — Tenofovir Disoproxil Fumarate, TDR — Transmitted Drug Resistance, UNAIDS — United Nations Programme on HIV and AIDS, WHO — World Health Organization.

Introduction. The HIV epidemic in Eastern Europe and central Asia has grown by 30% since 2010, reflecting insufficient investment in National AIDS responses across much of the region. The Russian Federation has the largest HIV epidemic and home 70% of people living with HIV in the region. In 2019, the incidence-prevalence ratio of 10.1 was higher than in any other region [1]. By mid-2020, 1.47 million people had been diagnosed with HIV in Russia. However, this does not equate to the number of people currently living with HIV since it does not account for acquired immune deficiency syndrome (AIDS) — related deaths or undiagnosed HIV-infected people [2–4].

The use of highly active antiretroviral therapy (HAART) in patients infected with HIV-1 has been recommended for suppressing virus replication, as it often results to a substantial recovery of impaired immunological function, thereby reducing HIV-linked morbidity and mortality apart from enhancing the quality of life of HIV/AIDS-infected people [5]. Since 2014 the United Nations Programme on HIV and AIDS (UNAIDS) introduced a new treatment target named 90–90–90 set for reducing the number of new HIV infections to 500,000 per year by 2020. These targets include increasing to 90% the proportion of people living with HIV who know their diagnosis, increasing to 90% the proportion of people living with HIV receiving antiretroviral treatment and increasing to 90% the proportion of people on HIV treatment who have an undetectable viral load [6]. The 2016 World Health Organization (WHO) guidelines on the

use of antiretroviral drugs for treating and preventing HIV infection recommend testing and treating everyone with diagnosed HIV, regardless of cluster of differentiation 4 (CD4) count [7, 8]. As the number of people newly infected with HIV is rising, the proportion of people living with HIV receiving antiretroviral treatment increases. Therefore, the proportion of individuals infected with HIV from people on ART with unsuppressed viral load or people not currently receiving treatment but with prior exposure to ARV drugs, because of mother-to-child transmission (PMTCT) or previous treatment, will increase [9].

Resistance to ARV is a major cause of treatment failure in individuals with HIV infection. It often develops in patients with incomplete viral suppression and has been associated with many factors include use of monotherapy, inadequate suppression of viral replication with suboptimal treatment regimens, nonadherence to ART, and initiation of therapy late in the course of HIV infection [10]. Resistant mutations that emerge from viral replication in individuals receiving ART known as acquired resistance. Transmitted drug resistance (TDR) occurs when previously uninfected individuals are infected with virus that has drug resistance mutations [8]. However, since the large-scale use of ARV there is an increasing trend in the prevalence of primary drug resistance (PDR) from 1.1% to 21% in the United States, Africa, and Europe [11].

Currently, HIV drug resistance testing prior to ART initiation is not being routinely performed in Russian Federation. However, recommendations have been developed to strengthen the monitoring of HIV drug-resistant strains at both the country and regional levels. The results of the studies are regularly sent to the electronic HIV drug resistance database of the reference center for monitoring HIV and HIV-associated infections of the central research institute of epidemiology of Rospotrebnadzor[1], to be considered for further decisions [12]. It has been conceptualized that the widespread use of ARV will result in the increased transmission of drug-resistant virus resulting in an increased prevalence of resistant variants in newly infected patients. Clearly, the transmission of resistant variants to uninfected individuals raises serious clinical and public health consequences [10].

In the current study, we determined the prevalence of mutations in the HIV-1 *pol* gene associated with resistance to antiretroviral agents in a cohort of 102 drug-naïve individuals newly diagnosed with HIV-1 in St. Petersburg and Leningrad region.

Materials and Methods

Study subjects. Plasma samples from 102 subjects newly diagnosed with HIV-1 infection aged ⩾18 years, hospitalized in the St. Petersburg Clinical Infectious Diseases Hospital named after S. P. Botkin from November 2018 to October 2019 were analyzed in this study. The selection criteria included documented HIV seroconversion within the previous 12 months or evidence of acute or early HIV infection. Early HIV infection was defined by a positive enzyme immunoassay (EIA) for HIV with an evolving result on Western blotting within 90 days after the onset of an acute retroviral syndrome (i.e., viral symptoms consistent with acute HIV infection). A detectable HIV ribonucleic acid (RNA) or p24 antigen defined acute HIV infection, in the absence of HIV antibody detectable by EIA, with subsequently documented HIV seroconversion. Stored plasma from 2 individuals infected who had a plasma HIV RNA level of less than 500 copies per milliliter were identified and were therefore excluded of the analysis. All patients had not previously received antiretroviral therapy at the time of their base-line evaluation. Then, a total of 100 subjects met the entry criteria and were included in the analysis and all study participants signed a written informed consent form before enrollment.

Specimen's collection. From each patient, 8 ml of venous blood was collected in 2 dipotassium ethylenediaminetetraacetic acid (K2EDTA) vacutainer tubes by the lab technician. Out of the 8 ml, 1 ml blood was used for HIV RNA detection. The remaining 7 ml blood was centrifuged in a bench top centrifuge within the vacutainer vial, at 2500 relative centrifugal force (RCF) for 10 minutes at room temperature for obtaining the plasma. The plasma was aspirated using a Pasteur pipette and stored in separate vials at -70° C freezer. The prepared plasma samples were then genotyped for studying the primary drug resistance.

Drug resistance genotyping. The AmpliSense® HIV-Resist-Seq kit (InterLabService Ltd., Russia) was used to detect mutations of HIV drug resistance in the protease gene (pro), reverse transcriptase gene fragment (rev) and HIV integrase gene. Viral RNA was extracted from 200 μl of plasma using the AmpliSens® RIBO-sorb nucleic acid extraction kit according to the manufacturer's instructions. The HIV-1 reverse tran-

[1] http://hivresist.ru.

scriptase gene was reverse transcripted to complementary deoxyribonucleic acid (cDNA) by using HIV-1 specific downstream primer RT-3 and superscript IV M-MLV Reverse Transcriptase (Bioneer Corporation, Republic of Korea). A base pair of the protease gene and reverse transcriptase gene fragment from cDNA were then used as templates in a 40-μl amplification reaction catalyzed by TaqF DNA Polymerase (JSC GenTerra, Russia). The method is known as «two-step reverse transcription polymerase chain reaction (RTPCR)». Conditions for the first round of PCR were 45° C for 30 minutes followed by 94° C for 10 minutes, followed by 30 cycles for 2 min 30 seconds: 95° C for 30 seconds, 50° C for 30 seconds, 72° C for 1 min 30 seconds, with a final elongation step at 72° C for 5 minutes. The nucleotide sequence of either reverse transcriptase, integrase or protease genes in individual viral RNA molecule was determined as follows: cDNA was synthesized and amplified by nested PCR via two stages. In Stage, I, the primers used were HIV-RT1-R (5'-GGA CTA CAG TCY ACT TGT CCA TG-3') and HIV-RT1-F (5'-ATG ATA GGG ATG GGA ATG GGT TT-3'), which was added to a PCR master Mix (Invitrogen). Amplification was performed under conditions as described herein. In Stage II, the template DNA was the PCR product from Stage I, the primers used were HIV-RT2-R (5'-TTA AAA TCA CTA RCC ATT GYT CTC C-3') and HIV-RT2-F (5'-GAC CTA CAC CTG TCA ACA TAA TTG G-3'), and the same PCR master mix (Invitrogen) was used. Amplification was conducted using the same PCR settings are those used in stage I. The DNA product of PCR amplification was purified by using AmpliSens® DNA-sorb-AM nucleic acid extraction kit (InterLabService Ltd., Russia).

Before setting up the sequencing reaction, an electrophoresis of nested PCR products was performed to evaluate the concentration of purified amplification products (PCR products) using a colorant EPh, 200F detection agarose kit (InterLabService Ltd., Russia), set to 800 volts for 1.5 minutes.

Purified product (15-μl) was used for sequencing, using forward primer and big dye terminator ready reaction mix (ABI Big Dye Terminator v1.1/v3.1 Cycle Sequencing Kit, Applied Biosystems, USA) according to the manufacturer's instructions. For sequencing reaction temple, forward primer 5'- GCC TGA AAA TCC ATA TAA CAC TCC-3' reverse primer 5'- CCA TCC AAA GAA ATG GAG GTT C-3' (2 μl concentration) of the *pol* gene, PCR buffer (2 μl) were added to 1 μl AmpliSense® HIV-Resist-Seq 5, 6, 7, 8 (InterLabService Ltd., Russia). A volume of 5 μl of the reaction was set up for sequencing and the samples were subjected to thermocycler GeneAmp PCR System 2700 (Applied Biosystems, USA). The reaction mixture was brought at 96° C for 1 min, followed by 25 cycles for 4 min 15 seconds: 96° C for 10 seconds, 50° C for 5 seconds, 60° C for 4 min and then at −16° C for 3 days prior for further use. Purification of the sequencing reaction mix was carried out according to version used. The samples were then loaded on to the sequencing machine, 3130xl genetic analyzer (Applied Biosystems, USA).

Sequencing was done according to manufacturer's instructions. For quality control of HIV-1 sequencing, low-positive and high-positive control samples were run with every batch. The positive controls ensured the RT-PCR and sequencing success. To ensure good sequence quality, the high-positive control was sequenced precluding editing mistakes. The sequencing results were analyzed to identify mutations and amino acid changes in the RT gene. The sequence was aligned and edited using a computer with DEONA 1.7.0 (Build11) software package (RMBIT, Russia). The type and position of mutations and amino acid changes were determined by comparison to a reference sequence. The sequence was submitted to the Stanford University HIV Drug Resistance Database. HIV-1 subtyping was performed using the COMET HIV-1 [13] and REGA Subtyping Tool v3.0 [14] and subsequently confirmed by phylogenetic analysis[1].

The first data processing using Microsoft Excel. The data that has been collected was then analyzed using the Statistical Package for Social Sciences (SPSS) IBM version 21 for Windows (SPSS Inc., Chicago, IL, USA). The normality of distribution of continuous variables was tested by Shapiro–Wilks test. Continuous variables with normal distribution were presented as mean (standard deviation [SD]); non-normal variables were reported as median (interquartile range [IQR]). The logistic regression analysis was used to identify risk factors associated with PDR. For all statistical tests, a value of $P<0.05$ was considered statistically significant.

Results

HIV-1 Subtypes. Analysis of the *pol* sequences from 100 HIV-1 infected patients confirmed the expected spectrum of viral subtypes in St. Petersburg and Leningrad region, which included 87 viruses (87%)

[1] http://dbpartners.stanford.edu:8080/RegaSubtyping/stanford-hiv/typingtool/

classified as sub-subtype A1, 11 (11%) as subtype B, and 2 (2%) as CRF06_cpx recombinant (Figure).

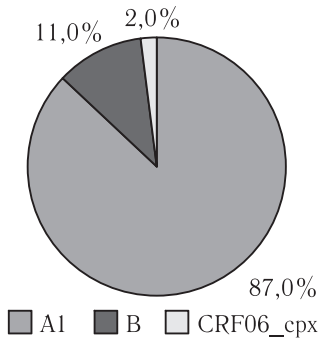

Figure. Distribution of HIV-1 genotypes among the 100 newly diagnosed HIV-1 infected patients in St. Petersburg
Рисунок. Распределение генотипов ВИЧ-1 среди впервые выявленных пациентов с ВИЧ-1 (n=100) в Санкт-Петербурге

Characteristics of the subjects. A total of 33 study subjects were men (32.4%) and 69 women (67.6%). The median age of the respondents was 39.5 (IQR=11.3) years. The highest level of education of the respondents was specialist degree and only 33 study subjects (32.4%) had a job. Most of the risk factors for HIV-1 infection/transmission mode were heterosexual (68.6%), followed by intravenous drug use (30.4%) and 1% of the cases was homosexual. Co-infection was found in 69 subjects (67.6%), with the highest co-infection being hepatitis C (51%), followed by hepatitis B (9.8%) and tuberculosis (TB) — 6.9%. Only 7 subjects (6.9%) had an acute retroviral syndrome; symptoms occurred after an identified episode of high-risk sexual exposure and needle use (1 case). Then a total of 9 subjects (8.8%) were at asymptomatic stage (stage 2) and 86 subjects (84.3%) were at an advanced stage (stage 3). The median CD4 count was 77.36 (IQR=239.1) cells/μl, the median HIV RNA level was 379590.5 (IQR=1041158.0) copies per milliliter. Antiretroviral-susceptibility testing was performed before treatment began. A total of 61 subjects began receiving an antiretroviral regimen with a median of 22 days from the date of HIV diagnosis and had adequate follow-up (data for analysis not shown) (Table 1). The choice of initial regimen was

Table 1

Demographic and clinical characteristics of the participants

Таблица 1

Демографические и клинические характеристики пациентов

Characteristic	Number of Individuals (%), n=102
Age — years old.	
Median	39.5
Range	21–81
20–29	5 (4,9)
30–39	46 (45)
40–49	37 (36,3)
50–59	11 (10,8)
>60	3 (3)
Job status:	
Working	33 (32.4)
Unemployment	69 (67.6)
Gender:	
Male	33 (32.4)
Female	69 (67.6)
Mode of infection: Homosexual contact	1 (1.0)
Heterosexual contact	70 (68.6)
Injection drug use	31 (30.4)
Laboratory variables	
Initial plasma HIV RNA level — copies/ml	379590.5
Median	238–10000000
Range	
Initial CD4 count — cells/μl	77.36
Median	1.00–1407.45
Range	
Elapsed time — days	
From HIV infection to start of treatment	22
Median	8–65
Range	

based on the standard of care according to the WHO consolidated guidelines on the use of ARV for treating and preventing HIV-infection. First-line ART for adults should consist of two nucleoside reverse-transcriptase inhibitors (NRTIs) plus a non-nucleoside reverse-transcriptase inhibitor (NNRTI) or an integrase inhibitor (INI). Tenofovir disoproxil fumarate (TDF) + Lamivudine (3TC) or Emtricitabine (FTC) + Efavirenz (EFV) as a fixed-dose combination was recommended as the preferred option to initiate ART [15].

Genotypic Analysis of Drug Resistance. Out of the 102 plasma RNA samples studied, 100 samples (who had a plasma HIV RNA level of more than 500 copies per milliliter) could be amplified and analyzed by direct DNA sequencing. Virus from 8 subjects (8%) was found to harbor resistance-conferring mutations to NRTIs. Four of these were found to harbor mutations associated with zidovudine resistance. One subject harbored virus with multiple resistance mutations, including zidovudine-related resistance mutations K70R (E) (n=1), D67N (n=1), L74I (n=1), and M184V (n=1) in combination with NNRTIs-related resistance mutations (V106M, G190S). Isolated zidovudine resistance mutation K70R (n=1) could be identified in virus from only 1 individual. An isolated amino acid substitution D67N that is associated with resistance to zidovudine only in context with other resistance-conferring mutations (i.e., M41L, K70R, and T215Y) was found in 1 isolate. A62V (n=4) could be identified isolated in 3 subjects and once in combination with NNRTIs resistance mutations V179D (n=1). The A62V amino acid substitution in HIV-1 reverse transcriptase (RT) gene is an important adaptive mutation in multi-drug-resistant viruses, and is not known to be a resistance-conferring mutation [16].

Resistance-conferring mutations to NNRTIs were seen in 5 individuals (5%). Four of these were found to harbor mutations associated with rilpivirine and etravirine resistance: E138A (n=2), V179D (n=1) and V106I (n=1). Similarly, four subjects had a virus with etravirine resistance conferring mutations. The amino acid substitutions V106M (n=1) and G190S (n=1) were observed in 1 individual also harboring a population of virus with the M184V, D67N, K70E, L74I substitutions.

Sequencing of the protease gene from the same 100 individuals detected virus harboring mutations in the protease coding sequence in 1 individual. That unique case of amino acid substitution M46I (n=1), known to be non-polymorphic could be identified in association with E13A, and was associated with resistance to atazanavir and lopinavir. No other primary protease inhibitor resistance conferring mutations were identified among the remaining 99 newly HIV-infected individuals (Table 2).

Table 2

Overall resistance mutations in reverse transcriptase and protease genes among 100 subjects with newly diagnosed HIV-1 infection

Таблица 2

Мутации устойчивости в генах обратной транскриптазы и протеазы среди 100 впервые выявленных пациентов с ВИЧ-1

Mutations	Number of Individuals (%)
Primary NRTIs, total	8 (8.0)
A62V	4 (4.0)
T69T_I	1 (1.0)
K70R	1 (1.0)
T215L*	1 (1.0)
T215L*	1 (1.0)
K219Q*	1 (1.0)
K70E#	1 (1.0)
D67N#	1 (1.0)
L74I#	1 (1.0)
M184V#	1 (1.0)
Primary NNRTIs, total	5 (5.0)
E138A	2 (2.0)
V179D	1 (1.0)
V106I	1 (1.0)
V106M†	1 (1.0)
G190S†	1 (1.0)
Primary protease inhibitors, total	1 (1.0)
M46I	1 (1.0)

Note: * — Resistance mutations found in the same subject; # — Multiple resistance mutations in one subject, in combination with NNRTIs-related resistance mutations (V106M, G190S); † — Multiple resistance mutations in one subject, in combination with NRTIs-related resistance mutations (D67N, K70E, L74I, M184V).

Примечание: * — Мутации устойчивости, обнаруженные у того же пациента; # — Множественные мутации устойчивости у одного пациента в сочетании с мутациями устойчивости к ненуклеозидным ингибиторам обратной транскриптазы (ННИОТ) (V106M, G190S); † — Множественные мутации устойчивости у одного пациента в сочетании с мутациями устойчивости к нуклеозидным ингибиторам обратной транскриптазы (НИОТ) (D67N, K70E, L74I, M184V).

The multinomial logistic regression analysis for risk factors of PDR showed that the HIV-1 genotype was a potential influencing factor associated with PDR. CRF06_cpx strains had a lower risk of PDR (Table 3).

Discussion

This cohort is predominantly heterosexual (68.6%) men and women, followed by persons who inject drugs (PWID) — 30.4% from St-Petersburg and others urban centers of Leningrad region. According to the national official report, heterosexual contact has

Table 3

Multinomial logistic regression analysis for risk factors of PDR

Таблица 3

Анализ мультиномиальной логистической регрессии для факторов риска ПЛУ

Factor	p	OR	95% CI for OR
Age	0.42	0.905	0.822–0.996
CD4	0.279	0.997	0.992–1.002
HIV-1 transmission route:			
Heterosexual	0.765	0.168	3.478
Homosexual	0.728	1.038	0.288–5.947
IDUs	—	—	—
HIV-1 genotype:			
A1	0.993	1.378	0.00
B	1.00	0.729	0.00
CRF06_cpx	—	—	—

OR — odds ratio, CI — confidence interval.

become the predominant risk factor for HIV epidemic in Russian Federation [2]. Under reporting of risks, especially same-sex behavior, given existing laws banning the sharing of information related to homosexuality in the country may explain the insignificant number of gay men in our study (only one self-reported case) [17, 18]. Patients aged 30–39 years had the highest prevalence of HIV in this study. This is similar to the finding of the Federal AIDS Center surveillance, in which the highest prevalence of HIV was among the same age group [2]. Several studies from different parts of the world, especially in China and South Africa reported similar findings [19, 20].

Our study showed that subtype A (sub-subtype A1 — 87%) still the major HIV-1 subtype followed by subtype B (11%) and CRF_cpx (2%), similar to what was obtained in other recent studies [21, 22]. A variety of subtypes C, G, F1, CRF02AG and CRF03_AB recombinants have been found but still rare in the region. Subtype A remains the most prevalent strain in parts of East Africa, Russia and former Soviet Union countries. The sub-subtype A1, which is dominant in St. Petersburg and Leningrad region was also found to be related to the east African strains (Uganda/Rwanda) and A2 spread considerably to some extent in central Africa [23]. Subtype B became prevalent in almost all parts of Europe and the Americas [24].

The overall prevalence of genotypes associated with drug resistance to any antiretroviral agent was 11% and HIV-1 strain with PDR mutations to drugs from more one classes (multidrug resistance) was found in 2%. However, we consider that there may be resistance-conferring mutations that have not been identified yet. Many patients didn't know when they had got HIV-1 infection and some patients might have been infected for a relatively long time. Therefore, this cohort represents a subset of newly diagnosed HIV-1 infection and may not reflect the prevalence of resistance in newly acquired HIV overall. According to WHO, the drug resistance prevalence in a geographical area can be categorized as <5%, 5–15% and >15% [8]. Thus PDR in our study can be categorized as moderate prevalence according to WHO criteria. Recent cohort studies have identified a frequency of about 10–24% of primary or transmitted drug resistance (TDR) in the USA during the past decade [25] comparing to 8.3%, according to a study carried among HIV-infected individuals from 26 countries in Europe who were newly diagnosed between 2008 and 2010 [26].

In some countries has been observed a lower rate of TDR (3.6% in China), as the duration of access to ART was much shorter [27]. However, comparisons of the rates of transmission of drug resistance across studies are not always straightforward because there could be differences in the methods of testing for drug resistance, geographic variability in patterns of antiretroviral-drug use, variations in the risk factors for exposure to HIV, also the subset of resistance mutations included in the assessment can vary by study.

In the present study, the transmission of virus with antiretroviral resistance has been reported in 8 cases of sexual and 3 parenteral transmission of HIV. The increasing prevalence of single and multidrug-resistant virus among patients with established HIV infection may be associated with more frequent transmission of drug-resistant virus to newly infected persons and has crucial implications for prevention and treatment strategies. In this study, HIV with drug resistance has been more prevalent among heterosexual

risk group than in other risk groups (intravenous drug users, homosexuals). Reports of increasing rates of unsafe sex in the incidence of sexually transmitted infections (STIs), including HIV infection in Russia suggest that risky behavior may result in an increased incidence of HIV infection and thereby an increased frequency of drug resistance [28].

In some regions of the Russian Federation, the level of TDR has already exceeded 5%, which indicates the monitoring need of HIV-1 resistance to ARVs among naive patients in each region [29]. The highest prevalence of TDR was found in patients from the central district (10.3%), which is most likely explained by the longer and wider use of ART compared to other districts. Earlier studies revealed that the prevalence of TDR has significantly increased in past few years due to ART becoming more available and has already reached 6,1% in 2016 [30]. Comparatively, higher prevalence of TDR was found in Eastern European countries. In Croatia, despite being a country with a low prevalence of HIV-1 infection (<0.04%) was reported a prevalence of 16.4% for TDR, with a predominant epidemic among men who have sex with men (MSM) [31]. Other regional countries reported substantially lower TDR prevalence, such as Slovenia (2.4%), Bulgaria (5.2%) and Greece (6.0%), while in Serbia (8.8%), Romania (14.8%), and Hungary (17%) TDR prevalence is higher than Russian setting.

In the current study, the prevalence of transmitted drug resistance was found to be moderate whereas earlier studies from Russian Federation on transmitted drug resistance have shown lower level of prevalence [32]. This may be due to differences in various factors, likely, study methodologies for testing, HIV-1 transmission, period of infection, HIV-1 subtype, the reference list of analogue resistance mutations (RAMs) used to evaluate the presence of relevant mutations, the use of suboptimal ART regimens and ongoing difficulties with adherence and tolerability that could lead to the accumulation of drug resistance. Despite these differences and limitations, the present study does reflect the rise in drug resistance in St. Petersburg, which deserves attention.

Conclusion

The prevalence of PDR mutation in this study was not higher than the national figures but needs a constant monitoring due to the challenges it presents towards HIV-prevention and antiretroviral treatment. HIV is one of the most genetically diverse pathogens due to its high-mutation. The current study showed that subtype A1, which remains the most prevalent strain in parts of East Africa, predominates in St. Petersburg and Leningrad region with 87.2%, followed by subtype B (11.8%) and CRF06_cpx (2%). This study also demonstrated that HIV-1 genotype was a potential influencing factor associated with PDR mutation, the reason why these findings may contribute to enhance the knowledge of molecular, epidemiological characteristics of HIV-1 in the eastern region of Africa where a missing subtype data and low sequence sampling levels are still a challenge. However, due to the small sample size of the study, further research in a larger population still necessary.

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