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Prevalence of resistance-associated substitutions (RAS) in hepatitis C virus in the Former Soviet Union countries

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ABSTRACT

Objective The emergence of resistance-associated substitutions (RASs) poses a significant challenge to the effective treatment of hepatitis C virus (HCV) infection using direct-acting antivirals. This study's objective was to observe the prevalence of HCV genotypes and RAS within the Former Soviet Union (FSU) countries.

Methods We analysed 60 NS3, 313 NS5A and 1119 NS5B sequences of HCV deposited in open-access databases from 11 FSU countries for the prevalence of genotypes and the presence of RAS using the Geno2Pheno software.

Results The following NS3 RASs were revealed through our analyses: 156P/S/T, 168del, 80K, 55A and 174S. The most prevalent NS5A RAS was 30K (12.69%) in genotype 3a, associated with resistance to daclatasvir, elbasvir and ledipasvir, followed by 62S (8.96% in genotype 3a), linked with resistance to daclatasvir, and 93H (3.95% and 6.72% in genotypes 1b and 3a, respectively), conferring resistance to daclatasvir, ombitasvir, elbasvir, ledipasvir and velpatasvir. The NS5B RASs found in this study were 451S and 556G, associated with resistance to dasabuvir.

Conclusion The high prevalence of HCV genotypes 1b and 3a in the FSU region and the presence of specific RASs should be considered when determining the most effective treatment regimen for HCV-infected individuals in the FSU countries.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Direct-acting antivirals (DAAs) are the current standard treatment for hepatitis C virus (HCV) infection.
⇒ Resistance-associated substitutions (RASs) can compromise DAA treatment effectiveness.
⇒ Limited data are available on HCV genotype distribution and RAS patterns in Former Soviet Union (FSU) countries.
⇒ Understanding regional RAS patterns is crucial for optimising treatment strategies.

WHAT THIS STUDY ADDS

⇒ Comprehensive analysis of HCV genotypes and RAS patterns across FSU countries.
⇒ Identified predominance of genotypes 1b and 3a in the FSU region.
⇒ Documented specific RAS patterns: NS5A RAS 30K (12.69% in genotype 3a) as most prevalent; multiple NS3 RAS including 156P/S/T, 168del, 80K, 55A and 174S; limited NS5B resistance (only 451S and 556G variants).
⇒ Offers baseline data for resistance patterns in a previously understudied region.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study provides evidence-based guidance for clinicians in FSU countries to optimise DAA treatment selection based on local genotype and resistance patterns. The findings support the need for resistance testing before treatment initiation, particularly in genotype 3a patients.
⇒ Healthcare policymakers can use this data to develop region-specific treatment guidelines and resistance monitoring strategies.
⇒ The established baseline also enables future surveillance of resistance pattern evolution in the FSU region.

INTRODUCTION

Blood-borne viral hepatitis C virus (HCV) infection is a significant contributor to liver disease-related deaths globally and remains a pressing global health issue.[1] The prevalence of HCV is estimated to be around 50 million worldwide.[2] The advent of direct-acting antivirals (DAAs) has transformed the treatment of HCV. Between 2015 and 2022, approximately 10 million cases of HCV were successfully cured, thanks to the improved accessibility of these medications.[2 3] In response to this advancement, the WHO has set a target to eliminate HCV by 2030.[4]

HCV, a member of the Hepacivirus genus, is an RNA virus with a genome comprising approximately 9.0 to 9.8 thousand nucleotides that encode structural and non-structural proteins. The structural proteins—core, envelope glycoproteins E1 and E2—form viral particles. The non-structural proteins—NS2, NS3, NS4A, NS4B, NS5A and NS5B—play important roles in viral polyprotein processing and viral replication.[5] HCV has 8 genotypes and approximately 105 subtypes.[6]

Distinct distributions of HCV genotypes are observed across various geographical regions. The prevalence of genotype 1 is the highest worldwide, responsible for 83.4 million cases; whereas genotype 2 is primarily endemic in West Africa,[7] genotype 3 in South Asia, genotype 4 in Central Africa and the Middle East, genotype 5 in South Africa, genotype 6 in Southeast Asia, genotype 7 in Canada[8] and genotype 8 recently discovered in India.[9] This global distribution of HCV genotypes is generally influenced by trends of human migration.[10] Numerous studies have demonstrated the significance of HCV genotype distribution in the management of patients with chronic HCV infection and disease progression, where certain genotypes have been linked with rapid progression and development of liver decompensation, hepatic cirrhosis, hepatic steatosis and hepatocellular carcinoma.[11]

Currently, four classes of DAAs are approved for the treatment of HCV. These drugs have three specific therapeutic targets: non-structural NS3/4A protease inhibitors, NS5A replication complex inhibitors and nucleoside/non-nucleoside NS5B RNA-dependent polymerase inhibitors.[5] The primary goal of antiviral treatment using DAAs is to achieve a sustained virological response (SVR), which is defined as the absence of detectable HCV RNA in the blood 12 weeks after completing the antiviral treatment.[12] Antiviral regimens typically involve the combination of at least two different drug classes that have distinct antiviral mechanisms. These recommended regimens have demonstrated high rates of SVR, ranging from 90% to 96%. The specific SVR rates may vary depending on factors such as the stage of liver disease and the viral genotype (GT).[12] Three pan-genotypic combination regimens—sofosbuvir/velpatasvir, sofosbuvir/velpatasvir/voxilaprevir and glecaprevir/pibrentasvir—have demonstrated effectiveness in treating all genotypes of HCV, including in patients with cirrhosis or co-infection with HIV. These DAAs have the potential to simplify medical care and promote the global expansion of HCV treatment.[13] Resistance-associated substitutions (RASs) are naturally occurring polymorphisms that evolve in response to DAAs and result in varying levels of resistance. These substitutions pose a significant challenge to the effective DAA treatment of HCV.[14] The prevalence of naturally occurring RASs varies across genotypes and sub-genotypes of HCV and also exhibits geographical variability.[15]

In HCV, recombination between genotypes/subtypes has also been observed, which may affect the efficacy of regimens based on multidrug combinations.[16 17] Similarly, DRMs in different genotypes of HCV NS3, and NS5A/5B can interfere with the DAA therapy. For example, DRMs D168 and A156, which emerge in relative frequency in different genotypes, affect the efficacy of all currently available protease inhibitors against HCV. Similarly, DRMs M28A/T/V, Q30E/H/K/R, L31M/V and Y93C/H/N (genotype 1a), L31I/M/V and Y93H (genotype 1b) and A30K and Y93H (genotype 3) are the most clinically significant DRMs in HCV NS5A region that can affect the efficacy of DAA. The use of combinational therapy, adherence to therapy, genotyping and viral load monitoring is important to control/prevent DRMs in HCV.[18]

Following the collapse of the Soviet Union in 1991, 15 countries gained independence, including Russia, Ukraine, Belarus, Kazakhstan, Kyrgyzstan, Uzbekistan, Tajikistan, Turkmenistan, Moldova, Estonia, Lithuania, Latvia, Georgia, Armenia and Azerbaijan. Since gaining independence, these countries have faced challenges in their political, social and economic transition.[19] Open border policies within the region have promoted travel and trade and facilitated infectious disease transmission.[19]

In the Former Soviet Union (FSU) region, the prevalence of HCV infection varies across 15 countries. The highest reported prevalence was in Ukraine at 3.40%, followed by Uzbekistan at 3.02% and Azerbaijan at 3.01% (online supplemental table 1).[4 20–22] Russia, Armenia and Kyrgyzstan recorded a prevalence of 1.85%, 0.70% and 2.51%, respectively, while Kazakhstan reported 2.02%. Tajikistan reported 2.57% prevalence, while Belarus showed a range of 2.12%.[4 21 23–25] The estimated prevalence in Moldova and Georgia was, respectively, 2.12% and 2.26%.[26 27]

HCV prevalence was reported at 5.8% in Central Asia, consisting of countries such as Armenia, Azerbaijan, Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, Uzbekistan, Mongolia and Georgia.[28] Among these regions, the predominant genotype was GT1, accounting for 70.4% of cases, followed by GT3 (19.6%) and GT2 (8.6%). Instances of mixed genotypes were infrequent, with no reported cases of GT4, GT5 and GT6.[29] Another study suggested that although genotype information was not available for Kyrgyzstan and Turkmenistan, a pooled analysis of data from Kazakhstan, Tajikistan and Uzbekistan indicated HCV genotype 1 (accounting for 53% of infections) and genotype 3 (representing 38% of infections) to be the predominant circulating strains. Additionally, there was a significant presence of genotype 2, comprising 9% of infections.[4]

The aim of this study was to examine the prevalence of HCV genotypes in FSU countries, investigate the association between the prevalence of RAS in DAA-failing patients, and review the treatment guidelines for HCV management in these countries.

METHODS

To observe the prevalence of HCV genotypes and subgenotypes in FSU countries, all the available HCV NS3, NS5A and NS5B sequences from the 11 FSU countries, that is, Armenia, Azerbaijan, Belarus, Kazakhstan, Kyrgyzstan, Russia, Tajikistan, Turkmenistan, Ukraine, Uzbekistan and Georgia were retrieved from the Los Alamos Hepatitis C Immunology Database (https://hcv.lanl.gov/components/sequence/HCV/search/searchi.html, data available as of 25 August 2023).[30] To increase the number of sequences for certain FSU countries with a

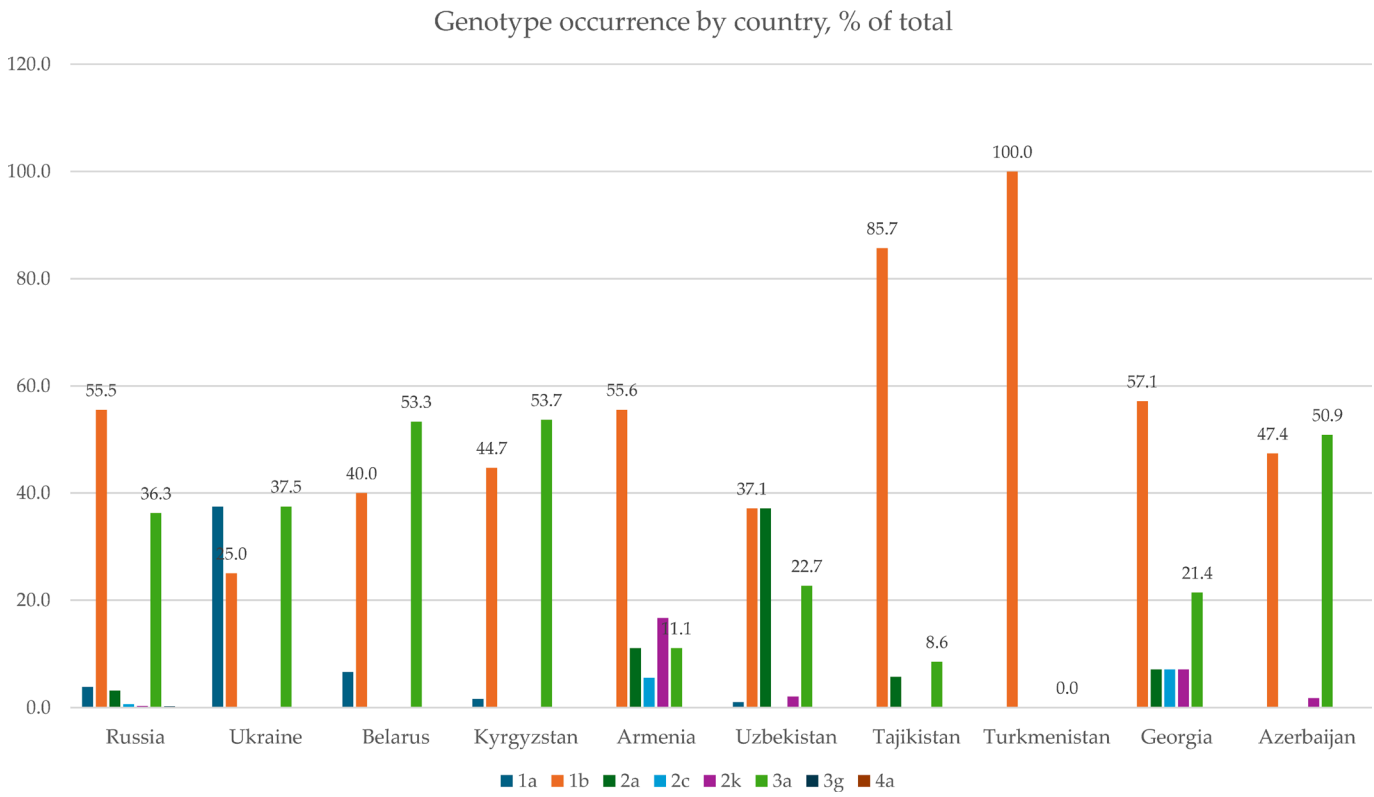

Figure 1 Genotype frequency as a percentage of the total, calculated based on the combined hepatitis C virus NS3, NS5A and NS5B sequence data from various Former Soviet Union countries.

limited count of NS5B sequences, the NS3 and NS5A genes were incorporated alongside the NS5B gene for subtyping and confirming the consistency of these findings. In the end, a total of 60 NS3, 313 NS5A and 1119 NS5B nucleotide sequences were downloaded and used to perform HCV genotyping using the Geno2Pheno software.[31] The Geno2Pheno software was also used to detect RASs in all NS3, NS5A and NS5B nucleotide sequences.[31] This tool has also been implemented in other studies that investigated the association between the prevalence of RAS among DAA-failing patients.[32 33]

RESULTS

Our analysis shows that overall, genotype 1b was the most prevalent HCV genotype in the FSU region, followed by genotype 3a (figure 1 and online supplemental tables 1 and 2). Russia, Belarus, Ukraine and Kyrgyzstan also showed the presence of genotype 1a, while Armenia had the most genotype diversity comprising genotypes 1b, 2a, 2c, 2k and 3a (figure 1 and online supplemental table 2).

Analysis of RASs (table 1) revealed three NS3 sequences with RASs: one from Ukraine with RAS 156P, 156S, 156T and 168del conferring resistance to voxilaprevir, an NS3/4 protease inhibitor; one from Russia with RAS 80K, associated with resistance to simeprevir, another NS3/4 protease inhibitor; and one from Russia with RASs 55A and 174S, reported to confer resistance to boceprevir, an NS3/4 protease inhibitor.[31]

In the NS5A sequences, a total of 80 RASs were identified (table 1). The most common RAS in the genotype 3a NS5A region was 30K, present in 17 out of 134 sequences (12.69%). Another frequently occurring RAS in genotype 3a NS5A sequences was 62S, identified in 12 out of 134 sequences (8.96%). Among the 1119 NS5B sequences in this study, only one sequence from Russia exhibited RASs: 451S and 556G, associated with resistance to dasabuvir.

DISCUSSION

This study aimed to observe the prevalence of HCV genotypes and RAS within the FSU countries. Our analysis shows that the most prevalent HCV genotype in the FSU region was genotype 1b, followed by genotype 3a. These findings align with our earlier investigation, where we examined the transmission of HCV in the FSU region using sequences from the same database.[34] The study identified a predominance of the same genotypes across several FSU countries, including Russia, Uzbekistan, Azerbaijan, Tajikistan and Georgia. However, one study found a higher prevalence of genotype 3a than subtype 1b among injection drug users in St. Petersburg, Russia.[35]

Treatment regimens in FSU countries

According to the American Association for the Study of Liver Diseases and the Infectious Diseases Society of America, the simplified treatment for treatment-naïve patients without cirrhosis or with compensated cirrhosis involves glecaprevir/pibrentasvir, sofosbuvir/

Table 1 RAS prevalence in the hepatitis C virus NS5A sequences by country, genotype and associated drug resistance

	Prevalence of RAS											
	Country				Genotype		Associated drug resistance					
	Russia, n=248	Belarus, n=25	Ukraine, n=7	Kyrgyzstan, n=32	1b, n=152	3a, n=134	DCL	OMB	EBR	LED	VEL	PIB
30Q	10 (4.03%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	10 (6.58%)	0 (0.00%)	+	+	–	–	–	–
30K	7 (2.82%)	10 (40.0%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	17 (12.69%)	+	–	+	+	LS	LS
30V	1 (0.40%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (0.75%)	+	–	+	+	+	–
31M	5 (2.02%)	2 (8.00%)	1 (14.29%)	0 (0.00%)	8 (5.26%)	0 (0.00%)	–	–	+	+	–	–
32L	1 (0.40%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (0.66%)	0 (0.00%)	+	+	–	+	–	–
58T	1 (0.40%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (0.66%)	0 (0.00%)	–	–	–	+	–	–
62S	6 (2.42%)	6 (24.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	12 (8.96%)	+	–	–	–	–	–
62L	3 (1.21%)	5 (20.00%)	0 (0.00%)	1 (3.13%)	0 (0.00%)	9 (6.72%)	LS	–	–	–	–	–
62T	0 (0.00%)	2 (8.00%)	0 (0.00%)	2 (6.25%)	0 (0.00%)	4 (2.99%)	+	NA	–	–	–	–
62M	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (3.13%)	0 (0.00%)	1 (0.75%)	+	–	–	–	–	–
92T	1 (0.40%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (0.66%)	0 (0.00%)	+	+	–	+	–	–
93H	6 (2.42%)	4 (16.00%)	1 (14.29%)	4 (12.5%)	6 (3.95%)	9 (6.72%)	+	+	+	+	+	–

'+': well-characterised resistance-associated mutation, '–': susceptible, 'LS': association with resistance, insufficient evidence for clinical outcome, 'NA': drug not licensed for the predicted genotype.[31]
DCL, Daclatasvir; EBR, Elbasvir; LED, Ledipasvir; OMB, Ombitasvir; PIB, Pibrentasvir; RAS, resistance-associated substitution; VEL, Velpatasvir.

velpatasvir.[36] Additionally, treatment-naïve patients with decompensated cirrhosis are recommended sofosbuvir/velpatasvir with ribavirin (genotype 1–6), ledipasvir/sofosbuvir with ribavirin (genotypes 1, 4, 5, 6). The re-treatment of DAA non-responsive patients should include glecaprevir/pibrentasvir plus sofosbuvir and ribavirin or sofosbuvir/velpatasvir/voxilaprevir and ribavirin.[36]

As per the treatment guidelines in Russia, treatment-naïve patients without cirrhosis, previous liver transplantation or with compensated cirrhosis are prescribed with velpatasvir/sofosbuvir, glecaprevir/pibrentasvir, daclatasvir/sofosbuvir (online supplemental table 3).[37] In addition, patients with no response to PegIFN (pegylated interferon)/ribavirin/sofosbuvir or sofosbuvir/ribavirin, patients with HIV/HCV coinfection should be prescribed with the same pangenotypic regimens. Determining the HCV genotype is only necessary if pangenotypic treatment is unavailable.

In Kazakhstan, the identification of the HCV genotype precedes the commencement of antiviral treatment. Treatment-naïve patients infected with HCV genotypes 1–3, without cirrhosis or with compensated cirrhosis, or coinfected with HIV/HCV are prescribed with sofosbuvir/daclatasvir, sofosbuvir/velpatasvir or glecaprevir/pibrentasvir (online supplemental table 3).[38] In addition, ledipasvir/sofosbuvir, elbasvir/grazoprevir, ombitasvir/paritaprevir/ritonavir and dasabuvir regimens are available options specifically for HCV infection genotypes 1a and 1b. HCV-positive patients with decompensated cirrhosis are prescribed ribavirin and sofosbuvir/daclatasvir or sofosbuvir/velpatasvir pangenotypic regimens, while for genotypes 1a and 1b a ledipasvir/sofosbuvir and ribavirin regimen is also available. For treatment-experienced patients without cirrhosis or with compensated cirrhosis sofosbuvir/velpatasvir/voxilaprevir, glecaprevir/pibrentasvir, sofosbuvir and glecaprevir/pibrentasvir are recommended, while in the presence of decompensated cirrhosis a preferred regimen is sofosbuvir/velpatasvir and ribavirin (online supplemental table 3).

Regarding Kyrgyzstan, treatment-naïve patients with HCV infection without cirrhosis or with no response to PegIFN and ribavirin are prescribed pangenotypic or genotype-specific regimens. These include daclatasvir/sofosbuvir (genotypes 1–4), ledipasvir/sofosbuvir (genotypes 1, 4–6) or sofosbuvir/ribavirin (genotypes 2, 3) (online supplemental table 3).[39] On the other hand, patients with treatment failure should receive sofosbuvir/daclatasvir, sofosbuvir/ledipasvir, ombitasvir/paritaprevir/ritonavir and dasabuvir, sofosbuvir/simeprevir.

In Belarus treatment-naïve patients with HCV are managed based on the genotype of HCV, using sofosbuvir/ledipasvir (genotype 1), ombitasvir/paritaprevir/ritonavir (genotype 1b), sofosbuvir/daclatasvir (genotypes 1–3), sofosbuvir/velpatasvir (genotypes 1–3).[40] Treatment-experienced patients are prescribed with ribavirin and sofosbuvir/ledipasvir (genotype 1), ombitasvir/paritaprevir/ritonavir (genotype 1), sofosbuvir/daclatasvir (genotypes 1–3), ombitasvir/paritaprevir/ritonavir and dasabuvir (genotypes 1a and 1b), sofosbuvir/velpatasvir (genotypes 1–3) (online supplemental table 3).

In Ukraine, treatment-naïve patients with HCV infection without cirrhosis or exhibiting no response to PegIFN and ribavirin are managed with the following

pangenotypic regimens: sofosbuvir/ledipasvir (genotypes 1, 4–6), sofosbuvir/velpatasvir (genotypes 1–6), sofosbuvir/daclatasvir (genotypes 1–6).[41] Additionally, patients with treatment failure should receive sofosbuvir, ombitasvir/paritaprevir/ritonavir and dasabuvir with ribavirin or glecaprevir/pibrentasvir with sofosbuvir (online supplemental table 3).

In Uzbekistan, the management of chronic HCV in patients without liver cirrhosis and compensated cirrhosis includes glecaprevir/pibrentasvir and sofosbuvir, sofosbuvir/velpatasvir/voxilaprevir, while patients with decompensated liver cirrhosis are treated with sofosbuvir/velpatasvir and ribavirin.[42] Furthermore, patients with multidrug resistance are recommended glecaprevir/pibrentasvir and sofosbuvir (online supplemental table 3).

In Armenia, treatment-naïve patients infected with HCV, without cirrhosis or with compensated cirrhosis, are managed with sofosbuvir/velpatasvir (genotypes 1–6), glecaprevir/pibrentasvir (genotypes 1–6), sofosbuvir/velpatasvir/voxilaprevir (genotype 3), sofosbuvir/daclatasvir (genotypes 1–6), sofosbuvir/ledipasvir (genotypes 1, 4–6), glazoprevir/elbasvir (genotypes 1, 4), ombitasvir/paritaprevir/ritonavir (genotype 1).[43] Patients with treatment failure should receive sofosbuvir/velpatasvir/voxilaprevir, sofosbuvir with glecaprevir/pibrentasvir and ribavirin, sofosbuvir/velpatasvir and ribavirin regimens (online supplemental table 3).

In Georgia, the antiviral treatment regimens depend on the HCV genotype: sofosbuvir/ledipasvir with or without ribavirin (genotypes 1, 4), sofosbuvir/velpatasvir (genotypes 1–4).[44] In Azerbaijan, treatment-naïve HCV-infected patients without cirrhosis or with compensated cirrhosis, coinfected with HIV/HCV are managed according to the genotype of HCV: sofosbuvir/velpatasvir (genotypes 1a, 1b, 2, 3, 4, 5, 6), glecaprevir/pibrentasvir (genotypes 1a, 1b, 2, 3, 4, 5, 6), sofosbuvir/velpatasvir/voxilaprevir (genotypes 3, 1l, 4r, 3b, 3g, 6u, 6v), grazoprevir/elbasvir (genotypes 1a, 1b, 2, 4, 5, 6). On the other hand, treatment-experienced patients should receive sofosbuvir/daclatasvir with or without ribavirin.[45] Previous studies reported that Tajikistan refers to the guidelines in Russia for the management of chronic hepatitis C infection (online supplemental table 3).[40]

Prevalence of the HCV genotypes and RASs in the FSU countries

In this study, we identified genotype 1b as the most prevalent genotype in the FSU region. Among 1492 sequences, we detected 3 NS3, 46 NS5A and 1 NS5B sequences carrying RASs.

For the RAS identified in the NS3 region, the 80K substitution in a Russian sequence had previously been recognised as a natural polymorphism associated with simeprevir resistance, particularly common among genotype 1a patients.[46 47] Additionally, the previously reported NS3 RAS L153I, associated with boceprevir resistance, was detected in Russia.[48]

Previous studies on the natural occurrence of NS5A polymorphisms revealed that the 30K variant was present in 9.5% of patients at baseline.[49] In this study, this RAS was observed in sequences from Russia (n=7) and Belarus (n=10), and is associated with resistance to the NS5A inhibitors daclatasvir, elbasvir and ledipasvir. Notably, this mutation has been reported to confer a high level of resistance to daclatasvir in genotype 3a.[50 51] Furthermore, this particular RAS was found to be associated with a mutation at site 62 (62S or 62T), which might have a reinforcing effect on drug resistance.[52]

The 62S RAS, another frequently occurring mutation, was detected in sequences from both Russia (n=6) and Belarus (n=6) and is known to confer resistance to daclatasvir. Additional NS5A RASs reported in the FSU regions include M28V, associated with genotype 1a and resistance to ombitasvir, ledipasvir, velpatasvir and pibrentasvir (table 1).[53] Furthermore, mutations such as C316N and F37L in the NS5A gene, along with Y56F, Q168 and I170 mutations in the NS3 region, have been documented in HCV sequences from Kyrgyzstan.[23]

In this study, only one sequence from Russia exhibited RASs: 451S and 556G. The 556G mutation has previously been linked to an 11-fold increase in EC50 towards dasabuvir.[46] Moreover, earlier studies have consistently highlighted the infrequency of RASs in this region of HCV sequences. For instance, a study analysing HCV genotype 3 in southwestern China have revealed the rareness of NS5B-specific RASs as well.[54] Furthermore, the analysis of the European prevalence of RASs among 938 patients infected with HCV demonstrated a lower prevalence of NS5B RAS for all genotypes except for GT11b and GT4d.[55] Another study involving 17 patients infected with HCV genotype 4 in Saudi Arabia observed no mutation associated with resistance among NS5B sequences.[56] Other NS5B RASs reported in the FSU region encompass L159F and Q309R, associated with ribavirin resistance, as well as E237G.[48 57]

One of the highly prevalent RASs in both genotypes 1b and 3a was 93H, accounting for six sequences (3.95%) and nine sequences (6.72%), respectively. This RAS was observed in sequences from Russia (n=6), Belarus (n=4), Kyrgyzstan (n=4) and Ukraine (n=1). Notably, prior research has indicated a lower prevalence of the 93H RAS in Russia compared with the regions of Asia, Europe and North America.[57] Specifically, in genotype 1b, the prevalence of the 93H RAS was 6%, contrasting with 15% in Asia. Similarly, in genotype 3a sequences, the occurrence of 93H was 2%, compared with 6% in North America.[57] The 93H RAS has been documented in various HCV genotypes in earlier studies.[58] This RAS is particularly significant as it imparts resistance to a majority of existing NS5A inhibitors, including daclatasvir, ombitasvir, elbasvir, ledipasvir and velpatasvir. Additionally, it has been associated with resistance against all NS5A inhibitors in vitro and has been linked to reductions in SVR rates in HCV genotype 3-infected patients treated with sofosbuvir/velpatasvir.[59 60] In a separate study, the presence of RASs

NS5A 30K and NS5A 93H led to 30 to 44-fold and 1000 to 2154-fold increases, respectively, in the 50% effective concentration (EC50) of daclatasvir, offering a potential explanation for decreased responsiveness to daclatasvir among patients with these RASs.[40 61] Furthermore, 93H was found to synergize with other NS5A RASs, enhancing their impact on treatment failure. Specifically, the coexistence of RAS 62L with 93H in vitro resulted in a threefold increase in the resistance associated with 93H compared with the presence of 93H alone.[62–64]

With respect to HCV management in the FSU countries, the existing guidelines do not recommend screening for RASs before the initiation of treatment. Nevertheless, the identification of RASs could significantly improve treatment efficacy. In the absence of routine RAS screening, the findings from this study may help guide treatment decisions based on the prevalence of specific RASs in the region. For example, in Russia, a pan-genotypic treatment regimen includes daclatasvir, while a genotype 3a-specific regimen incorporates elbasvir (table 1). However, it is crucial to note that the most prevalent NS5A RAS in genotype 3a identified in this study was 30K, observed in sequences from Russia (n=7) and Belarus (n=10). This RAS is associated with resistance to daclatasvir, elbasvir and ledipasvir (table 1). Hence, the selection of the management strategy should favour NS5A inhibitors apart from the previously mentioned DAAs to avoid the possible drug resistance. Furthermore, another highly prevalent NS5A RAS is 93H, which is assumed to confer resistance to most of the NS5A inhibitors, except for pibrentasvir (table 1). Thus, treatment regimens containing pibrentasvir are preferable to other DAAs. For example, Russian guidelines include a pangenotypic regimen of pibrentasvir/glecaprevir, which may be more frequently recommended than other options. Additionally, the presence of the NS5A RAS 62S, associated with resistance to daclatasvir—a commonly used DAA in FSU country guidelines (table 1)—suggests that alternative NS5A inhibitors should be considered for effective HCV infection management.

We anticipate certain limitations of the study. First, small sample sizes may impact the statistical assessments and trends observed for RASs across the FSU countries, especially for NS3 and NS5 genes. The smaller sample sizes may limit the detection of rare variants; however, the sample sizes are still sufficient to detect common RAS, particularly those above 5% prevalence.[65 66] We must note that we analysed all available sequence data from FSU countries. Subsequent studies with large sequences, as they become available, can provide valuable insights about the prevalence of RAS, especially rare variants, in this previously unstudied region. Second, the study sequences had an uneven representation of different FSU countries, which may affect HCV genotype prevalence rates and RAS patterns. Although this geographical heterogeneity may impact the generalisability of our findings to under-represented regions, this study provides the first comprehensive analysis of RAS patterns in this region using all available sequence data. These results establish an important baseline for understanding HCV resistance patterns in the FSU countries and identify the need for enhanced surveillance and sequencing efforts in under-represented countries to improve geographical coverage in future studies. Finally, crucial data pertaining to patient demographics, routes of infection, treatment response, coinfection with HBV/HIV and the presence of comorbidities (mild or severe liver disease, cirrhosis, liver transplant, etc)—information that could contribute significantly to our analyses and to the restructuring of treatment guidelines—was unavailable. This constraint is significant because when selecting the appropriate treatment plan, it is essential to consider these factors on a case-by-case basis to tailor the treatment approach for each individual with HCV infection in FSU countries.

CONCLUSION

Overall, the study revealed a high prevalence of genotype 1b, followed by genotype 3a, with Armenia displaying the highest genotype diversity. Analysis of RAS showed that NS5A sequences from FSU countries had 80 RASs, with 30K being the most prevalent. On the other hand, among 1119 NS5B sequences, only one sequence from Russia exhibited RASs (451S and 556G) associated with dasabuvir resistance, suggesting a relatively low prevalence of NS5B resistance mutations in the region. Currently, there is insufficient data on the presence of RAS to DAAs in the FSU region. As a result, the findings of our study can help bridge the knowledge gap regarding the occurrence of RAS in FSU countries. The identified RAS patterns establish a baseline for monitoring resistance emergence in the region, which is crucial as treatment access expands. This data will help optimise first-line therapy selection and guide retreatment strategies for the small percentage of patients who fail initial DAA therapy.[67] These practical applications of this study can help clinicians make evidence-based decisions for HCV treatment in the FSU region, potentially improving cure rates and reducing treatment failures.

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