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Etiology of Four Waves of the COVID-19 Pandemic in Ukraine according to the SARS-CoV-2 Virus Genome Sequencing Data: A Pilot Study

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Abstract: The COVID-19 pandemic in Ukraine, from March 2020 to June 2022, witnessed distinct waves, each characterized by an increase in cases and fatalities. Next-generation sequencing has been used to understand the impact of viral variants on the pandemic situation in Ukraine. We analyzed SARS-CoV-2 genome sequencing data to identify viral variants circulating during each wave. By integrating epidemiological information, we established associations between viral variants and disease spread. The adoption of next-generation sequencing for SARS-CoV-2 surveillance in Ukraine, despite limited resources, yielded adequate and trustworthy results, reflecting the pandemic situation. After the Russian military invasion of Ukraine in February 2022, a large number of refugees crossed the border with neighboring countries. Mutation analysis on sequencing data from Ukraine and Poland was used to estimate the exchange of SARS-CoV-2 variants between the countries during this period. Omicron subvariants detected in both countries were similar. The analysis of SARS-CoV-2 sequences from Poland and Ukraine revealed shared nucleotide mutations that can be used to identify the directions of spreading.

Keywords: SARS-CoV-2; next-generation sequencing; mutation analysis

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1. Introduction

The emergence of the novel severe respiratory syndrome coronavirus 2 (SARS-CoV-2), causing Coronavirus Disease 2019 (COVID-19), radically and permanently changed life on the planet. Initially, COVID-19 was reported in December 2019 as a cluster of cases of viral pneumonia in Wuhan, Hubei Province, China, and later spread worldwide. On 30 January 2020, the World Health Organization (WHO) announced COVID-19 as an emergency situation, representing a serious threat to global public health. On 11 March 2020, the WHO declared a pandemic. As of 7 January 2024, over 774 million confirmed cases and over seven million deaths have been reported globally [1].

At an early stage of the pandemic, covering the period before the quarantine in Ukraine (until 17 March 2020), COVID-19 cases were detected among people who had recently traveled abroad and later among people who had contact with infected people. However, the introduction of a strict quarantine, the suspension of air and other transportation

between countries, the suspension of public transport, the cancellation of face-to-face education in all educational institutions, and other restrictive measures significantly slowed down the development of the first wave of the pandemic in Ukraine, affecting the incidence rates, which were fundamentally lower in other countries of the world at this time. A significant increase in morbidity began at the end of August—in September 2020. The incidence reached its peak in November 2020. It was the first high wave of the pandemic in the country. The second wave began in the western regions of the country in February 2021, peaked in April, and ended in June 2021. The third wave of cases began in September 2021, with a peak in November and a decline in December 2021. And as soon as January 2022, the fourth wave of the pandemic began, which was characterized by a sharp increase in morbidity, the peak of which fell in February 2022.

In Ukraine, the first COVID-19 cases were registered in March 2020, with 5,520,483 cases and 109,918 deaths reported by 2 July 2023 (the latest data are not available) [2].

This study was devoted to identifying the peculiarities of the pandemic course in Ukraine in the period from its beginning to the first half of 2023. During this time, various spikes in the number of disease cases and the emergence of different variants of SARS-CoV-2 were identified and characterized. The situation related to the military invasion in Ukraine on 24 February 2023 was considered separately.

2. Materials and Methods

2.1. Sample Collection

Nasopharyngeal swabs were obtained from patients with COVID-19 between May 2020 and May 2022. Informed voluntary consent from all patients was obtained using an approved consent form from the Ministry of Health of Ukraine (Form of Primary Records 003-6/o). Patient samples were primarily obtained from hospitalized patients but also, to a lesser extent, from non-hospitalized patients under observation in sentinel healthcare institutions. Patient samples were obtained from multiple cities in Ukraine, including Kyiv, Dnipro, Ivano-Frankivsk, Lviv, Uzhhorod, Rivne, Khmelnytskyi, Chernivtsi, Vinnytsia, Kharkiv, and Lutsk. Samples were collected using cotton-tipped applicators (300298, DeltaLab, Barcelona, Spain) in viral transport medium (VTM) and handled by trained healthcare workers as described in [3].

2.2. Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR)

Viral RNA was extracted from 140 µL of a patient sample using the QIAamp Viral RNA Mini Kit (Qiagen, Holden, Germany).

Patient infection was confirmed from patient samples using RT-PCR. The TaqPath COVID-19 CE-IVD RT-PCR Kit (Thermo Fisher Scientific, Pleasanton, CA, USA) was used to confirm positive samples. Samples were analyzed on a 7500 Real-Time PCR System (Applied Biosystems, Singapore). Positive samples containing SARS-CoV-2 RNA with a Ct value less than 30 were selected for whole-genome sequencing. A total of 167 samples were sequenced during the pandemic; 114 of them were sequenced at the Department of Respiratory and Other Viral Infections, and 53—by virtue of cooperation with CNR Virus des Infections Respiratoires—at France SUD. A complete list of samples is provided in Supplementary S2.

2.3. Illumina Next-Generation Sequencing (NGS)

We used different approaches in different periods of the study to sequence 114 samples.

The Respiratory Virus Oligo Panel (Illumina, San Diego, CA, USA) in combination with Illumina RNA Prep with Enrichment (Illumina, San Diego, CA, USA) was used to prepare libraries from 18 samples that were collected during May–August 2020. The preparation was performed according to the manufacturer's protocol.

Seven samples (collected in February–March 2021) were sequenced according to the ARTIC protocol (version 1 and version 2 primers) [4,5]. The ARTIC protocol (version 3 primers) was used for 89 samples that were collected from November 2021 to June 2022 [6].

Libraries were prepared using Illumina RNA Prep with Enrichment (Illumina). Amplicon-based approaches (ARTIC approaches) simplified the methodology and reduced library preparation time; however, well-known issues of this protocol were also observed; namely, when sequencing some SARS-CoV-2 variants, certain primers did not work well, resulting in amplicon drop-off and a lack of sequence coverage across some areas of the genome. This occurred despite a rather high average level of coverage of the genome in general; to combat this, primer sets were regularly updated to correspond to the most common virus variants at the time of sequencing [7].

The concentration of prepared libraries was assessed using the NEBNext® Library Quant Kit for Illumina® (New England Biolabs, Ipswich, MA, USA). NGS was performed on the iSeq 100 (Illumina) platform. Sequencer Fastq files were analyzed using the DRAGEN RNA Pathogen Detection App and DRAGEN COVID Lineage programs on Illumina's Basespace online platform. All sequences were uploaded to the GISAID EpiCoVTM global database [8].

2.4. Bioinformatics and Data Analysis

Bioinformatics and data processing were performed using the Interactive Phylogenetic Data Visualization tool. A total of 1081 sequences were analyzed during this study; 155 of our sequences and 926 additional sequences from Ukraine were downloaded from the GISAID database [8]. Data on the used sequences are given in Supplementary S3. Two SARS-CoV-2 reference genomes (Wuhan-Hu-1/2019, GISAID ID: EPI_ISL_402125 and Wuhan/WH01/2019, GISAID ID: EPI_ISL_406798) were used, and Wuhan/WH01/2019 was used to root the phylogenetic trees generated. Sequences were analyzed using the Nextstrain Augur pipeline, a Python script wrapper for multiple bioinformatics programs [9]; Mafft [10] was used for multiple sequence alignment and mutation identification, IQTree [11] to build phylogenetic trees, and TreeTime [12] for generating representations of changes over time and annotation of phylogenetic trees. All analyzed sequences met Augur's default quality criteria: a genome length of more than 27,000 base pairs with no more than 3000 unrecognized nucleotides. Sequence metadata were manually adjusted to include official transliterations of city names compatible with Augur's geographic representation, and the sampling dates were consolidated into a single format. A configuration profile that included all selected sequences in the phylogenetic tree was generated based on the SARS-CoV-2 Nextstrain assembly on Github [13].

Classification of viral clades and their frequency, phylogeny, and sequence differences in relation to geography and time were defined and further visualized using the Auspice Javascript web application [14].

2.5. Mutation Analysis of Refugee Situation

Sixty-seven genomes sequenced by us from samples collected in Ukraine between January and June 2022 (Supplementary S4), as well as 18,747 genetic sequences of virus samples from Poland collected during the same period, which were taken from the GISAID database (Supplementary S5), were used for analysis. Regarding these sequences, we searched for mutations using CoVsurver [8]. Thereafter, metadata on the sequence data were combined with the results of the mutation search. The data combined in this way were used for analysis and comparison. Data processing was carried out using custom scripts in the R programming language. The source code of these scripts is available at [15].

3. Results

3.1. COVID-19 Infection in Ukraine: March 2020 and June 2023

The course of the pandemic was analyzed based on 167 sequenced samples, which we selected from patients from multiple cities across Ukraine during the entire period (Supplementary S1). The combination of sequencing results with the available number of cases made it possible to confirm the relationship between the dominance of certain SARS-CoV-2 variants and the corresponding waves of disease growth in Ukraine. It also

helped to determine the time limits of the detected waves. Table 1 presents systematized data on the SARS-CoV-2 variants detected in the country based on our own studies for the entire observation period. The largest number of sequenced viruses belonged to the Omicron variant. This was likely due to laboratory capacity and the availability of patient samples, both of which increased over the course of the study.

Table 1. SARS-CoV-2 variants that circulated in Ukraine between May 2020 and June 2022. Viruses are differentiated by clade and genetic lineage.

	1st Wave (March 2020–January 2021)	**2nd Wave (February–August 2021)**	**3rd Wave (September–December 2021)**	**4th Wave (January–June 2022)**
WHO name	Wuhan and its first variants	Alpha	Delta	Omicron
Nextstrain clade	19A, 20A, 20B, 20D	20I	21A, 21I, 21J	21K, 21L, 21M, 22A, 22B, 22C
GISAID clade	G, GR, GV, O, V	GRY	GK	GRA
Pangolin lineage	B.1, B.1.131, B.1.527, B.1.1, B.1.1.1, B.1.1.243, B.1.1.325, B.1.1.351, B.1.1.374, B.1.1.398, B.1.1.485	B.1.1.7	B.1.617 + AY	B.1.1.529 + BA
Overall samples from Ukraine used in bioinformatics analysis (n = 1081)	246	69	440	326
Samples collected and sequenced by authors of this research (n = 167)	25	37	28	77

The first peak of COVID-19 incidence in Ukraine occurred in November 2020. The second wave began in February 2021, starting in the western regions of the country, peaked in April, and subsided in June 2021. The third wave began in September 2021, peaked in November, and subsided in December 2021. The fourth wave began in January 2022 and was characterized by a sharp increase in case numbers, then started to fall in February 2022. All these waves are visualized in Figure 1.

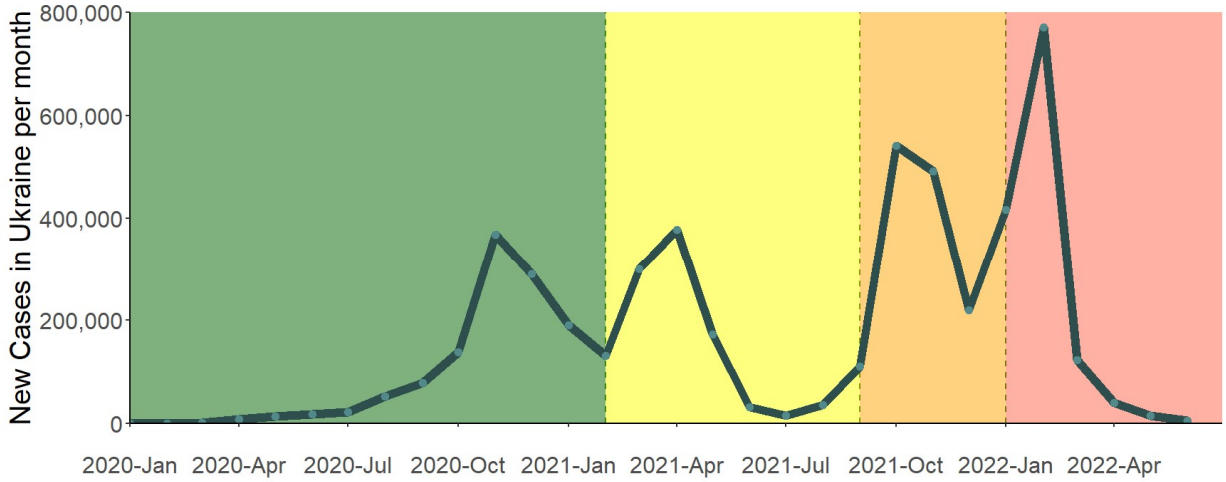


Figure 1. Official statistical data describing the number of COVID-19 cases during the four SARS-CoV-2 waves in Ukraine between March 2020 and June 2023 [2]. Each wave represented by different colors.

The initial wave of the pandemic in Ukraine (March 2020–January 2021) was caused by the SARS-CoV-2 Wuhan variant. The biggest number of new cases per month was

in November 2020. Twenty-five viral sequences were analyzed in our study; of these, three were G clade, and the remaining twenty-two were GR clade. Viruses analyzed were collected across multiple Ukrainian cities (Kyiv, Kharkiv, Dnipro, Lviv, Chernivtsi, Ivano-Frankivsk, and Lutsk) and did not represent a specific geographic region.

The second SARS-CoV-2 wave in Ukraine began on 15 February 2021 (Figure 1) and was initially identified in western Ukraine. This second wave was caused by the introduction of SARS-CoV-2 Alpha, first detected in the United Kingdom, and was characterized by a shorter incubation period and, thus, an increased incidence rate over the Wuhan strain [16,17]. Thirty-seven samples of SARS-CoV-2 from this wave were analyzed (Table 1), all of which were genetic lineage B.1.1.7 (Alpha) containing the Spike (S) protein mutation N501Y. These Alpha variant samples were collected from six cities in Ukraine (Kyiv, Dnipro, Ivano-Frankivsk, Kharkiv, Lviv, and Khmelnytskyi).

The third SARS-CoV-2 wave was identified on 15 September 2021 due to the rapid increase in the number of new cases (Figure 1) and increase in disease severity; mortality increased up to 18,246 in November 2021 compared to the maximum value registered for the previous wave (11,260 in April 2021) [2]. This wave was caused by SARS-CoV-2 Delta, first detected in India [18,19]. Twenty-eight SARS-CoV-2 Delta variant viruses were detected in our study, all of which belonged to the GK clade and genetic lineage B.1.617 + AY. Samples from this wave were obtained from patients in Kyiv and Dnipro.

The fourth wave in our study began in January 2022 and was caused by the highly contagious Omicron variant [20]. Omicron was initially detected in Botswana, Hong Kong, and the Republic of South Africa; however, while highly infectious, symptoms and disease course overall were less severe, with patients not requiring supplemental oxygen as often as those infected with previous variants [20]. Seventy-seven samples were analyzed during the fourth wave, all of which belonged to the GRA clade (B.1.1.529 lineage). Samples were obtained from patients in Kyiv, Dnipro, and Khmelnytskyi.

3.2. Mutational Analysis of SARS-CoV-2 in Ukraine

For bioinformatics analysis, we also used the extended list of 1081 samples from Ukraine, 155 of which originated from us and others from GISAID (Supplementary S2). Based on these data, a phylogenetic tree was built (Figure 2), and the dynamics of virus nucleotide and amino acid mutations was also evaluated (Figures 3 and 4).

The phylogenetic tree highlights the evolutional basement of previously determined waves associated with the prevalence of different viral variants in different pandemic periods. The main drivers and indicators of these changes over time are mutations. The Nextstrain Augur pipeline allowed us to visualize a significant increase in viral mutations (Figure 3a). While the initial outbreak and first wave showed a relatively low number of mutations in each analyzed sequence compared to the reference genome, viruses obtained from patients at the end of May 2022 showed 50 to 56 amino acid substitutions compared to the original strain; the divergence coefficient increased as well.

Viruses detected during this first wave showed fewer nucleotide substitutions and, respectively, fewer amino acid substitutions than those detected later. In our analysis, the viral population detected increasing divergence. While the divergence coefficient within the viral population ranged between 9 and 39, the majority had a divergence coefficient of 24–26, suggesting that they deviated strongly from the initial sample. For the second wave, the divergence coefficient ranged from 30 to 45 in the population of these viruses, which is bigger than in the initial wave. In the case of the fourth wave, sequences from this wave showed a divergence coefficient in the population of the Omicron variant viruses ranging from 55 to 74.

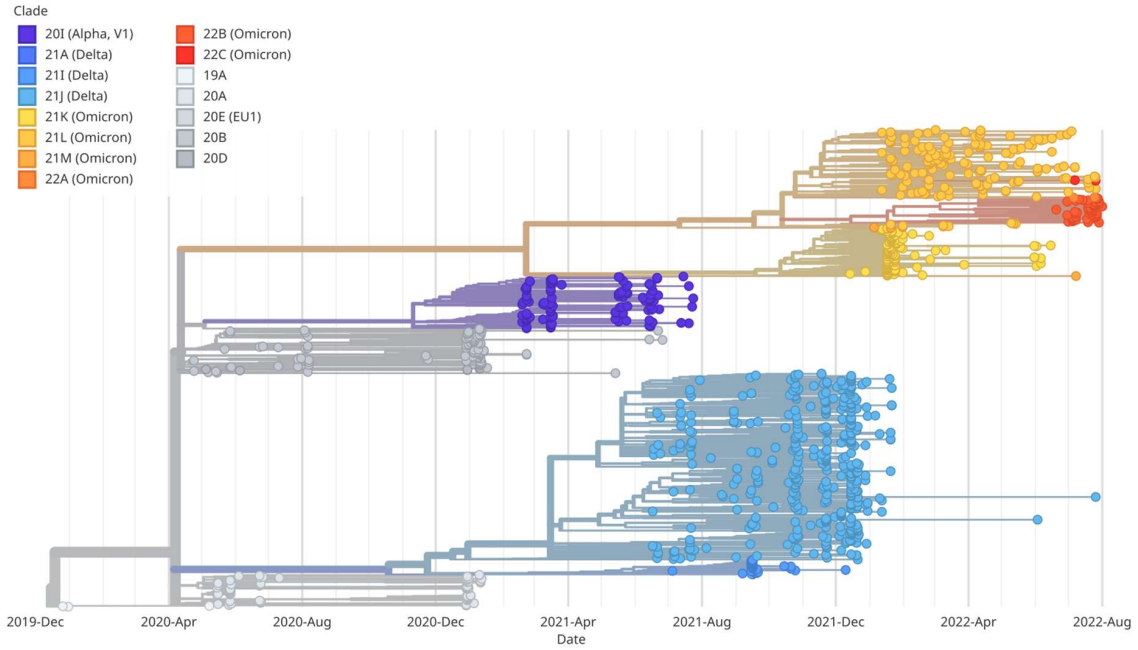
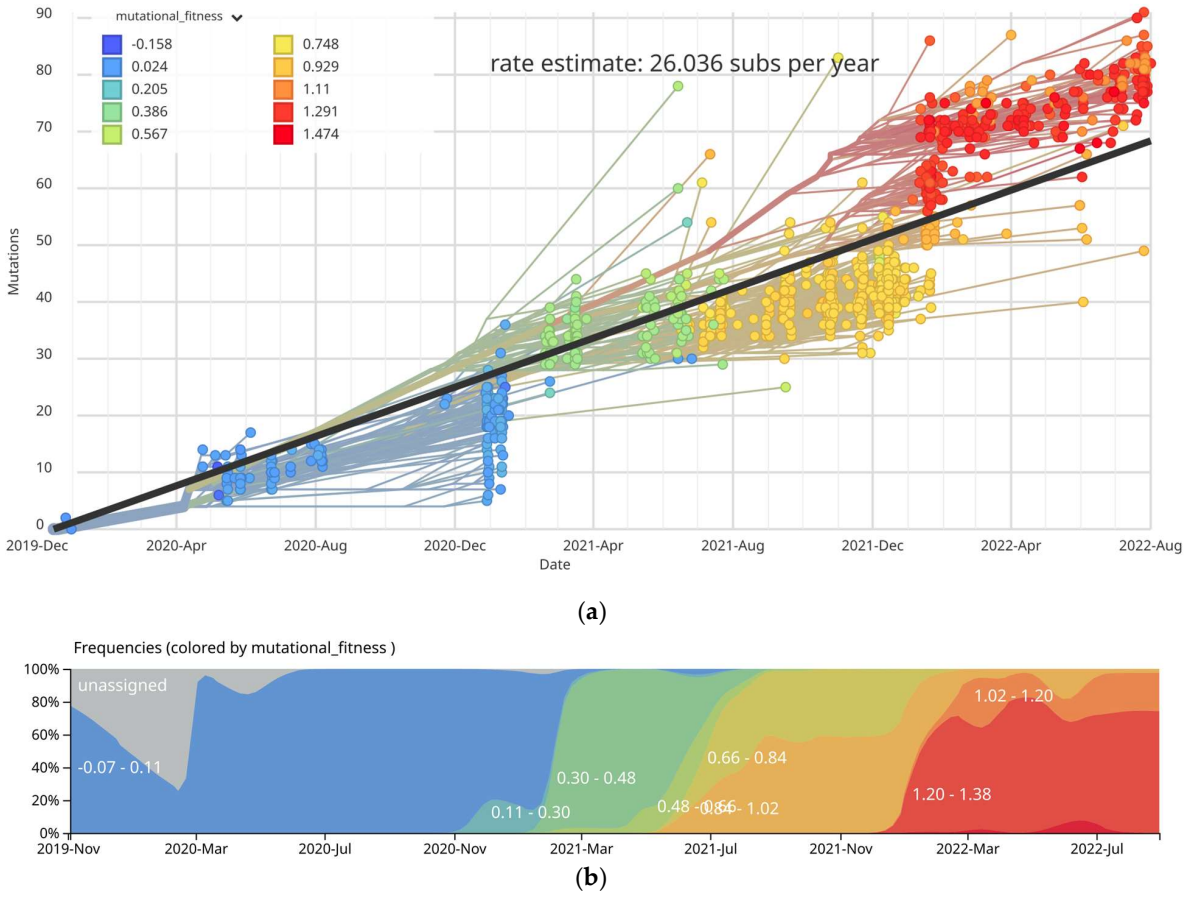


Figure 2. Phylogenetic tree of SARS-CoV-2 variants in Ukraine from May 2020 to June 2022. Virus clades are indicated by color: 20I Alpha (purple, 21J Delta (blue), 21K Omicron (orange), and 21L Omicron (red). Data are arranged according to the date of sample collection.



(**a**)

(**b**)

Figure 3. (**a**) Dynamics of number of mutations (and mutational fitness) in Ukrainian SARS-CoV-2 genomes over time compared to reference genome; (**b**) the mutational fitness frequency as a percentage over time.

Figure 4. SARS-CoV-2 spike protein amino acid mutations during all four waves of the pandemic in Ukraine.

As seen from Figure 3b, the mutational fitness of the SARS-CoV-2 variants in Ukraine, expressed as a percentage, reaches up to 100% rate at the peak of the circulation of a certain variant. At the same time, a constant increase in the mutational fitness coefficient was observed from 0.07 at the beginning of the pandemic in March 2020 to 1.37 in June 2022, which indicates the progressive evolution of this pathogen. And considering the extremely high intensity of the epidemic process caused by this virus, it is obviously actively adapting to the human population and taking root.

Functional SARS-CoV-2 S protein mutations were also analyzed over time. While minimal S protein mutations were observed in the first two waves, the third, and especially the fourth, waves showed an increase in the number of protein mutations compared to the reference genome (Figure 4).

3.3. Cross-Border Movement of SARS-CoV-2 Variants between Ukraine and Poland in Period of Military Invasion

The military invasion of troops from the Russian Federation into Ukraine on 24 February 2022 caused an immense number of refugees to cross the borders of neighboring countries. In the context of the COVID-19 pandemic, this resulted in a unique epidemic situation for study. We attempted to estimate the spread of SARS-CoV-2 variants between Ukraine and Poland after the start of the full-scale war. An important obstacle is that the

number of viruses sequenced in Ukraine was significantly lower than in Poland (67 and 18,747, respectively). We tried to assess the possibility of using information on mutations that were detected in the virus during the period of intensive movement of refugees. At the beginning of the war, the same subvariants of SARS-CoV-2 (BA.1, BA.2, with a predominance of the latter; Figure 5) were detected in Ukraine and Poland. In this case, more rare and unique mutations than those that characterize the known variants of the virus may be considered as a possible tool for tracing the flows of spreading.

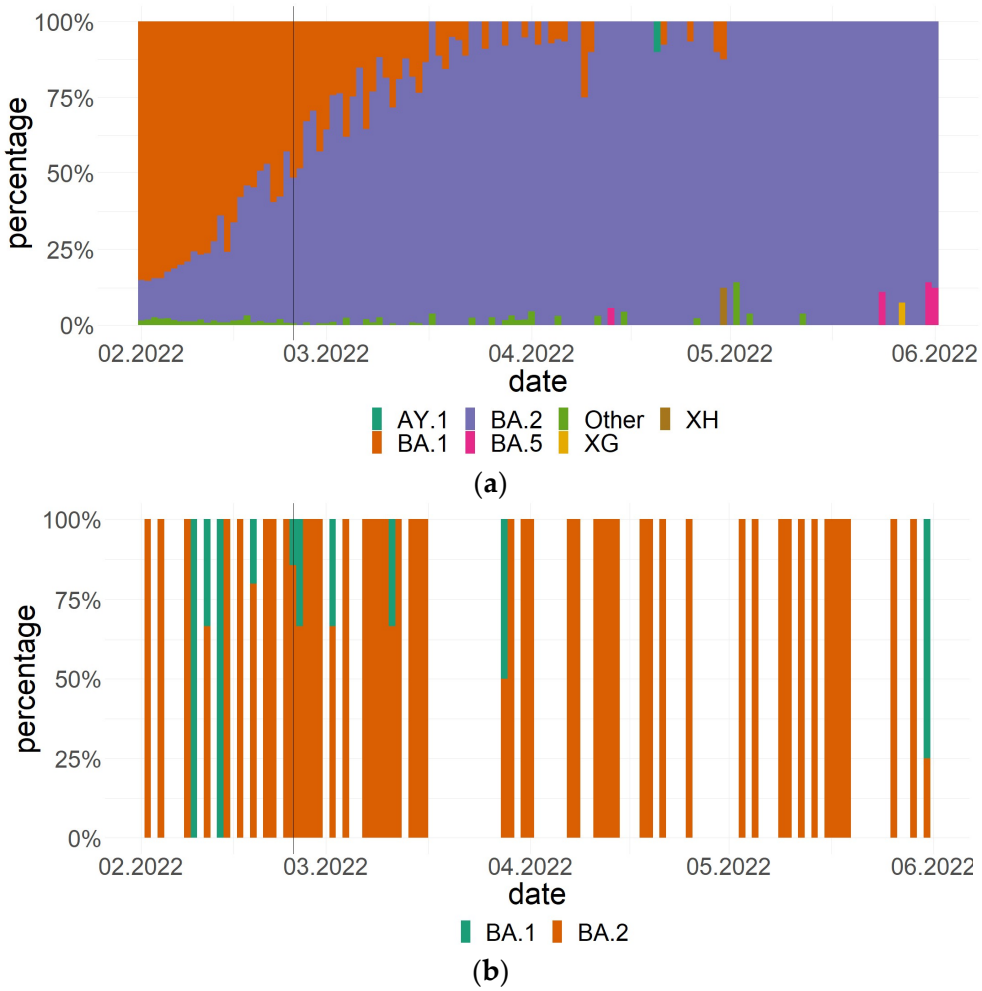


Figure 5. Percentage distribution of the prevalence of virus variants in Poland (**a**) and Ukraine (**b**) in the period from February to June 2022. The black line indicates the date of the start of the full-scale war (24 February 2022).

The analysis revealed that a large proportion of sequences from Poland and Ukraine shared common nucleotide mutations. Over the time period analyzed, SARS-CoV-2 Omicron subvariants that were detected in both countries were similar. Mutations were selected that (1) occurred at the nucleotide level, (2) occurred at <10% in both Ukraine and Poland data sets, (3) were present in both Ukraine and Poland data sets, and (4) occurred more than one time in the Ukraine data set. This selection resulted in a total of 31 mutations selected (Supplementary S6). However, a small number of samples did show uncommon and unique mutations, including C8991T, C10507T, C25317T, C7772T, C5274T, C9430T, C21595T, C26571T, and C5284T (Figure 6).

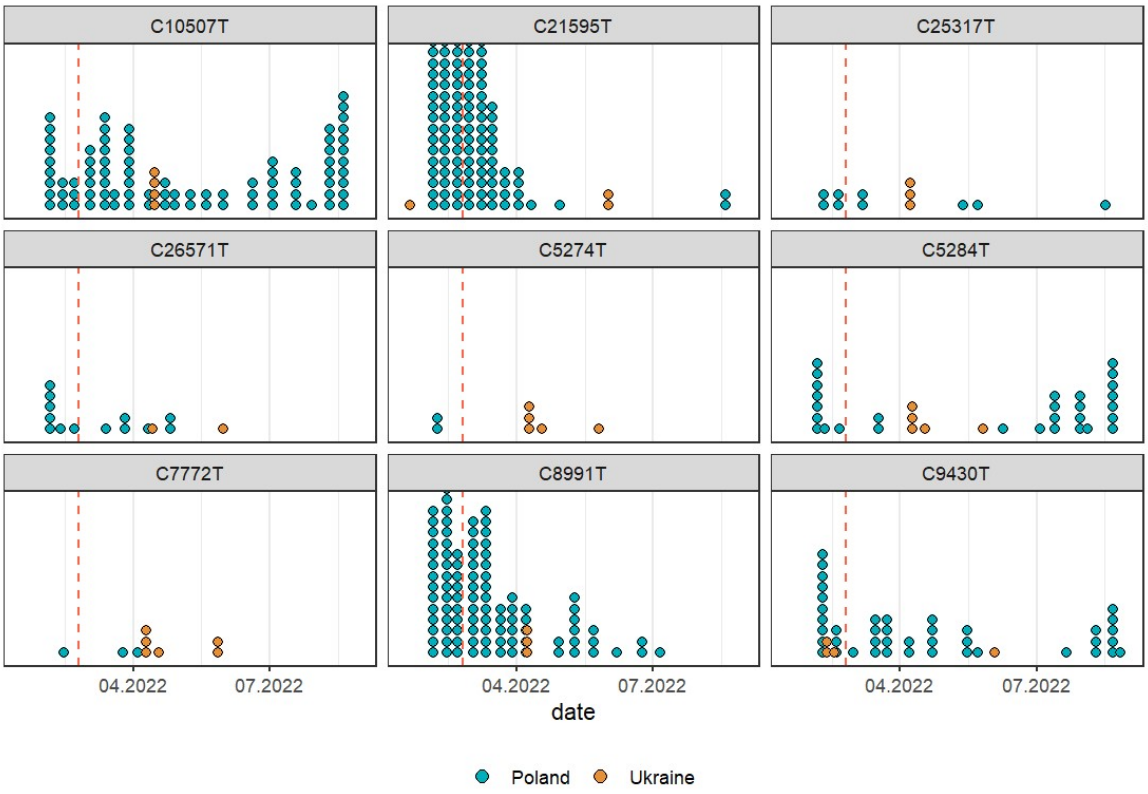


Figure 6. Dynamics of detection of several mutations in SARS-CoV-2 in Ukraine and Poland over time. The red dashed line indicates the date of the start of full-scale war (24 February 2023).

4. Discussion

The active adoption of next-generation sequencing as an important tool for epidemiologic surveillance was a new experience for Ukraine. There were a limited number of instruments, people, and institutions that were involved in NGS monitoring of SARS-CoV-2 variants during the pandemic. Despite the small number of sequenced samples, the surveillance was adequate, it can be trusted, and it clearly reflected the situation in Ukraine.

Next-generation sequencing was a valuable tool for understanding the etiology of these waves and for comprehending the virus's dynamics and the impact of various factors on the pandemic. During the early stage of the pandemic in Ukraine, prior to the quarantine being instituted (17 March 2020), COVID-19 cases were detected in people with recent international travel and those who had contact with infected patients. On 17 March 2020, Ukraine initiated unprecedented strict quarantine measures during the COVID-19 pandemic, including flight bans, travel restrictions, and other quarantine measures. It is known that quarantine and social distancing also had another influence on the health of people in the world, especially their mental health, and the availability of medical care through the repurposing of medical institutions for COVID-19 pandemic purposes [21–23]. These drastic measures reduced or ceased population movement and had a significant impact on the spread of SARS-CoV-2 in Ukraine. The other consequence of quarantine was the postponed peak of incidence that appeared only in November of 2020. These measures likely contributed to the fact that only four SARS-CoV-2 main variants were found in Ukraine: the initial COVID-19 strain (Wuhan), Alpha, Delta, and Omicron.

Throughout the development of the COVID-19 pandemic in Ukraine, we observed changes in viruses that occurred due to the accumulation of mutations in their genome. Today, several main variants of SARS-CoV-2 are known, in particular: Alpha (B.1.1.7), the first variant of concern described in the United Kingdom (UK) in late December 2020; Beta (B.1.351), first reported in South Africa in December 2020; Gamma (P.1), first reported in Brazil in early January 2021; Delta (B.1.617.2), first reported in India in December 2020;

and Omicron (B.1.1.529), first reported in South Africa in November 2021 [24,25]. Beta and Gamma variants of SARS-CoV-2 did not circulate in Ukraine. The variants Epsilon, Eta, Iota, Kappa, Lambda, and Mu, which are less widespread all over the world [26], were not presented in Ukraine. After the Omicron variant appeared in the world, one of its subvariants steadfastly changed others [27].

Moreover, the emergence of new pathogen variants in the country was associated with their introduction from other countries and not with their formation within our country. The majority of mutations that occur in the viral genome do not lead to changes in its properties, but there are also the most successful variants that are able to spread massively in the human population, which are usually characterized by a certain set of mutations that is characteristic of one or another virus variant [28].

On 24 February 2022, the Russian Federation began a full-scale invasion of Ukraine. This led to numerous tragic and catastrophic upheavals in Ukrainian life, including significant and unprecedented migration within the country and abroad. The airspace of Ukraine has been closed for civilian airspace users [29]. As a result, the states bordering Ukraine served not only as the key shelters for refugees but also played the role of a transit zone when the destination was a country that does not share a common border with Ukraine. The number of border crossings from Ukraine to Poland per day reached up to more than 140,000 on the first days of March 2022 [30]. According to the UN Refugee Agency [31], the number of entries of Ukrainian refugees as of 23 April 2022 was 2,899,713 in Poland, 433,214 in the Republic of Moldova, 774,094 in Romania, 489,754 in Hungary, 354,329 in Slovakia, and 602,339 in other countries of the region.

Thus, Poland became the country with the biggest number of border crossings and overall number of refugees. Such significant migration was expected to cause challenges for the healthcare system in Poland, especially in the context of the ongoing COVID-19 pandemic [32,33].

According to the data on crossing the border from the Ukrainian side to Poland, the most active refugee movement was in the period between the beginning of the war and the end of March 2022. Before the war, in Poland and in Ukraine, there was a tendency toward a decreasing number of new cases of the coronavirus infection. But because of this sudden refugee situation, we can expect the slowing down of this trend in Poland because of the possible importation of new cases with refugees from Ukraine, among whom there was a certain number of infected people. Moreover, the movement itself was often accompanied by a large crowd of people, which probably increased the reproduction number and strengthened the effect of the influx of refugees on the situation with COVID-19 in Poland. Estimating this dependence reliably is a difficult task due to the lack of necessary data.

In both countries, the situation with the circulating SARS-CoV-2 variants was very similar before the military invasion (Figure 5). That is why we used rare mutations as an instrument to determine the spread of the virus between countries. Rare mutations, which often do not give the virus any evolutionary advantages, may not accumulate in the virus population and can quickly disappear after their emergence. This complicates the application of these mutations for monitoring the geographic spread of the virus, as it is difficult to estimate true spread from independent emergence in different locations. Therefore, in addition to the probability of the very fact of geographical distribution, it is also worth considering the time interval that separates two cases of detection of the same mutation. Obviously, the shorter this interval between the cases, the more probable the connection between them. Therefore, in our study, we tried to determine this criterion, taking as a basis the incubation period of the disease, the time from infection to the possibility of diagnosis, and the period of infectivity of the Omicron variant of SARS-CoV-2 (BA.1 and BA.2).

Thus, the smallest interval between two cases can be when the virus acquires a certain mutation in a particular patient and is transmitted to another human. In essence, this is the time of the infection process itself. However, since in our study, we used sequencing data, which are based on material in which the virus is detected in sufficient quantities, then, in fact, it is not just the time of infection but the period from infection to the possibility

of diagnosis. For Omicron subvariants, this parameter is 3.7 days [34]. Thus, this is the minimal time interval between two directly related cases of mutation detection. If the time interval between the cases is shorter, then these cases are not consecutive but parallel.

The situation of transmission through one carrier should be considered as an additional time criterion. This is when a mutated virus enters a human's body; he or she becomes ill and, at the last moment of transmissibility, passes it on to another person. This means that we should also use the sum of the incubation period and the period of transmissibility. The incubation period for the Omicron variant of SARS-CoV-2 is 3.61 days [35,36]. The generalization of various studies indicates that in the case of the Omicron variant, the highest contagiousness was observed within 5 days after the onset of symptoms, although it could last even longer [37]. In conclusion, the maximum time of stay of the virus in the body of one human before transmission is added to the predetermined minimum time interval between cases (3.7 days) (3.61 + 5 = 8.61 days). Therefore, this criterion is defined as 12.31 days, which, for ease of use, we accepted as 12 days.

These time criteria make it possible to filter out cases more carefully where mutations in different countries occurred sequentially, first in one and then in another country, but due to the large time interval the relationship between their occurrence is difficult to distinguish from just random independent emergence of the same mutations in different countries.

Based on our approach, we can identify situations when mutations first appear in one country and then in another that can be a sign of spreading (Figure 6). But in general, our approach has shown limited applicability. For a more reliable use of such methodology to monitor border crossings of the virus, a larger amount of sequencing data is needed from the countries that we studied. It is important to reconcile these mutational data with many other types of data: properties of viral infection, human movement rates, vaccination rates, etc. Only given many heterogeneous data may it be possible to create reliable models for monitoring and forecasting the development of epidemic situations.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/microbiolres15020065/s1, Supplementary S1: List of Supplementary Files; Supplementary S2: Metadata sequenced samples collected by authors of this article; Supplementary S3: Metadata of sequenced samples from Ukraine used for bioinformatics analysis; Supplementary S4: Metadata of Ukraine samples used for mutation analysis of refugee situation; Supplementary S5: Metadata of sequenced samples from Poland used for mutation analysis of refugee situation; Supplementary S6: List of mutations selected for mutation analysis of refugee situation.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Sequencing data are available on GISAID (https://gisaid.org/ (accessed on 15 September 2023)). Information about exact sequences used in different parts of this research is provided in Supplementary Materials.

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