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Professional paper

MOLECULAR DIAGNOSTICS OF COVID-19 IN SELECTION REGIONS IN NORTH MACEDONIA

Nadica RITOVKA[1], Mije REÇI[2], Sheval F MEMISHI[2], Ismail FERATI[3] Vlatko TANEVSKI[4]

[1]*Institute of Microbiology and Parasitology, Saint Cyril and Methodius University, Faculty of Medicine, Skopje, NMK*
[2]*Department of Biology, Faculty of Natural Sciences and Mathematics, University of Tetova-Tetovo, NMK*
[3]*Department of Food, Faculty of Food Technology and Nutrition, University of Tetova, NMK*
[4]*VT diet, Bitola, NMK*
[*]*Corresponding Author: e-mail: mije.reci@unite.edu.mk*

Abstract

The purpose of this study was to assess the incidence and dynamics of positive cases with SARS-CoV-2 during the period from 01.07.2021 to 31.06.2022 at the level of some sampling points in North Macedonia**.** The laboratory analyses were carried out at the Institute of Microbiology and Parasitology, Faculty of Medicine in Skopje. In total, 33,095 samples were processed from patients with acute respiratory symptoms, taken from the points in Skopje (Čair and Čento), as well as from the Public Health Centers in Ohrid-Debar, Strumica, and Štip-Probištip. RNA extraction was performed with the abGenix kit, while detection of the SARS-CoV-2 virus was performed with the RT-PCR method, using the SARS-CoV-2/SARS-CoV Multiplex REAL-TIME PCR kit. The analysis showed a significant fluctuation in the number of positive samples, with distinct peaks in September-October 2021 and January 2022, which corresponded to the waves of the pandemic and the circulation of new variants, including Omicron. The number of tests and positive cases showed a general upward trend, although with monthly variations influenced by epidemiological and seasonal factors. During the analyzed period, distinct waves of SARS-CoV-2 infection were observed, characterized by different rates of spread that appear to be related to different epidemiological factors, including the circulation of viral variants and the level of immunization in the population. Preventive measures and the level of vaccination have played an important role in controlling the spread of the virus in certain periods. Even though the pandemic has ended, maintaining capacities for continuous laboratory monitoring with molecular techniques remains important for rapid response to future infectious threats. It is also recommended to follow the circulation of new variants of respiratory viruses and dynamically adapt public health measures in accordance with epidemiological trends.
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Keywords: Coronavirus, SARS-CoV-2, COVID-19, Pandemic, Molecular diagnostic, Infection.

1. Introduction

Coronaviruses are a group of enveloped, positive-sense, non-segmented RNA viruses that belong to the family *Coronaviridae*—a monophyletic cluster within the order *Nidovirales*—and infect three classes of vertebrates: mammals (coronaviruses and toroviruses), birds (coronaviruses), and fish (bafiniviruses) (International Committee on Taxonomy of Viruses [ICTV], 2012). This group includes several important pathogens, including SARS-CoV (Severe Acute Respiratory Syndrome), MERS-CoV (Middle East Severe Acute Respiratory Syndrome), and SARS-CoV-2—the virus responsible for the global COVID-19 pandemic (Zhu *et al*, 2020; Lu & Zhang, 2020).

SARS-CoV-2 was first identified in late 2019 in Wuhan, China, and erupted into a global pandemic that has profoundly affected public health, economy, and social life (Wu & McGoogan, 2020). This virus has an average incubation period of 4 to 5 days; however, it can vary from 2 to 14 days (Lauer *et al*, 2020). Clinical manifestations range from mild forms with fever, cough, fatigue, and difficulty breathing, to severe conditions involving respiratory failure and death (Guan *et al*, 2020).

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In the fight against the pandemic, molecular diagnostic methods have played a crucial role. Reverse transcriptase polymerase chain reaction (RT-PCR) is the standard method for the detection of SARS-CoV-2 RNA in respiratory samples (Corman *et al*, 2020). This method has helped confirm cases, isolate infected individuals, and track the spread of the virus in real time. In North Macedonia, efforts to control the pandemic have involved the use of RT-PCR tests in various public and private laboratories. However, there have been variations in the reporting of positive results, raising concerns about the accuracy of the tests (Balkan Insight, 2022). Peshnachka et al. (2022) evidenced the importance of molecular technology for the detection of SARS-CoV-2 and its variants, as well as for epidemiological surveillance. Also, previous studies have shown that the sensitivity and specificity of RT-PCR tests can be affected by technical factors such as sample quality and RNA extraction protocols (Popova *et al*, 2021).
Considering these challenges and the importance of the molecular approach in the control of the pandemic, this study aimed to analyze the results of RT-PCR tests for SARS-CoV-2 performed in several regions of North Macedonia during the period from April 1, 2021 to August 31, 2022. The objectives of the study include: monthly analysis of the samples taken and processed, calculation of the percentage of positive results by month, and identification of time intervals with significant changes (increase or decrease) in the percentage of positive results in relation to the total number of processed samples during the research period. This analysis aims to provide a valuable overview of the state of molecular diagnostics at the regional level during the pandemic in the country.

2. Materials and methods

2.1 Material: The material used in this study consisted of nasopharyngeal and oropharyngeal swab samples from patients with acute respiratory symptoms, of various ages and genders. The samples were collected from collection points in Skopje (Čair and Čento), as well as from Public Health Centers in Ohrid-Debar, Strumica, and Shtip-Probištip, during the period from 01.04.2021 to 31.04.2022 (13 months).
All samples were transported according to the guidelines of the World Health Organization and were processed at the Faculty of Medicine, Institute of Microbiology with Parasitology, Skopje, in the laboratory for molecular diagnostics.
2.2 Method: For processing the collected samples, the Reverse Transcription Polymerase Chain Reaction (RT-PCR) method was used. For this method, amplification reagents were used: SARS-CoV-2/SARS-CoV Multiplex PCR REAL-TIME REAL ("DNA-Technology Research & Production" LLC, Russia), which are intended for routine diagnostics and research purposes. For RNA extraction from the samples, an extraction kit was used — ab Genix extraction plate (AITbiotech Pte Ltd, 25 Pandan Crescent, #05-15, TIC Tech Centre, Singapore).
RNA extraction procedure from samples: The abGenix kit for RNA and DNA extraction is used for the rapid extraction of high-quality nucleic acids (DNA and RNA) from serum, plasma, and other liquid samples. The purified product can also be used for various other molecular biology applications. This kit is stable and can be stored for 12 months at room temperature. The extraction process is automated, and the abGenix extractor is used for this purpose.
RNA extraction was performed according to the manufacturer's protocol. The procedure included the following steps:

1. Mixing the reagents in the extraction plate by inversion rotation.
2. Remove the aluminum foil from the surface of the plate.
3. Place 200 µl of the sample into specific wells of the plate, one sample per well.
4. Addition of 10 µl internal control from the amplification kit.
5. Placing the plate in the extractor in the designated position (cut edge at the bottom left).
6. Mounting the extensions for the metal pipettes on the extractor.

7. Selection of the VIR-19 (COVID-19) protocol for viral RNA extraction.
8. After completion of the process, the extracted RNA was carefully transferred to sterile microtubes (Eppendorf) from the respective column.

RT-PCR amplification: For the detection of SARS-CoV-2, the SARS-CoV-2/SARS-CoV Multiplex REAL-TIME PCR kit was used, an in vitro method based on PCR amplification with fluorescence detection. This kit contains four specific probes, each associated with a specific fluorescent dye and with different target genes: The Fam probe (green) identifies SARS-CoV-like coronaviruses; probe Hex (yellow) is used as an internal control; the Rox probe (orange) detects the SARS-CoV-2 coronavirus E gene; while the Cy5 probe (red) is bound to the SARS-CoV-2 N gene.

The PCR mix was prepared with ready-made tubes complete with primers, where 15 µl "mastermix" (including 0.5 µl Taq polymerase and buffer), 20 µl mineral oil to prevent evaporation, and 10 µl extracted RNA were added to each tube. The tubes were closed, centrifuged, and placed in the DT-PRIME RT-PCR device for amplification and analysis.

The fluorescence intensity was measured at each reaction cycle and analyzed with the appropriate software for virus identification and quantification.

3. Results

During the period from April 1, 2021 to April 30, 2022, RT-PCR tests for the detection of SARS-CoV-2 were performed at the Institute of Microbiology and Parasitology at the Faculty of Medicine in Skopje, on samples collected from the testing points in Čair and Čento (Skopje), as well as from the Public Health Centers in Ohrid (including Dibra) and Štip (including Probištip). A total of 36,065 samples were processed for SARS-CoV-2 detection. The monthly number of performed tests is presented in Table 1, and the general testing trend is visualized in Figure 1.

Table 1. Monthly number of RT-PCR tests for SARS-CoV-2, April 2021 – April 2022

Month	**Number of tests**
April 2021	1859
May 2021	1541
June 2021	644
July 2021	425
August 2021	2736
September 2021	3985
October 2021	4912
November 2021	3979
December 2021	3699
January 2022	3777
February 2022	3523
March 2022	3172
April 2022	1813

Total	36,065

From the data presented in Table 1, there was a significant increase in the number of tests between August and October 2021, with the peak observed in October (4,912 tests). This increase coincides with a major wave of infections during that period. The testing activity remained relatively high until March 2022, reflecting a continued need for diagnostics, influenced by circulating viral variants and public health interventions. In contrast, the periods with the lowest testing volumes (June–July 2021 and April 2022) correspond to epidemiologically calmer phases with lower virus circulation.

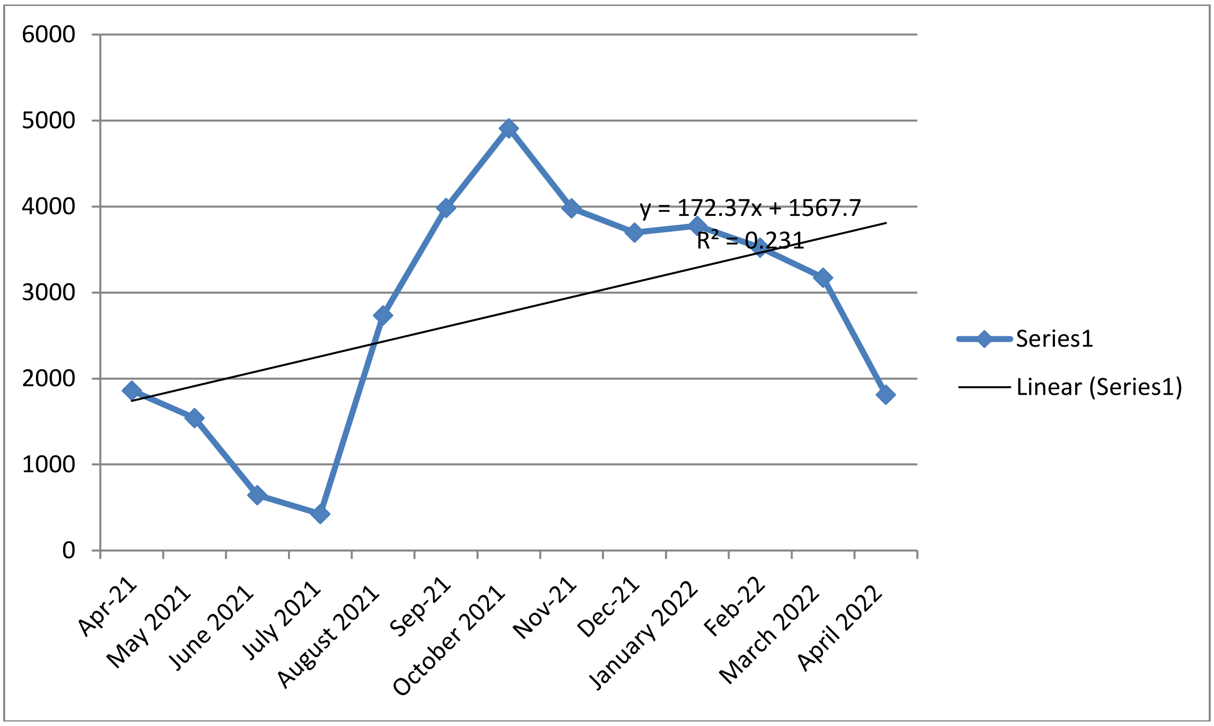

Figure 1. Monthly trend of RT-PCR testing for SARS-CoV-2, April 2021 – April 2022

Figure 1 presents the linear trend line of monthly testing for COVID-19 during the period April 2021 – April 2022. The equation of the trend line is $y = 103.05x + 379$, while the coefficient of fit $R^2 = 0.2974$, suggesting an overall trend of increasing testing, but with weak correlation over time.

This means that, although there is a slight upward trend in the number of tests, monthly changes are dependent on other factors such as the increase in new cases, seasonal changes, testing strategies, and epidemiological measures.

In conclusion, Figure 1 shows an overall increasing trend in testing during the analyzed period, but the intensity of testing has varied significantly from month to month, reflecting the dynamics of the pandemic and the demand for diagnostics in certain periods.

In Table 2, the monthly numbers of positive samples identified during this period are presented.

Table 2. Monthly number of positive RT-PCR results and positivity rate for COVID-19 (April 2021 – April 2022)

Month	**Positive cases**	**number of tests**	**positivity rate (%)**
April 2021	709	1859	38.14
May 2021	301	1541	19.53
June 2021	31	644	4.81
July 2021	43	425	10.12
August 2021	1368	2736	50.00
September 2021	1547	3985	38.82
October 2021	1339	4912	27.26
November 2021	1446	3979	36.33
December 2021	1250	3699	33.80
January 2022	2284	3777	60.48
February 2022	2190	3523	62.17
March 2022	1289	3172	40.65
April 2022	508	1813	28.01
Total	**14,305**	**36,065**	—

Note: Positivity rate is calculated as the percentage of positive cases relative to the number of tests for each month. It reflects the incidence trend and testing intensity in the respective periods. The data in Table 2 show that the number of positive samples for SARS-CoV-2 has had a noticeable fluctuation during the period analyzed. The months of June and July 2021 mark very low levels of positive cases (31–43 cases), which coincides with a quiet epidemiological phase. The number of cases began to rise sharply from August 2021, with peaks in September (1547) and October (1339), reflecting a new wave of infection. Another major peak in cases was recorded in January 2022, with 2,284 positive cases, which may be linked to the circulation of the Omicron variant of SARS-CoV-2. Although we do not possess molecular data or sequencing results from our own samples, national and international reports indicate that Omicron was the dominant variant in Albania and the surrounding region during this period. According to a report by *Telegrafi*, the Omicron variant was present in Albania at the end of 2021 and the beginning of 2022 (Telegrafi, 2022). Following this peak, the number of positive cases shows a steady declining trend, with a notable drop in April 2022 (508 cases).

The monthly data for the number of positive samples for SARS-CoV-2 in Table 2 are presented in Figure 2. Figure 2 shows the general linear trend of these positive cases during the analyzed period.

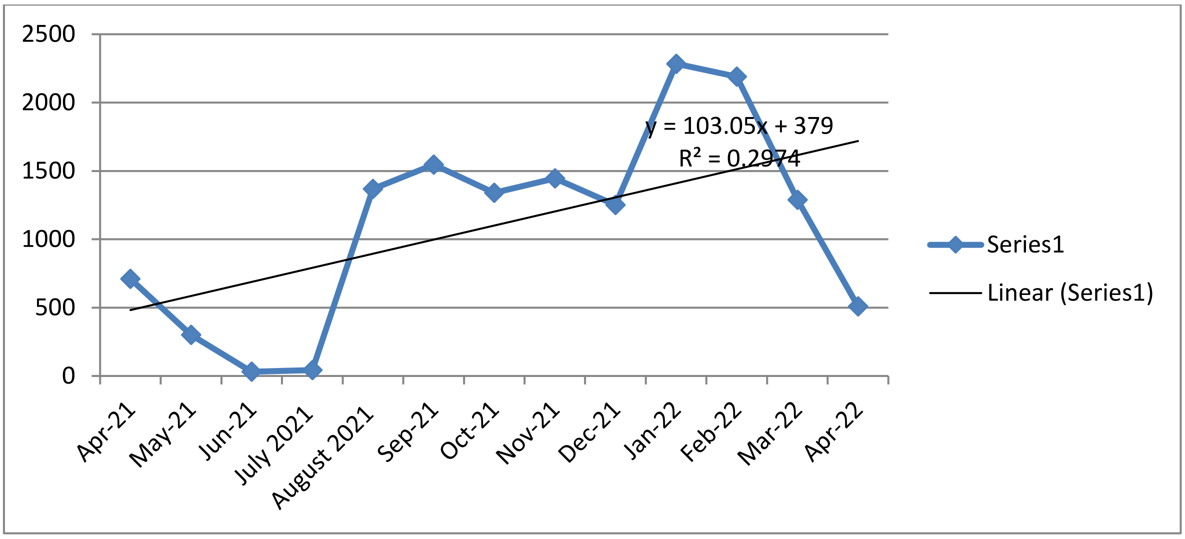

Figure 2. Monthly number of COVID-19 positive cases detected by RT-PCR at the Institute of Microbiology and Parasitology, April 2021 – April 2022

Figure 2 illustrates the monthly distribution of COVID-19 positive cases confirmed by RT-PCR at the Institute of Microbiology and Parasitology over the period from April 2021 to April 2022. The linear trend line displayed in the figure (y = 103.05x + 379) indicates a gradual upward trend, suggesting an average monthly increase of approximately 103 positive cases. However, the coefficient of determination ($R^2 = 0.2974$) reveals a weak correlation between time and the number of positive cases. This suggests that only about 30% of the variation in case numbers can be explained by the passage of time, highlighting the role of additional influencing factors such as the emergence of new virus variants, seasonal effects, public health measures, and vaccination coverage within the population.

The figure also shows some pronounced peaks of infection, especially during the months of September-October 2021 and January-February 2022, which coincide with the largest waves of infections both nationally and globally. Meanwhile, summer months (June–July 2021) and April 2022 represent quieter periods of the pandemic, with a lower number of positive cases.

In summary, although the overall trend from April 2021 to April 2022 shows a gradual increase in positive cases, the rise is not steady. Instead, it is shaped by external epidemiological factors. The pronounced peaks in autumn 2021 and winter 2022 reflect clear waves of infection, while the lower-case numbers during certain months suggest periods of improved virus control.

To assess the monthly trends in the number of positive cases for COVID-19 detected through the RT-PCR method, the analysis of dynamics indices was applied according to two comparative approaches: (1) with a stable basis, where monthly data are reported in relation to the reference month - April 2021, and (2) with a variable basis (chain index), in which each month is compared with the previous month. These indices allow for a more detailed assessment of the rate of increase or decrease in positive cases over the studied period. The data are presented in Table 3.

Tabela 3. Monthly dynamics indices of positive COVID-19 cases, April 2021 – April 2022

Month	**Number of positive samples**	**Stable-base index (%)**	**Variable-base index (%)**
April 2021	709	57%	/
May 2021	301	24%	42%
June 2021	31	2%	10%
July 2021	43	3%	139%
August 2021	1368	109%	3181%
September 2021	1547	124%	113%
October 2021	1339	107%	87%
November 2021	1446	116%	108%
December 2021	1250	100%	86%
January 2022	2284	183%	183%
February 2022	2190	175%	96%
March 2022	1289	103%	59%
April 2022	508	41%	39%

The results of Table 3 show that the highest peak of the dynamics index with a stable base was recorded in January 2022 (183%), compared to the base month (December 2021, 100%). This implies a new wave of infections after a stable period. The lowest value was registered in June 2021 (only 2%), compared to the base month of December 2021 (100%).

Regarding the variable-based index, the highest increase was recorded in August 2021, when the index reached 3181% compared to July of the same year, a month characterized by very low incidence (only 43 cases, or approximately 139%). This sudden surge reflects the onset of a new epidemiological wave following a relatively calm period.

In conclusion, the dynamic indices clearly reflect the fluctuations in the course of the pandemic. August 2021 and January 2022 represent peaks in infection rates, while June 2021 and April 2022 are characterized by a low intensity of virus transmission.

To better understand the rate of change in positive COVID-19 cases, the monthly growth and decline between successive periods has been analyzed. The corresponding data are presented in Table 4.

Table 4. Rate of increase and decrease of positive COVID-19 cases in two-month periods, 2021–2022

Period	**Number of cases**	**Type of change**	**Rate (%)**
May – June 2021	301 → 31	Decrease	90%
July – August 2021	43→ 1368	Increase	3081%
December – January 2022	1250 →2284	Increase	83%
February – March 2022	2190 →1289	Decrease	41%
March – April 2022	1289 →508	Decrease	61%

The data presented in Table 4 reflect the most pronounced rates of increase and decrease in positive COVID-19 cases during the study period. The largest increase was recorded from July to August 2021, with a 3081% increase, corresponding to the onset of a new wave of virus transmission. Conversely, the most significant decline occurred between May and June 2021, with a 90% decrease, following a relatively calm phase after the first wave of infections.

The significant increase in cases from December 2021 to January 2022 (83%) coincides with the winter season and the reported emergence of the Omicron variant at the national level (Telegrafi, 2022).

In conclusion, the dynamic changes in positive COVID-19 cases during the analyzed periods clearly illustrate the progression of the pandemic's epidemiological waves. These fluctuations are influenced by several factors, including seasonality, the emergence of new variants such as Omicron, and public health measures implemented at different times. Due to the lack of direct sequencing data in this study, these associations should be interpreted as informed contextual inferences based on available external reports.

4. Discussion

This study analyzed the results of RT-PCR tests for SARS-CoV-2 conducted from April 2021 to April 2022, involving a total of 33,095 samples processed at the Institute of Microbiology and Parasitology in Skopje and several regional laboratories in North Macedonia. The data revealed significant fluctuations in the percentage of positive cases during this period, with notable peaks in September–October 2021 and January 2022, while the summer and spring months showed a marked reduction in virus spread.

This pattern of infection is consistent with other national and regional reports, as previously noted by Kiprijanovska et al. (2023) and Sredkova et al. (2024), who highlighted the impact of viral variants and seasonal factors on infection dynamics in the Balkan region. The January 2022 peak coincides with the spread of the Omicron variant, which is characterized by very high transmissibility but lower morbidity and hospitalization rates. These findings align with global and regional literature, emphasizing that the Omicron variant led to a sharp rise in infection numbers but with milder clinical manifestations (Guan et al., 2020; Kiprijanovska et al., 2023). This is also supported by studies from Saito et al. (2022), which demonstrate that Omicron has higher transmissibility but lower hospitalization rates.

The analysis of the linear trend showed a gradual increase in the number of tests and positive cases, but with a low correlation coefficient ($R^2 = 0.2974$), suggesting that external factors such as public health measures, seasonality, and vaccination coverage had a greater impact on incidence variation than the number of tests alone. According to ECDC data, North Macedonia had relatively low vaccination coverage during the study period, with approximately 40–45% of the population fully vaccinated by early 2022, which may have contributed to the observed fluctuations in infection rates (ECDC, 2022). These findings are consistent with the observations of Peshnachka et al. (2022), who emphasized that the effectiveness of diagnostic methods and sampling protocols is key to the accuracy and duration of virus detection.

The monthly dynamics of positive cases identified August 2021 and January 2022 as periods with the highest transmission, while June 2021 and April 2022 marked quieter phases. These trends are comparable to those reported in regional and international literature (Sredkova et al., 2024; Dimzova et al., 2022) and indicate the significant impact of emerging variants and environmental conditions on periodic outbreaks (He et al., 2020).

When evaluating the limitations of this study, it should be noted that the results depend on the quality and representativeness of the collected samples, as well as the testing policies and capacities during the research period. The lack of detailed clinical data limits the ability to correlate laboratory findings with clinical manifestations, while the absence of genomic sequencing to identify specific variants restricts a full interpretation of the factors driving epidemiological fluctuations. This is in line with the findings of Li et al. (2021), who stress the importance of genomic surveillance in understanding the spread and behavior of new viral variants.

In future research, it is recommended that similar studies incorporate molecular components for variant identification and integrate clinical and epidemiological data to allow for a more comprehensive assessment of transmission patterns. Furthermore, analyzing the impact of

vaccination and naturally acquired immunity at the population level would enhance the understanding of different phases of the pandemic (Dimzova et al., 2022).
Additionally, future studies should explore the role of socioeconomic factors in the dynamics of virus transmission and control, as emphasized by Ahmed et al. (2023).
In conclusion, this study provides valuable insight into the transmission dynamics of SARS-CoV-2 in North Macedonia during a critical phase of the pandemic and contributes to the development of a data-driven foundation to support planning and response to future public health challenges.

5. Conclusions

During the 12 months analyzed (April 2021 – April 2022), more than 33,000 samples were tested for COVID-19 at the Institute of Microbiology and Parasitology, Faculty of Medicine in Skopje, using RT-PCR on material collected from various locations across the country. The collected data demonstrated a marked dynamic in the spread of SARS-CoV-2, with evident fluctuations in both the number of positive cases and the monthly testing volume.
Statistical and visual analyses revealed several clear peaks in infection during this period—most notably from August to October 2021 and in January 2022—which corresponded with known national and global epidemiological waves. These peaks reflected the influence of factors such as the circulation of new viral variants (e.g., Delta and Omicron), seasonal patterns, and changes in public health policies.
The assessment of the dynamic indices with stable and variable bases highlighted particularly sharp changes, including a 90% decrease in June 2021 and a dramatic 3081% increase in August 2021. These shifts reflected interruptions and re-emergence in infection cycles, underscoring that the presence of the virus in the community was more strongly influenced by external factors (such as control measures and variant spread) than by the simple passage of time—an interpretation supported by low model fit coefficients in the trend analysis.
Testing also followed a general upward trend during months of heightened epidemiological activity, although with considerable variability. This variability reflected the immediate demand for diagnostic capacity during critical phases. The highest number of tests was recorded in October 2021, while the months with the lowest testing volume coincided with quieter phases of the pandemic.
In conclusion, the results of this analysis demonstrated that the spread of SARS-CoV-2 during the study period followed a pattern of recurring waves, characterized by rapid increases and more gradual declines in case numbers. These waves were strongly influenced by factors such as seasonality, viral genetic changes, the timing of public health interventions, and vaccination coverage. Continuous epidemiological surveillance and adaptive public health strategies proved essential for effectively managing the pandemic and remain critical for responding to future public health threats.

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