



## PCR TESTING FOR SARS-COV-2 AMONG STUDENTS OF MEDICAL UNIVERSITY-PLEVEN DURING THE FOURTH WAVE OF COVID PANDEMIC

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### ABSTRACT:

**The aim** of the study was to determine the spread of SARS-CoV-2 among students of Medical University-Pleven during the fourth wave of the COVID pandemic by PCR testing and to analyze epidemiological data.

**Material and methods:** Cross-sectional epidemiological study was conducted for the period November-December 2022. A sociological method of data collection (interview) was used, and a questionnaire was developed for this purpose. Nasopharyngeal swab was taken from the students and RT-PCR analysis of SARS-CoV-2 was performed.

**Results:** An interview was conducted with 200 students from various specialties at Medical University-Pleven. Clinical symptoms were reported by 24 (12%) of respondents, the leading ones being runny nose and cough; with comorbid conditions were 10 participants (5%) with predominant chronic lung diseases. Three students reported contact with coronavirus-positive patients. Six of the participants had travelled abroad in the past 14 days. Eighty-four students (42%) were immunized: 25 with one dose, 43 with two doses and 16 with three doses. Only two tested samples (1%) were positive for SARS-CoV-2. Both subjects reported complaints at the sampling time and were unvaccinated.

**Conclusion:** The results of the study show a low percentage of infected, mostly non-immunized persons, which coincides with the trend in Bulgaria during the fourth wave of the COVID pandemic.

**Keywords:** COVID-19, PCR testing, students,

### INTRODUCTION

The COVID-19 pandemic caused significant problems in different fields of human society. The necessity for adaptation according to the worsened reality affected even stable sectors such as healthcare and education [1]. Medical universities and university hospitals restricted their activities – because of the high risk of infection. The restrictive measures had a negative impact on the quality of the medical education. The professional readiness of the medical students worsened, and their training often was prolonged [2, 3]. Clinical teaching is a specific field of medical education. There is a higher risk of infection and requirements for strict protective standards for which the medical students must be well trained. At the same time, the medical students as a part of the population also were involved in the epidemiological process for transmission of the infection. There are few studies on the prevalence of COVID-19 among medical students [1-4, 5]. Although, during the pandemic, new variants of SARS-CoV-2 have appeared and the severity of infection decreased, the high contagiousness of the virus and the increased risk for working medical professionals, including students training in medical specialties, still remain. Based on these concerns, we aimed to determine the spread of SARS-CoV-2 among students of Medical University-Pleven during the fourth wave of the COVID pandemic by PCR testing and to analyze epidemiological data.

### MATERIALS AND METHODS

#### Data collection

A cross-sectional epidemiological study was conducted for the period November-December 2022. Students from all specialties of the Medical University-Pleven were invited to participate in the study according to the preliminary prepared schedule. A sociological method of data collection (interview) was used, and a questionnaire was developed for this purpose. The questionnaire was in accordance with SARS-CoV-2 guidelines by the National

Center for Infectious and Parasitic Diseases and included questions in four areas: personal information (age, sex, specialty), current clinical symptoms (fourteen symptoms were listed to choose), risk factors (comorbid conditions), epidemiological data (data on contact with COVID-19 patient, residence abroad, recurrence of COVID-19 infection, tests used to confirm the diagnosis, vaccination status). Clinical and epidemiological data were obtained for each student. In this study, we adhered to ethical guidelines and obtained ethical approval from the Ethical Commission of Medical University - Pleven (Protocol No.725/2022). Prior to participation, written informed consent was obtained from all participants.

### Nasopharyngeal samples

Nasopharyngeal swabs were collected from 200 students at the Medical University of Pleven. Sampling was carried out at the Laboratory of Epidemiology, Parasitology and Tropical Medicine by laboratory technicians who had undergone proper training. After collection, the nasopharyngeal swab specimens were placed in a viral transport medium and kept at +4° C.

### PCR testing

Detection of SARS-CoV-2 was carried out in the Virology Laboratory of the Medical University – Pleven by real-time PCR testing. Viral RNA was extracted using manual isolation (NucleoSpin Dx Virus, Macherey-Nagel,

Germany) or automated extraction (MagCore Viral Nucleic Acid Extraction Kit, RBC Bioscience, Taiwan). RT-PCR analysis of SARS-CoV-2 was performed using the GeneFinder COVID-19 Plus RealAmp Kit (OSANG Healthcare, Korea), which detects the RdRp gene, the E gene, and the N gene. A positive control and a negative control were included in each PCR run to monitor assay performance. An internal control targeting the RNase P gene was used to ensure that nucleic acids were present in each sample. A specimen was considered positive for SARS-CoV-2 if there was amplification for the RdRp, E, and/or N gene, with Ct values not higher than 40.

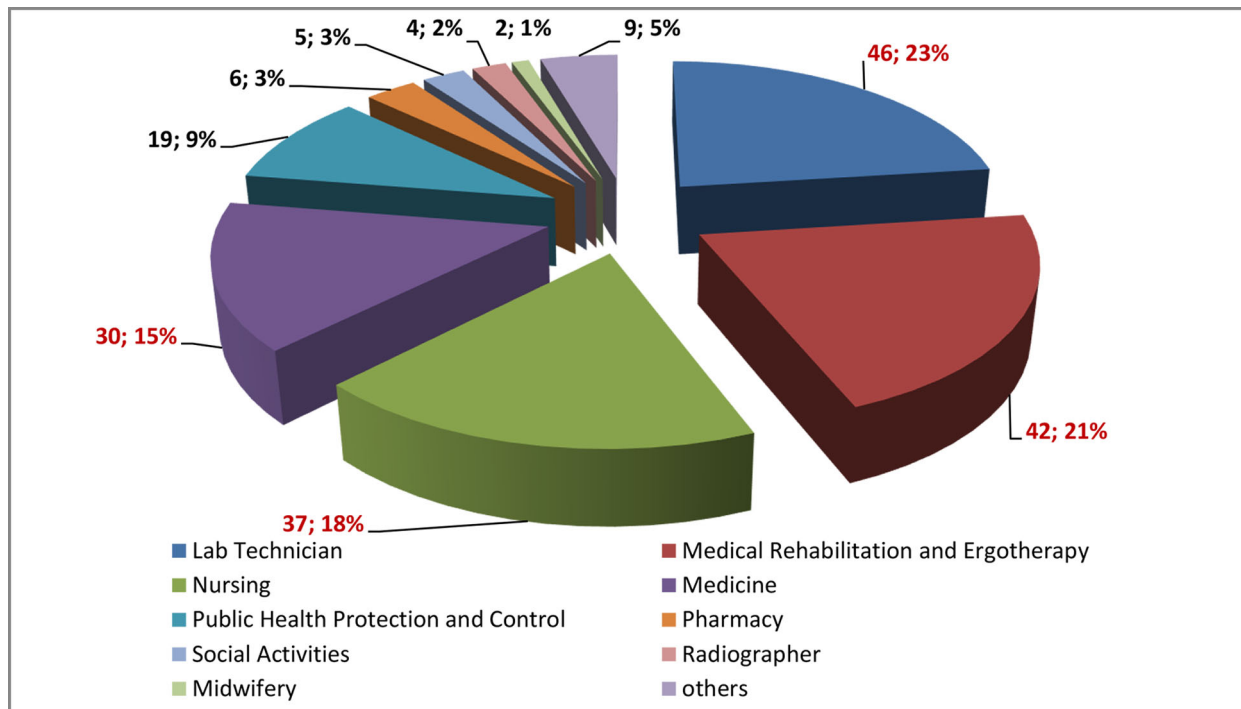
### Statistical analysis

Data were entered into an electronic database (Microsoft Excel v. 2010) and analyzed using statistical software (SPSS Statistics 19.0). The variables were analyzed by t-test and  $\chi^2$  test (for parametric and non-parametric distributions, respectively;  $p < 0.05$  was considered significant).

### RESULTS:

An interview was conducted with 200 students from various specialties at Medical University-Pleven. The highest number of tested students was registered in specialties of Laboratory technician (n=46, 23%), Medical rehabilitation and ergotherapy (n=42, 21%), Nursing (n=37, 18%) and Medicine (n=30, 15%) (fig. 1).

Fig. 1. Structure of the tested participants

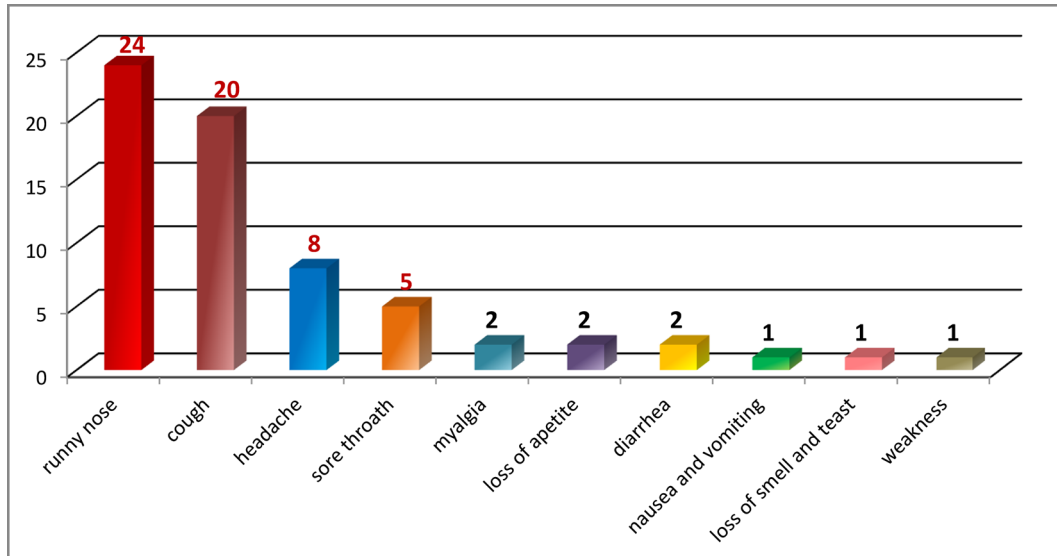


Participants ranged in age from 18 to 58 years, with a median age of 21 years. The distribution by gender shows female dominance – 150 (75%).

Clinical symptoms were reported by 24 (12%) of respondents, the leading ones being runny nose and

cough. Also, students reported headaches, sore throats, muscle aches, loss of appetite, diarrhea, loss of smell, and more (fig. 2). With comorbid conditions were 10 participants (5%) with a predominance of chronic lung diseases (asthma and chronic bronchitis).

**Fig. 2.** Clinical symptoms of the tested participants at the time of sampling



Epidemiological data from the interview are summarized in Table 1.

**Table 1.** Epidemiological data of the tested participants

| Characteristic   | n (%)        |
|--|--------------|
| Contact with a COVID-19-positive patient in the last 14 days | 3/200 (1.5%) |
| Traveling abroad in the last 14 days                         | 6/200 (3%)   |
| Destination (country)  |              |
| Romania  | 2            |
| Turkey   | 2            |
| Hungary  | 1            |
| Egypt  | 1            |
| Previous infection with SARS-COV-2                           | 98/200 (49%) |
| Number of episodes   |              |
| 1 episode  | 70 (35%)     |
| 2 episodes   | 24 (12%)     |
| 3 episodes   | 4 (2%)       |
| Confirmation of diagnosis                                    |              |
| By rapid test  | 55/98 (56%)  |
| By PCR   | 33/98 (34%)  |
| No confirmed   | 10/98 (10%)  |
| Vaccination against SARS-COV-2*                              | 84/200 (42%) |
| Number of doses  |              |
| 1 dose   | 25/84 (30%)  |
| 2 doses  | 43/84 (51%)  |
| 3 doses  | 16/84 (19%)  |

\* Administered vaccines: AstraZeneca, Janssen, BioNTech/Pfizer, Moderna

SARS-CoV-2 was detected in 2 (1%) of tested nasopharyngeal specimens. Both participants with positive PCR test were unvaccinated. They were with COVID-19 infection, twice confirmed by PCR. At the sampling time, a 34-year-old woman from a specialty Laboratory technician reported headache as a single symptom. The other SARS-CoV-2 positive student, a 19-year-old man from the same specialty, reported cough as a single symptom.

## DISCUSSION:

Our cross-sectional epidemiological study aimed to establish the circulation of SARS-CoV-2 during the fourth epidemic wave in the country. The survey was voluntary, and 200 (6.66%) students responded to it out of the 3,000 students. We associate the low interest reported by us with the low morbidity that is registered in this period in the country and the milder course of the disease. On the other hand, some of the students were either immunized or recovered from COVID-19 infection and had the attitude that they were protected from the virus. The fact that during the pandemic, the restrictive measures, the regular testing of personnel in medical and educational institutions had an adverse psychological effect on people should not be underestimated. Visible from the structure of the participants, students from the specialty "Laboratory technician" predominate (23%). We believe that their direct participation in sampling and previously conducted additional training before the start of the study played a role in their motivation. Public Health Protection and Control students also responded, most likely motivated by their professors. Students from the other specialties participated with fewer representatives. Participants ranged in age, with an average of 24 years (median age 21 years). A similar age distribution was mentioned by Wouambo RK et al. [6]. The gender distribution showed female prevalence (150/200; 75%,  $p < 0.0005$ ) and was consistent with data from other studies [7-9]. Multiple studies have shown that females were more sensitive to eventual adverse health consequences of the pandemic, and therefore, they are more likely to comply with preventive measures, including testing for SARS-CoV-2 [10]. Regarding complaints at the time of sampling, students were purposefully questioned about symptoms suggestive of an infectious disease and those specific to COVID-19. The number of people with symptoms is not unusual for the season, as are the reported runny noses and coughs that occur with most respiratory infections. Among the respondents, 17 participants were found to have accompanying diseases, mainly chronic lung diseases.

According to the recommendations of the Regional Committee for Europe to the WHO and the European Infectious Disease Surveillance System (TESSy), and the European Center for Disease Prevention and Control (ECDC), the case classification system is three-level and

includes the following categories of cases: suspected (any person, meeting the clinical criteria), probable (any person meeting the clinical criteria and having an epidemic contact or any person meeting the diagnostic imaging criteria) and confirmed (any person meeting the laboratory criteria: nucleic acid or antigen detection of SARS-CoV-2 in clinical samples) [11, 12]. Considering the clinical criteria of the definition of a case of COVID-19, namely – any person with at least one of the following symptoms: cough; fever; difficulty breathing; sudden onset of anosmia, ageusia, or dysgeusia, at the questionnaire level, we defined as a "suspected case" for the disease in 21 of the participants. Analysis of epidemiologic data showed that only three participants reported contact with a COVID-19 positive in the past 14 days. Of these three, only one had symptoms (cough and headache) on the day of the study. According to the Case Classification System (TESSy), we can conclude that we have one "probable case" of COVID-19 disease in the study sample. Of the students who travelled abroad, only one had a sore throat. Similar to the results of other studies, as well as data on the prevalence of infection, half of the participants had encountered the virus (49%). Most of them had been sick once. Recurrent episodes of past infection among study participants confirm the fact of short-lived immunity after relapse. Rapid tests were used for the diagnosis in 56% of cases, but in 33%, it was confirmed by PCR.

It is known that a means of building herd immunity in the population is its vaccination. This is especially important for the most vulnerable groups who are at high risk of contracting and transmitting the virus. Medical professionals, including those studying at medical universities, are among the target groups for vaccination. Of the participants in our study, 42% reported having been immunized, with the majority of them immunized with two doses. Despite the many questions that have arisen regarding vaccine safety, duration of immunity, and others, students at our university have achieved good immunization coverage, but compared to the students at other European universities, significantly lower [5]. Complete vaccination is essential to achieve a good protective effect.

The present study was completed with a laboratory examination of nasopharyngeal secretions in the Virology laboratory. The two positive samples found, which is 1%, shows a decrease in the circulation of the virus during the fourth epidemic wave. The few similar studies available in the literature in Europe and America show comparable exposure to SARS-CoV-2 [8, 9, 13-15]. This is not in concordance with studies in Asia and Africa, which recorded higher infection rates of 13% and over 20%, respectively [6, 7].

As an exception to the studies cited so far, we should note one by a team of authors from South Korea



at the beginning of the pandemic, in which none of the studied students and staff were found to be infected with SARS-CoV-2 [16]. The positive samples in our study were among students of one specialty, which suggests transmission of the virus through close contact. Also, the involvement of both genders suggests equal susceptibility in men and women, which has been reported in other studies. A positive PCR result for SARS-CoV-2 means infection with the virus but not yet illness [17]. Our students had complaints, and the necessary anti-epidemic measures (treatment, isolation, monitoring) were taken against them. We believe that the leading risk factor is the lack of immunization in both students.

## CONCLUSIONS:

The results of the study show a low percentage of infected, mostly unimmunized persons, which coincides with the trend in the country during the fourth wave of the COVID-19 pandemic. The spread of SARS-CoV-2 depends on the immune status of the population. It is necessary to stimulate the application of booster doses of vaccine to groups at risk. Also, conducting screening tests gives a momentary assessment of the state of the population, for which purpose the application of PCR is the method of choice.

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