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Evaluation of the effectiveness of COVID-19 vaccination campaigns in Romania. A multidimensional analysis of immunization strategies.

SUMMARY OF DOCTORAL THESIS

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INTRODUCTION

Infectious diseases pose a serious threat to the health of millions of people. A greater understanding of the potential impact of infectious diseases could help in the optimal management of disease outbreaks [1]. Since the beginning of the 21st century, three previously unidentified zoonotic coronaviruses have emerged: SARS-CoV (causing Severe Acute Respiratory Syndrome), MERS-CoV (Middle East Respiratory Syndrome coronavirus), and SARS-CoV-2 (previously known as 2019-nCoV), which is responsible for the current COVID-19 pandemic. All three coronaviruses have caused epidemics and pandemics that have significantly impacted society and people worldwide. The SARS outbreak from 2002 to 2004 resulted in over 8,000 cases and 774 deaths, causing widespread anxiety and economic disruptions. MERS, first identified in 2012, had a lower total number of cases compared to SARS, but a much higher mortality rate. The ongoing SARS-CoV-2 outbreak, which began in 2019, has caused a global pandemic, with over 600 million confirmed cases and over 6 million deaths [2, 3].

The objective in the theoretical part of the thesis was to systematize the latest data from the specialized literature regarding the profile and characteristics of the Coronaviridae Family (symptoms, incubation period, reservoir of the host cell, and transmissibility of the coronaviruses that marked the beginning of this century) and the mechanism of action of mRNA vaccines and vector-based vaccines. The objectives associated with personal contributions were as follows: contributing to global and local efforts to control the COVID-19 pandemic by identifying vaccination status, the type of SARS-CoV-2 infection, and post-vaccination adverse events in several target groups of interest in Romania; evaluating the effectiveness of COVID-19 vaccination campaigns during the pandemic among healthcare professionals and the general population in Romania; comparative analysis of vaccination-associated events during the pandemic; exploring non-vaccination decisions; drawing useful conclusions for public health policies and strategies for optimal pandemic management. Thus, the studies conducted in the doctoral thesis aimed at the multidimensional analysis of COVID-19 vaccination-related events among pharmacists, medical staff, and the general population in correlation with the experience of having the disease or other possible medications administered for chronic conditions or co-administration of other vaccines.

The doctoral thesis is structured as follows: a general part (I) in which current knowledge from the scientific world regarding the three coronaviruses that have marked this century and the importance of vaccination to prevent future outbreaks were detailed, and an

experimental part (II) comprising four chapters analyzing the population's perception of COVID-19 vaccination. The thesis concludes with the conclusions and contributions made through the conducted studies.

I. GENERAL PART
1.CORONAVIRIDAE FAMILY

The first chapter includes information about the chronological landmarks for the Coronaviridae family, with a focus on the differences between the three coronaviruses of the 21st century regarding their symptoms, incubation period, reservoirs, and transmissibility. The term "coronavirus" first appeared in 1968, and in 1975, the International Committee on Taxonomy of Viruses established the Coronaviridae family as a member of the order Nidovirales, comprising positive-sense, single-stranded RNA viruses encapsulated [4]. Coronaviruses (CoV) belong to the Coronaviridae family, a family of positive-sense, single-stranded RNA viruses that are usually characterized by an enveloped, spherical particle with a crown-like appearance. The Coronaviridae family is classified into four genera: Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus [5,6].

Approximately 70% of emerging human pathogens originate from animals, and in recent decades, most epidemics and pandemics have been caused by RNA viruses, due to their higher mutation rates and the ability to produce unique genetic changes. The RNA viruses with the greatest impact on humans are CoVs, influenza virus, and the human immunodeficiency virus (HIV) [7].

Until 2002, CoVs were mainly studied for scientific or veterinary purposes, due to the mild symptoms associated with human CoVs. However, the world's perception of CoV virulence changed in 2002 when a zoonotic betacoronavirus called SARS-CoV appeared in southern China and caused a global epidemic with over 8,000 cases and 774 deaths (mortality rate: 9.5%), ending in 2004 [8]. Ten years later, another zoonotic betacoronavirus called MERS-CoV (Middle East Respiratory Syndrome coronavirus) emerged in the Middle East, causing 2,521 cases and 919 deaths (mortality rate: 35%). Using genome sequencing and molecular epidemiology, it was proven that both epidemics were based on the spread of viruses from animals to humans, followed by secondary transmission from human to human.

Severe Acute Respiratory Syndrome (SARS-CoV)

The definition of SARS-CoV has undergone several modifications over the years as new information has been discovered. However, the definition established by the WHO at that time included 2 criteria for establishing a possible diagnosis of SARS-CoV [9]:

- Person who presented after November 1, 2002 with a history of fever over 38 °C, cough or difficulty breathing, with exposure in the last 10 days before the onset of symptoms. Exposure was defined as appropriate contact with SARS-positive or possibly positive person who traveled to an area with possible local transmission of the virus or lives in an area with local transmission;
- Person with unexplained acute respiratory illness leading to death, after Nove,ber 1, 2002, who did not undergo an autopsy but had exposure during the 10 days before onset of symptoms;

In addition to the respiratory tract, SARS-CoV can infect multiple organs and cell types during the course of the disease, including intestinal mucosal cells, renal tubular epithelial cells, neurons, and cells of the lymphoid and reticuloendothelial system. The main clinical symptoms reported for SARS-CoV positive patients include high fever, chills, myalgia, dry cough, headache, general malaise, and diarrhea. Productive cough, sore throat, nausea, and vomiting were less commonly reported among confirmed SARS patients. Approximately 40%-70% of patients reported watery diarrhea about a week after the onset of the disease [9, 10]. Decreased appetite and confusion were predominantly reported among elderly patients, while SARS-CoV infection in children under the age of 12 presented with mild symptoms. Among pregnant women, SARS infection carries a high risk of mortality. No mortality cases were reported in children and adolescents. Asymptomatic infection was not common during the 2003 epidemic [11].

Middle East Respiratory Syndrome (MERS-CoV)

Ten years after the identification of SARS-CoV in China, another zoonotic and highly pathogenic coronavirus was identified in the Middle East (Middle East Respiratory Syndrome Coronavirus - MERS-CoV), marking the beginning of the second epidemic of the last century with a major global impact. In June 2012, the death of a 60-year-old man due to acute pneumonia and renal failure was reported in a hospital in Jeddah, Saudi Arabia [12]. In April 2012, a group of 11 severe respiratory disease cases was reported in a hospital in Jordan, which

was retrospectively diagnosed as MERS [12], followed by three more MERS cases in the United Kingdom in September 2012 [13]. By January 20, 2017, the World Health Organization (WHO) had received a total of 2,521 confirmed cases of MERS reported from 27 countries, with 919 deaths (mortality rate of 35%). Nearly 80% of the cases were linked to exposure in the Middle East [14].

Unlike SARS-CoV, the MERS epidemic had a slower course, lasting almost 4 years, with local outbreaks with a small number of cases identified in different regions of the Arabian Peninsula: 25 cases in Al-Hasa from April 1 to May 23, 2013, 255 cases in Jeddah from January 1 to May 16, 2014, 45 cases in King Fahad, Riyadh from March 29 to May 21, 2014, and 130 cases in King Abulaziz, Riyadh from June to August 2015 [15]. In May 2015, a person traveling from Saudi Arabia to South Korea was responsible for nosocomial transmission of the virus, resulting in an outbreak with 186 confirmed cases in 16 different hospitals [12]. According to the US Centers for Disease Control and Prevention (CDC), from the beginning of 2022 until December 5, 2022, six cases of MERS-CoV have been reported as follows: 3 in Saudi Arabia, 2 in Qatar, and 1 in Oman, including one death. All cases were primary cases, and all except one reported contact with camels [16].

The clinical spectrum of MERS-CoV infection varies from asymptomatic or mild respiratory symptoms to severe acute respiratory illness and death. A typical representation of MERS clinical picture includes: fever, cough, and difficulty breathing. Pneumonia is common, but not all MERS patients develop this condition. Gastrointestinal symptoms, including diarrhea, have also been reported. Severe respiratory illness can lead to respiratory failure requiring mechanical ventilation or care in an intensive care unit. Elderly individuals, those with weakened immune systems, and those with underlying chronic conditions such as kidney disease, cancer, chronic lung disease, hypertension, cardiovascular disease, and diabetes are at higher risk of developing severe disease [17]. The most common abnormalities observed on chest radiographs and computed tomography (CT) scans have been bilateral lung infiltrates, consistent with viral pneumonia and acute respiratory distress syndrome. Common laboratory findings at the time of diagnosis have included low hemoglobin, lymphopenia, thrombocytopenia, elevated aspartate aminotransferase (AST), and elevated lactate dehydrogenase levels [18].

Although MERS-CoV has predominantly infected adults, there has been a reported case of a 9-month-old newborn diagnosed with infantile nephrotic syndrome, leading to severe respiratory symptoms and multiple organ dysfunction until death. From a group of 11 children who tested positive for MERS-CoV, only two were symptomatic, and they had Down syndrome and cystic fibrosis, suggesting that symptomatic disease may occur in children with other comorbidities [18].

COVID-19 pandemic (Severe Acute Respiratory Syndrome 2 - SARS-CoV-2)

After the two epidemics of the 21st century (SARS-CoV and MERS-CoV), scientists emphasized that "it is likely that in the years to come, more CoV members will appear" [19]. Unfortunately, they were right, and a new highly contagious CoV called Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) emerged in 2019, causing the largest pandemic of the modern era known as Coronavirus Disease 2019 (COVID-19). Although it is associated with a lower mortality rate compared to previous CoVs, SARS-CoV-2 has caused 656 million cases and 6.67 million deaths to date, due to its high contagiosity and the ability to be transmitted by asymptomatic and pre-symptomatic individuals.

In December 2019, several cases of an unidentified form of viral pneumonia were reported in the city of Wuhan, Hubei province, China. In the following weeks, the SARS-CoV-2 virus spread rapidly worldwide, and scientists and doctors had no answers or solutions for its transmission or pathology. In March 2020, the WHO officially declared COVID-19 a pandemic, due to the rapid spread of the new CoV globally (over 80,000 cases were reported by February 24, 2020, with 2,700 deaths [20]). Unprecedented measures were taken to slow down the spread, including travel and gathering restrictions, closure of restaurants and shopping complexes, and school closures – the speed with which all these measures were implemented was a decisive factor in reducing the spread of the pandemic, but it had a dramatic economic and psychological impact. COVID-19 brought major challenges during its spread, from isolating the virus, detection, prevention, and treatment to vaccine development.

2. VACCINES, THE BEGINNING OF A NEW ERA IN INFECTIOUS DISEASE APPROACH

Chapter 2 highlights the importance of vaccines for global public health, as one of the most effective ways to save lives and prevent infectious diseases. This chapter is divided into two parts: the first part details the complexity of vaccine manufacturing technologies that have been

available for decades, while the second part focuses on the new manufacturing technologies that enabled the development of COVID-19 vaccines.

As a result of concerted and collaborative efforts by global health organizations, national institutions, rigorous scientific research, and awareness programs regarding the benefits of immunization to the population, the global vaccination rate has improved. Vaccination has become a significant concern for global health, currently representing the most efficient method of immunizing the body. Increasing vaccination rates has become a target for many countries, as statistical data over the years have shown a considerable reduction in the number of preventable deaths through vaccination.

The Complexity of Vaccine Manufacturing Technologies

A vaccine contains an agent that resembles the causative microorganism of the disease, typically made from a live or killed microbial entity, its toxins, or even surface proteins. In the pharmaceutical industry, vaccine manufacturing is one of the processes that presents the most challenges throughout its course until reaching the final product, making it one of the most costly processes [21]. The production time of a vaccine batch can vary from a few months, as in the case of the flu vaccine, to 3 years for combined pentavalent and hexavalent vaccines.

Vaccine manufacturing involves a multi-step process to reach the final product, as follows [22]:

- The first stage consists in obtaining the pathogen or a recombined protein from the pathogen, these proteins can be produced in bacteria, yeast or cell cultures. Viruses are grown on primary cells such as chicken fibroblasts or can be grown on continuous cell lines. This stage represents obtaining the raw material for the future production of vaccines, and a disruption of this process complicates obtaining the final product;

- The second stage continues with the release of the antigen from the substrate and the isolation from he medium used for its growth, followed by the purification of the antigen. For vaccines containing recombinant proteins, this step may incolve separation by column chromatography or ultrafiltration, and for inactivated vaccines, only virus inactivation without purification may occur;

Vaccines against COVID-19

The COVID-19 pandemic has put immense strain on healthcare systems worldwide, pushing for new therapies and preventive strategies. The race to find a vaccine against COVID-19 began in January 2020, after Chinese researchers shared the genomic sequence of the SARS-CoV-2 virus and with the help of previous studies on the structure of highly pathogenic coronaviruses

such as SARS-CoV and MERS-CoV. Several prophylactic vaccines against COVID-19 have been under development in multiple countries.

By mid-December 2020, the United States Food and Drug Administration (FDA) granted emergency use authorization for the Cominarty (Pfizer/BioNTech) and Spikevax (Moderna) vaccines [23, 24], as there were sufficient evidence that both vaccines could be effective in combating the COVID-19 pandemic. Subsequently, the European Medicines Agency (EMA) also granted authorization. On February 15, 2021, the AstraZeneca/Oxford vaccine (Vaxzevria), a viral vector vaccine isolated from chimpanzees, manufactured by the Serum Institute of India and SKBio, received emergency use authorization [25]. It was followed by the JCOVDEN vaccine (Ad26.COV2.S) on March 12, 2021, created by Janssen Pharmaceuticals (Johnson & Johnson), which is a recombinant human adenovirus that encodes the full-length spike protein of the SARS-CoV-2 virus, producing antibodies against infection [26], and is administered in a single dose.

All first-generation COVID-19 vaccines are based on the sequence of the S protein of the original strain identified in Wuhan. However, new variants of SARS-CoV-2 have emerged over time, as described in chapter 1, some with significant sequence variations in the N-terminal domain (NTD) and receptor-binding domain (RBD) of the S protein. These new strains have received considerable attention, as variations in the S protein sequence can affect the sensitivity of SARS-CoV-2 variants to vaccine-mediated immunity and may impact the vaccine's effectiveness against these variants.

mRNA Vaccines

Several approaches have been tested concurrently in the development of vaccines against COVID-19, including inactivated virus vaccines, live attenuated vaccines, recombinant protein vaccines, adenovirus vector vaccines, influenza virus vector vaccines, mRNA vaccines, and DNA vaccines. The revolutionary innovation was represented by mRNA vaccines, which played a unique role in controlling the COVID-19 pandemic. Both the Cominarty and Spikevax vaccines utilize modified RNA to encode the spike protein of the SARS-CoV-2 virus, mutate the mRNA to fix the spike protein in a three-dimensional shape that binds to cells even before interacting with human ACE-2 receptors on the cells to which virus-neutralizing antibodies must interact. Additionally, both vaccines use a lipid nanoparticle (LNP) delivery system. As mRNA vaccines are highly potent and can be rapidly and easily produced, they represent a new

class of vaccines that offer potential advantages over traditional viral vector vaccines, replicating or non-replicating, at a relatively low cost [27].

The successful development of mRNA vaccines is the result of many years of past research. The mRNA molecule was first described by Brenner and colleagues in 1961, but due to the highly unstable nature of the mRNA molecule, it was only in 1969 that the first protein was produced in vitro from isolated mRNA. In 1984, Krieg and colleagues were the first to use SP6 RNA polymerase to successfully transcribe and synthesize mRNA in vitro, laying the groundwork for further studies on in vitro mRNA. After years of investigation, in August 2018, the first therapeutic RNA interference drug (siRNA), Onpattro® - Alnylam Pharmaceuticals Inc., Cambridge, MA, USA, was approved by the FDA. mRNA vaccines for various infectious diseases such as rabies, influenza, Ebola, Zika, and dengue virus have entered preclinical research or clinical trials recently [28].

The fundamental mechanism underlying mRNA vaccine technology relies on a vehicle that allows the delivery of a nucleic acid molecule encoding the antigen of interest into the target cell of the human host, thereby enabling the host cell to manufacture the target protein and express the antigen to trigger the immune response. In this way, upon invasion by a pathogen carrying the antigen, the host's immune system can quickly trigger both humoral and cellular immune responses, thereby preventing the disease.

Viral Vector Vaccines

For the development of a viral vector vaccine, a safe virus, either replicating or non-replicating, is used to deliver specific components of the disease-causing virus, which can stimulate the immune system while remaining harmless. The viral vector carries the target viral proteins into the human body to enhance the immune response.

The Vaxzevria vaccine is a non-replicating viral vector vaccine in which an adenovirus from chimpanzees was used, modified to lose its ability to replicate. If a human adenovirus had been used for vaccine development, there was a possibility that some of the population would have immunity to it due to a previous infection, which could have reduced the efficacy of the vaccine by neutralizing the vector. The adenovirus's role is to transport a fragment of genetic information from the SARS-CoV-2 virus into cells at the site of vaccine injection, which will later synthesize the S protein of the virus. This protein will be recognized by the immune system as foreign to the body, leading to the production of antibodies and immune cells that will attack it [29]. Studies conducted in the United Kingdom, Brazil, and South Africa have

shown that the Vaxzevria vaccine had an efficacy of 81.3% in preventing symptomatic COVID-19 in participants who received two doses at a 12-week interval. Other research in the UK has shown that it provides 74.5% protection against the Alpha variant and 67.0% protection against the Delta variant. It is also 77.9% effective against the Gamma variant but only 10.4% effective against the Beta variant.

The JCOVDEN vaccine utilizes a recombinant, non-replicating human adenovirus type 26 (Ad26) as a vector, encoding a full-length protein linked to the membrane of the SARS-CoV-2 virus [30], in a stabilized conformation based on the original Wuhan strain of SARS-CoV-2, which has been authorized for emergency use worldwide.

II. PERSONAL CONTRIBUTIONS.
EXPERIMENTAL PART
4,STUDIES ON THE EFFECTS OF COVID-19 VACCINATION AMONG PHARMACISTS' COMMUNITIES IN ROMANIA.

Knowing in detail the involvement of pharmacists and their role in combating the pandemic at the national level, in the first chapter of personal contributions, we analyzed their perception regarding COVID-19 vaccination through a study conducted in four different regions in Romania (Iași, Bihor, Olt, and Bucharest). Pharmacists are trusted professionals for the general population and can enhance proper communication regarding medical information about the effectiveness and safety of medications. They can provide patients with accurate information about the importance of vaccination. The research involved 850 participants from community pharmacies in the four regions, and the results showed that the majority of pharmacists are vaccinated against COVID-19, and no serious adverse events were identified.

The research was conducted between January 2022 and March 2022 and was made possible through close collaboration with the National Colleges of Pharmacists in Iași, Bihor, and Olt, which facilitated the broad dissemination of the questionnaire. The study was a real effort of collegiality and professionalism among pharmacists from the three historical regions of Romania, aiming to highlight centralized collective national responses regarding the complex aspects of COVID-19 vaccination among pharmacy professionals in Romania.

Amid the global pandemic, research was also conducted using online platforms to enroll participants in studies. In this study, the questionnaire was distributed online through the Google Forms platform. For the counties of Iași, Bihor, and Olt, the link to the online questionnaire was distributed to the staff of community pharmacies through the Colleges of Pharmacists, while in Bucharest, the questionnaire link was distributed on social media platforms.

In the study, 850 questionnaires were distributed, of which 804 were considered for the final evaluation, as the rest did not allow for proper data analysis (incomplete questionnaires). The obtained data were coded, validated, and analyzed using Microsoft Office Excel (macOS) version 16.67 and GraphPad Prism Version 9.5.1(528). The statistical significance of the data was calculated using the Chi-Square test, Pearson's coefficient, and Fisher's test. The results of the applied statistical tests present a 95% confidence interval. Data were considered to represent a statistically significant difference for a value of $p<0.0001$ and a statistically significant difference for a value of $p<0.05$.

The results obtained in the study showed that 84.45% of the participants are vaccinated against COVID-19, but only 60.67% of the vaccinated individuals have received the complete vaccination schedule (2 doses and a booster dose). Even though over 60% of unvaccinated individuals (62.40%) had experienced the disease and its severity, they chose not to get vaccinated. Among these, 16.80% were infected twice, compared to vaccinated individuals who were protected against the virus, with less than 10% of them being infected twice (7.07%). The results of this study highlight that vaccination is the main means of protection against the virus, especially among the personnel in community pharmacies who are constantly exposed to the virus.

As a general observation regarding the safety and efficacy of COVID-19 vaccines, the obtained results identified a single adverse event that is not listed in the Summary of Product Characteristics, namely hypertension, reported by a pharmacist in Bucharest who received 2 doses of the Pfizer/BioNTech vaccine.

From the collected data in this study, it was evident that after administering the second dose, the percentage of individuals who experienced adverse reactions increased by one unit (93.98%) compared to the first dose (92.78%). The data also showed that there were no statistically significant differences regarding pain at the injection site between dose 1 and dose 2, as well as between dose 2 and dose 3, which coincides with the manufacturers' statements. Between dose 1 and dose 2, a significant difference was observed regarding the appearance of fatigue, a trend that was not maintained after administering dose 3, where there was no significant difference compared to dose 2. Moreover, over half of the individuals who experienced adverse reactions after the first dose did not take medication to alleviate them, a trend that was repeated for the second dose, and for dose 3, there was a significant decrease in the severity of adverse reactions, where the administration of medication was not as frequent.

5.PERCEPTION OF PHARMACY STUDENTS IN ROMANIA REGARDING COVID-19 VACCINATION

The COVID-19 pandemic had a significant impact on students; schools and universities closed during the state of emergency, and some continued with distance learning even after the state of alert was declared. This led to a series of challenges for students, including difficulties with online learning, social isolation, and educational disruptions. Given the situation of social isolation among students, we considered it relevant to initiate a study on the perception of pharmacy students, as future professionals in the field of health, regarding the novel coronavirus and COVID-19 vaccines. Understanding their opinions on the greatest pandemic of the century is important, as they can further encourage people in their social groups to obtain accurate information about COVID-19.

The conduct of the study was approved by the Research Ethics Committee of the "Carol Davila" University of Medicine and Pharmacy in Bucharest. The study was carried out using a questionnaire consisting of 26 questions, elaborated based on the information available in the Summary of Product Characteristics for each COVID-19 vaccine available in Romania at the time of the study, regarding vaccine adverse reactions, as well as based on data available in the specialized literature. The obtained data were coded, validated, and analyzed using Microsoft Office Excel (macOS) version 16.67 and GraphPad Prism Version 9.5.1(528). The statistical significance of the data was calculated using the Chi-Square test and the Fisher test. The data were considered statistically significant for a p-value <0.0001 and a significant difference for a p-value <0.05.

Pharmacy students are generally rigorous when it comes to vaccination aspects. The data obtained from the analysis regarding pharmacy students' perception of COVID-19 vaccination revealed the following:

- 76,03% of first-year students are vaccinated of which 23.91% are vaccinated with the full schedule
- 81,20% of second-year students are vaccinated of which 28,70% are vaccinated with the full schedule
- 90,60% of third-year students are vaccinated of which 24,44% are vaccinated with the full schedule
- 83,11% of fourth-year students are vaccinated of which 25,00% are vaccinated with the full schedule
- 93,98% of five-year students are vaccinated of which 16% are vaccinated with the full schedule

There is an almost 20% difference between the number of vaccinated students in the first year and those in the fifth year, favoring the fifth year. This suggests an evolution of knowledge in the medical field as well as in health education. This aspect is important to note because future pharmacists are future counselors to patients, and their opinions have a significant impact at the population level. Furthermore, from the gathered data, it was observed that the majority of students (77.60%) vaccinated immediately when the vaccine became available in Romania, suggesting that they took into account future exposure to the virus since they were about to start their practice period in community pharmacies beginning in February 2022.

Approximately one-third of unvaccinated students stated that the SARS-CoV-2 virus does not pose a risk to their health, and that is why they did not get vaccinated. This suggests that these students, as future professionals in the healthcare field, either have not acquired sufficient immunological and microbiological knowledge, or their youthful age gives them a psychological sense of invincibility. Moreover, 21% of them stated that they plan to get vaccinated in the near future, and all of them had a confirmed infection in the past, which further supports the conclusion that the decision to vaccinate is not based on medical knowledge, but rather on the traumatic experience of the disease

Considering that student social activities are highly active, vaccination in this type of community becomes extremely important as a means of disease prevention. Thus, the study results showed that for vaccinated students, even though they reported a first viral infection,

the recurrence (getting infected a second time) was statistically significantly lower (p<0.0001) compared to unvaccinated students.

Analyzing the frequency of adverse reactions (RA) reported after each vaccine dose among pharmacy students versus practicing pharmacists (previous chapter), a significantly higher frequency of "headache" RA was observed among students compared to practicing pharmacists, who reported "muscle pain" RA with a significantly higher frequency. A possible explanation could be the specific professional profile of student activities (mainly neurological) versus the risks associated with the pharmacist profession (pain associated with prolonged standing position).

Although the safety profile of the vaccines available in Romania at the time of the study did not demonstrate any impact on the population's health status, continuous monitoring of spontaneous adverse events is important for identifying new unknown events during clinical trials. Most data on drug safety, in general, are obtained from post-marketing data. Hence, it is crucial to encourage the reporting of adverse reactions. The data obtained in our study suggest that there is plenty of room for improvement regarding pharmacovigilance knowledge among pharmacy students, as only 17.68% of them reported adverse reactions following vaccination. It should be noted that Romania was not among the top countries actively reporting adverse events to EudraVigilance, and in this context, the results obtained by us are promising.

6.STUDIES REGARDING THE EFFECTS OF VACCINATION IN THE FIRST DECLARED COVID-19 CLINICAL INSTITUTE IN ROMANIA.

Chapter 6 presents the research conducted at the "Prof. Dr. Matei Balș" National Institute of Infectious Diseases regarding the experience of doctors with COVID-19 and vaccination. During the COVID-19 pandemic, doctors played a vital role in providing medical care and treatment to those infected with the SARS-CoV-2 virus, being the backbone of the pandemic response. Vaccinating doctors helps protect the healthcare system from being overwhelmed by a large number of staff falling ill or needing to be quarantined, which is why vaccinating medical personnel against COVID-19 is considered a global priority. The focus in this chapter was on the type and duration of adverse reactions, given their prolonged exposure to the virus and natural immunization through infection. Additionally, a comparative analysis was conducted between the perception of doctors and pharmacists regarding vaccination.

This study was made possible with the approval of the Bioethics Committee of the "Prof. Dr. Matei Balş" National Institute of Infectious Diseases. The research on the perception of medical personnel regarding COVID-19 vaccination took place from January 2022 to April 2022 within the Institute. The study was conducted using a questionnaire consisting of 17 questions formulated by a multidisciplinary team, including an infectious disease specialist, pharmacist, and epidemiologist, which represents the original element of the thesis, justifying and defining the complexity of the questionnaire. The questionnaires were distributed physically to medical staff and later collected to enter the responses into the database.

Vaccinating medical personnel against COVID-19 is considered a global priority as they are at increased risk of exposure due to close contact with infected patients. The data obtained showed positive results of the vaccination campaign carried out at the "Prof. Dr. Matei Balş" Institute of Infectious Diseases for medical personnel, with over 90% of the staff vaccinated against COVID-19.

Vaccination is one of the most effective methods of protection against COVID-19, reducing the risk of infection, severe illness, hospitalization, and death. This is the conclusion we reached based on the data collected in this study, where we identified that the majority of medical personnel were infected in 2020 when the vaccine was not yet available, with a substantial reduction in the number of cases in 2021 and 2022. The data also showed that in 2021 and 2022, no person experienced a form of illness that required hospitalization.

One of the major conclusions identified in this study refers to post-vaccination adverse events, where we identified the following reactions that are not included in the Product Characteristics Summary, one case of arterial hypertension was reported among pharmacists:

- **Rachialgia** for Cominarty Vaccine
- **SYncope** for Cominarty Vaccine
- **High blood pressure** for Spikevax vaccine
- **Tachycardia** for Spikevax vaccine

From the analysis of adverse reactions, a trend was observed after the administration of dose I and II, with the majority of adverse events being reported for the Vaxzevria vaccine, and likewise, the most reported gastrointestinal adverse events for this vaccine. These adverse events could also be attributed to the co-administration of medications or other vaccines. From the data obtained, it was noticed that during the administration of dose III of the vaccine, more

people concurrently received the flu vaccine, as it coincided with the flu season, and the adverse reactions after dose III cannot be entirely attributed to the COVID-19 vaccine.

From the comparative analysis conducted between medical personnel and pharmacists, results were obtained that are supported and in line with very recent specialized literature, which emphasizes the risks among healthcare professionals regarding exposure to the virus. The data showed that the majority of healthcare personnel have been infected with the SARS-CoV-2 virus at least once, which underscores the importance of reaching a vaccination rate of 100% in order to have medical and pharmaceutical support in combating the pandemic.

7.RESEARCH ON COVID-19 VACCINATION IN DIFFERENT POPULATION COMMUNITIES IN ROMANIA.

In chapter 7, we analyzed the perception of the general population, representing the majority of Romania's population. Major differences were observed in terms of vaccination status compared to healthcare professionals, with less than half of the total participants being vaccinated against COVID-19. The collected data confirm that the population's anxiety about the safety and efficacy of vaccines continues to hinder the achievement of vaccination standards set by the WHO.

The study related to this chapter was conducted from March to June 2022, using online platforms. The questionnaire for this study consisted of 25 questions and was distributed through the Google Forms platform on various social networks. The questionnaire was available to anyone willing to participate in this study and was not limited to specific target groups.

The results of this study revealed statistically significant differences compared to other groups of individuals analyzed, regarding the COVID-19 vaccination status among the general population. Although most participants had been vaccinated in childhood with other types of vaccines and had received the flu vaccine in the past, suggesting that they did not hold contrary views regarding the vaccination process in general, less than half of the participants enrolled in the study were vaccinated against COVID-19. These results demonstrated the hesitancy of the population to get vaccinated against COVID-19, which may be due to misinformation about the vaccine or philosophical beliefs based on speculations regarding its safety and efficacy.

According to the data collected during the study, a significant number of unvaccinated individuals stated that they refuse to get vaccinated because they believe the vaccine is not effective. These results suggest a lack of trust in the advancement of manufacturing technologies, as a causal relationship for their general vaccine refusal could not be established based on the collected data. Based on the data collected in this study, it can be concluded that addressing the reasons behind low vaccination rates and providing accurate information and resources to individuals and communities can help increase vaccination rates and ultimately end the pandemic.

CONCLUSIONS AND PERSONAL CONTRIBUTIONS

This work addressed a current international topic, being among the first researches conducted in Romania on the subject of COVID-19 vaccination.

The originality of the thesis is supported by:

- Conduncting studies in geographical areas of interest in Romania (Oltenia, Transylvania, Moldova and Muntenia) with relatively different ethnic and cultural-regional influences;
- Identification of population factors of vulnerability (vaccination, pharmacovigilance, polypharmacy) that require sustained education from specialists, in a population where high-school education is of average level;
- Identification of the vaccination status of COVID-19 at the level of Romania, both for the personnel of health system and for the geenral population;
- Determining the vulnerable points, during a pandemic, of the national strategies for communication and dissemination of information to the population in the decision regarding the COVID-19 vaccination;
- Identification of new post-vaccination side effects that are not found in the Summary of Product Characteristics (SmPC);
- Results regarding the reasons for non-vaccination, which can contribute to local authorities efforts to improve vaccination campaigns ;

Based on the studies conducted in accordance with the proposed objectives, the following conclusions have been drawn:

I.   Pharmacists' Perception of COVID-19 Vaccination in Romania

The obtained results showed that 84.45% of community pharmacy personnel are vaccinated against COVID-19, with only 60.67% of vaccinated individuals having completed the full vaccination schedule, which does not meet the WHO's objective of vaccinating 100% of healthcare workers. From the obtained results, it was observed that the majority of individuals consider that the SARS-CoV-2 virus does not pose a risk to their health, which is the reason why they have not been vaccinated.

The study results revealed a higher incidence of infections among unvaccinated individuals compared to vaccinated individuals, findings that are consistent with data from the specialized literature and confirm the vaccine's efficacy.

As a general observation regarding the safety of COVID-19 vaccines, the obtained results regarding post-vaccination adverse events identified only one adverse reaction that is not listed by the manufacturers of the four vaccines available in Romania at the time of the study, namely hypertension. For all four vaccines, the manufacturers reported local reactions as very common (pain at the injection site, swelling, itching, redness), which were also observed in our study. Participants frequently reported headaches and a general feeling of tiredness, which are also reported as very common by the manufacturers.

II.   Pharmacy Students' Perception of COVID-19 Vaccination

The results revealed the pioneering attitude of students in the early years of medical and pharmaceutical studies, with the lowest percentage of vaccinated individuals, as one-third of unvaccinated students claim that the SARS-CoV-2 virus does not pose a risk to their health, and that is why they have not been vaccinated. This suggests that these students, as future professionals in the healthcare field, have not yet acquired sufficient immunological and microbiological knowledge to understand the risks and severity of COVID-19.

Reporting adverse events helps identify any potential safety issues with medications or vaccines and tracks their overall safety profile, making it an important aspect in the pharmaceutical field in recent years. Our research provides valuable insights into this area, as less than one-third of participants reported experiencing adverse events. Therefore, it can encourage universities at the national level to offer additional courses in this new and significant domain.

III.   The perception of healthcare professionals regarding COVID-19 vaccination.

The major conclusion from this study was that vaccination is the only effective method of protection against severe forms of the disease. The collected data demonstrated that the majority of medical personnel were infected in the year 2020 when the vaccine was not yet available, but there was a substantial reduction in the number of cases in the years 2021 and 2022. Furthermore, the data showed that in the years 2021 and 2022, no person manifested a form of the disease that required hospitalization.

From the comparative analysis conducted between medical personnel and pharmacists, the results obtained are supported and consistent with very recent specialized literature, which emphasizes the risks among healthcare professionals regarding exposure to the virus. The data indicated that the majority of healthcare personnel have been infected with the SARS-CoV-2 virus at least once. Hence, achieving the vaccination threshold of 100% is crucial to have medical and pharmaceutical support in combating the pandemic.

IV.   The general population's perception regarding COVID-19 vaccination

The results of this study revealed significant statistical differences compared to other analyzed groups of individuals regarding the COVID-19 vaccination status among the general population. Despite thorough testing for safety and efficacy, it is worth noting that misinformation and vaccine conspiracy theories have fueled hesitancy towards vaccination, with the results showing that less than half of the participants enrolled in the study are vaccinated against COVID-19.

The research in this study showed that individuals decided not to get vaccinated because they perceive the vaccine as ineffective. Based on these results, it can be concluded that addressing the reasons behind low vaccination rates and providing accurate information and resources to individuals and communities can help increase vaccination rates and ultimately put an end to the pandemic.

The studies conducted and presented in this work are pioneering in Romania concerning targeted research on COVID-19 vaccination. These studies provide original insights in this direction, being the first research conducted on the vaccination status of the population in Romania.

In conclusion, the results of the original research presented in this work confirm the proposed objectives, offering important perspectives on COVID-19 vaccination from two points of view: (1) the current vaccination status among healthcare professionals and the general population, and (2) the results related to the reasons for non-vaccination can help shape public health policies and strategies to reach the vaccination threshold recommended by the WHO.

This doctoral thesis provides, at the end of the recent pandemic, a comprehensive analysis of the beneficial and preventive effects, as well as adverse events, and investigations regarding the reactivity of the general population and healthcare professionals after the rapid development and administration of new vaccines obtained through modern technologies and used at different stages of the pandemic. The conclusions of this thesis can provide predictive information for the development of correct strategies in managing potential infectious diseases with significant population implications.

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List of published scientific papers

A. ISI/BDI indexed papers

ISI indexed papers

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