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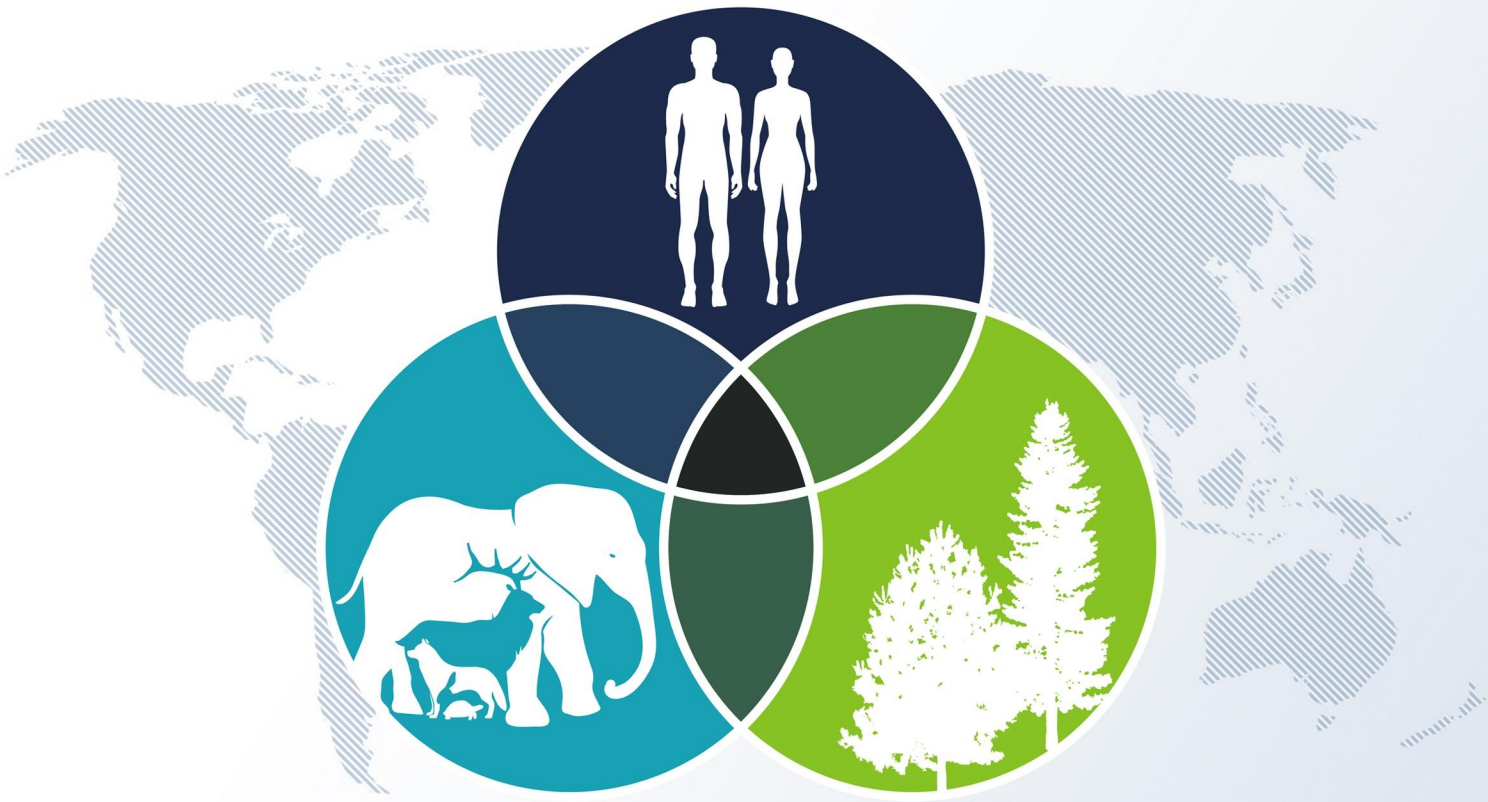

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The Moldovan Association for Biosafety and Biosecurity (MDBBA) is a scientific and practical, instructive and educational, non-governmental, apolitical and non-profit professional organization, created in 2017.

The main objective of the association is the development of good practices and culture in the field of biosafety and biosecurity and the promotion of knowledge within professional and research-innovation groups.

***Biosafety*** – includes security principles, technologies and rules to be followed to prevent unintended exposure to pathogens and toxins or their accidental release/leakage.

*"Protection of personnel, population from unintended exposure to pathogens/biohazardous material".*

***Biosecurity*** - includes a wide spectrum of measures (biosecurity policies, regulatory regime, scientific and technical measures) applied in an organized framework, necessary to minimize risks (prevention of actions, terrorist attacks by the intentional release of pathogens or toxins as well as loss, their theft or misuse).

*"Protection and prevention of theft, intentional misuse of pathologies/biohazardous material".*

***Risk management*** – is a decision-making process in which the results of risk assessment (the process of estimating workplace hazards) are integrated with economic, technical, social and political principles to generate strategies for risk reduction.

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# The One Health Concept: Working on a more efficient healthcare system

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One Health Concept of the world Health Organisation is not a new concept, but it was somewhat neglected over the years. However, the concept is a very important one. It recognizes that human health, animal health and environmental health are interconnected instead of separate subjects. Furthermore it is very important for how we (should) look at human health care. Besides these 3 main fields also food safety is part of the One Health Concept. A lot of the yearly illnesses derive from contaminated food or are transmitted by food. Some even say that more than half of the illnesses in human originate from food sources. Examples from recent history that started from food are the Ebola outbreak in West Africa by bush meat and the the SARS-CoV-2 pandemic that probably started at a food market in Wuhang, although for the last is still no conclusive evidence.

At the moment we treat patients in hospital that are ill as we did in the past and what we will keep on doing in the future. That is of course a good approach, but aren't we treating symptoms of a much bigger problem? To my opinion we don't tackle the actual source of the disease that mainly lies not in the patient itself but in his surrounding like the environment, life style, food safety (in the broadest sense of the word) or even the genetic composition of the patient. Sometimes, we even can't treat patients anymore because of the antibiotic resistance of microorganisms that is derived from the animal industry to provide us with food products.

With the strategy of the One Health Concept we can address and solve main causes of human diseases that lay outside of the health care system. It is an integrated approach in which experts from all fields that need to be involved will work together and find solutions for, not only human diseases, but also solutions for better food safety, animal health and environmental health including the climate changes we are suffering from right now. It is an integrated approach where no borders between all involved disciplines should be present and a new integrated way of thinking must be present. Main thing is that a lot of issues must be tackled and solved. Only with the integrated approach of the One Health Concept it should be feasible to reach the targets. However, for reaching the targets of the One Health Concept a lot of other people will be involved besides the experts. Without policy makers and politicians the One Health Concept will not work, because only with a global approach targets will be reached. Without the commitment of politicians from all countries problems will be tackled only locally, instead of the global approach needed for the One Health Concept.

For the One Health Concept a platform where information can be shared. One example is the journal One Health & Risk Management which regularly pays attention to subjects of the One Health Concept.

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RESEARCH ARTICLE – ARTICLES DE RECHERCHE

BIOLOGICAL SCIENCES

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# THE MICROBIAL STATUS OF BEE FAMILIES *APIS MELIFERA* DURING THE WINTER PERIOD

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***Keywords:*** *bees, bacteria, fungals, culture media, bacterial colonies.*

***Introduction.*** *Bees are very important to balancing different ecosystems around the globe. It's worth noting that, like animals and humans, bees are affected by different pathogens, such as bacteria, viruses, ecto- and endoparasites, and fungi. Therefore, the health of bee families depends on the veterinary sanitary measures undertaken by the beekeepers in close contact with the veterinarians. The aim of the research is to analyze the impact of the microbial status of bee families during the winter period.*

***Material and methods.*** *The research consisted of bee families from the experimental apiary of the Institute of Microbiology and Biotechnology of the Technical University of Moldova. Samples of dead bees, about 50 from each examined hive, were collected for bacteriological research. The morphological properties of the bacterial colonies were studied.*

***Results.*** *The obtained results demonstrate that a diverse microbial microflora prevails in bee families during the winter period. The results of microscopic investigations confirmed the presence of bacteria E. coli, accounting for over 50% of bacterial forms, followed by Streptococcus and Staphylococcus with a level of 30%, fungal flora 15%, and up to 5% bacteria of the genus Salmonella spp.*

***Conclusions****. The bacteriological research carried out in bee families after the winter period, reproduces the data of persistent bacteriocenosis in bee families during the cold period of the year and allows for predicting the risk of infectious diseases in bees.*

***Cuvinte-cheie:*** *albine, bacterii, fungi, medii de cultură, colonii bacteriene.*

## STATUSUL MICROBIAN AL FAMILIILOR DE ALBINE *APIS MELIFERA* ÎN PERIOADA IERNATULUI

***Introducere.*** *Albinele sunt incredibil de importante pentru a echilibra diferite ecosisteme de pe glob. De menționat faptul că albinele asemeni animalelor și oamenilor sunt afectate de diferiți agenți patogeni – bacterii, viruși, ecto și endoparaziți, fungi. Din acest considerent, sănătatea familiilor de albine depinde de acțiunile sanitare veterinare care le întreprind apicultorii în strânsă conlucrare cu medicii veterinari. Scopul cercetării – analiza impactului statusului microbian al familiilor de albine în perioada iernatului.*

***Material și metode.*** *Ca material de cercetare au servit familiile de albine de la stupina experimentală a Institutului de Microbiologie și Biotehnologii a Universității Tehnice din Moldova. Au fost prelevate probe de albine moarte, câte 50 de albine de la fiecare stup examinat, pentru cercetări bacteriologice. S-au studiat proprietățile morfologice ale coloniilor bacteriene.*

***Rezultate.*** *Rezultatele obținute demonstrează că la familiile de albine în perioada iernatului predomină o microfloră microbiană diversă. Rezultatele investigațiilor microscopice au confirmat prezența bacteriilor E. coli cu peste 50% din numărul formelor bacteriene, urmate de streptococi și stafilococi cu o pondere de 30%, flora fungică – 15% și până la 5 % bacterii din genul Salmonella spp.*

***Concluzii.*** *Cercetările bacteriologice efectuate la familiile de albine după perioada iernatuluiredau tabloul bacteriocenozei persistente la familiile de albine în perioada rece a anului și permit prognozarea riscului bolilor infecțioase la albine.*

## INTRODUCTION

Bees are extremely important for balancing different ecosystems around the globe. Due to bees, many species of plants are pollinated in forests, grasslands, and a multitude of eco-sys-tems; resulting in the production of fruits that serve as food for many animal species and hu-mans. At the same time, bees produce a lot of important products like honey, propolis, which are beneficial for the human health and find applications in pharmaceuticals, cosmetics, bakeries, etc. (1, 2).

We have to think that if bees stop pollinating the fruits and vegetables which we eat, the world would lose a special nutritional supply for animals, and also all the elements in the chain that are ultimately consumed by humans. There are numerous studies that try to explain why bee populations and honey production are declining. However, the reason for this is not yet fully known. It is important to mentioned that there are a significant number of diseases, including infectious (bacterial, viral, fungal, etc.). These diseases are dangerous and affect both adult bees and their brood (Salmonellosis, Collibacilosis, Viral septicemia, American and European foulbrood, Aspergyllosis, etc.). For this reason, the health of the bee families and the efficiency of their activity depends on the veterinary sanitary measures undertaken by the beekeeper in close contact with veterinarians (3, 4, 5, 6).

At the moment there are programs for monitoring and sanitary veterinary supervision of beekeeping units at national level, which provide for control and surveillance measures, or eradication measures in case of the appearance of infectious diseases in bees (6, 7).

Considering the above, *the aim* of the investigations was to analyze of the impact of the micro-bial status of bee families during the winter period.

## MATERIAL AND METHODS

The research material served the bee families from the experimental apiary of the Institute of Microbiology and Biotechnology of the Technical University of Moldova. After the winter period, three common samples were taken from the examined bee families. These samples comprised five hives selected through a random method. The general samples being composed of dead bees and remains from inside of the hives, for bacteriological research, in order to establish the presence and diversity of the bacterial flora from dead bees during the winter period.

For the isolation of bacterial and fungal forms from the collected samples, were used the common, selective and special nutrient mediums as: nutrient agar, RVS broth, XLD agar, Brilliance Salmonella Agar, Endo medium, Sabouraud medium. After incubation of the culture media at the thermostat (at 37±1˚C), were studied the morphological properties of the bacterial colonies that developed on the nutrient culture media. Smears were prepared from the obtained colonies and stained using the Gram method for microscopic investigations.

## RESULTS

After examination of bee families to assess their condition after the winter period (fig. 1), priority was given to families with unsatisfactory physiological status (reduced mobility of the bee swarm, excessive bee mortality, diarrheal matter on the walls of the hives and on beehives). Bee samples for laboratory investigations were then randomly selected according to these criteria (fig. 2).

The results of bacteriological investigations confirmed significant growth of bacterial colonies on general culture media, as well as on special nutrient culture media. The most intensive growth of bacterial colonies was on the Endo medium, where specific microbial colonies for the *E. coli* bacteria genus were observed. The bacte-rial colonies were morphologically characterized with spherical and oval shapes, dark – red color, having a gloss with a metallic appearance. Colonies on this medium (on this medium) were predominant with the highest growth intensity across all culture media (fig. 3).

On the peptone agar medium there was a noticeable and intense growth of colonies of *Streptococcus* and *Staphylococcus* bacterial forms with a gray-white color and spherical shapes, uniformly placed on the entire surface of the Petri dish (fig. 4).

In the case of insemination on the Salmonella Shigiella Agar medium, initially were observed an uneven discoloration of the nutrient medium fol-

lowed by a moderate development of *Salmonella* spp. colonies on the entire surface of the Petri plate. These colonies exhibited a light pink color and a wet appearance, with oval shapes (fig. 5).

Figure 1. Examination of bee hives after winter period.

Figure 2. Preparation of samples taken from bee hives for bacteriological investigations.

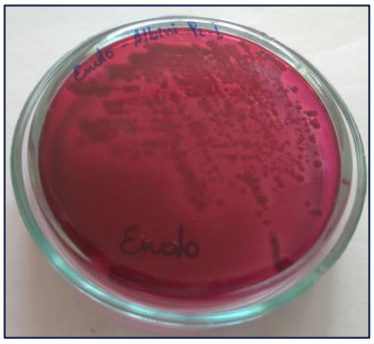


Figure 3. Colonies of E. coli on the nutrient medium Endo.

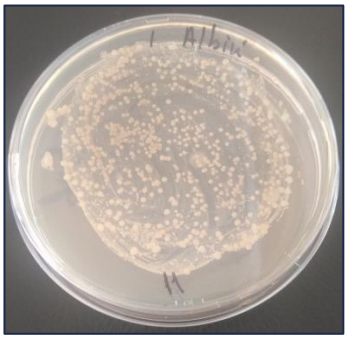

Figure 4. Colonies of Streptococcus and Staphylococcus on nutrient Agar.

Co-inoculations on Sabouraud medium showed an intensive growth of characteristic typical microscopic fungal colonies. These colonies developed over the entire surface of the Petri dish, had a characteristic filamentous-fluffy, with gray-white color and spherical shapes, placed on the surface of the substrate, concretized in the surface of the nutrient medium (fig. 6).

For the morphological-microscopic studies of microbial and fungal colonies with a typical, well-

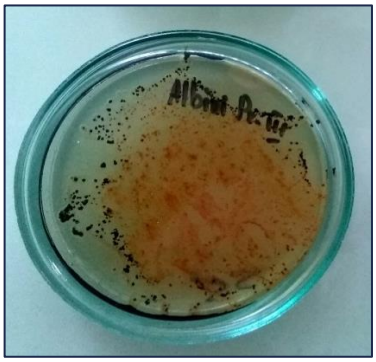

Figure 5. Colonies of Salmonella spp. on nutrient medium SSA.

Figure 6. Colonies of fungals on nutrient medium Sabouraud.

devel oped morphological structure, smears were prepared, stained according to the Gram method and examined under a biological microscope with a magnification of 10x100.

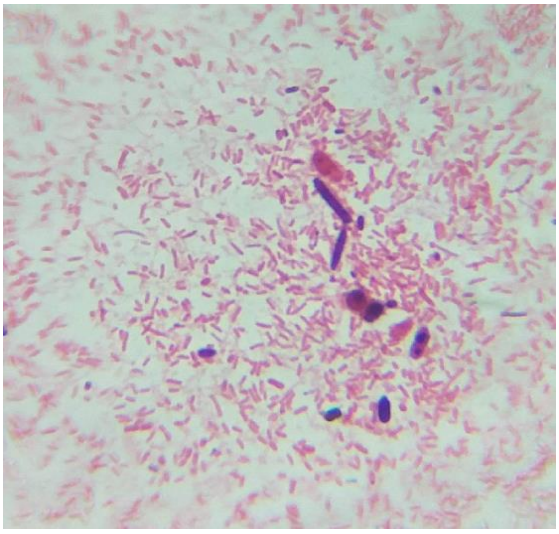

Figure 7. Association of microbial forms (*E. coli, Salmonella* spp., yeasts).

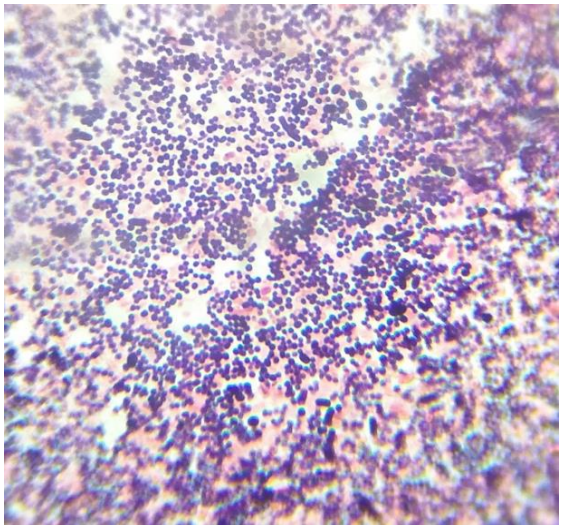

Figure 8. Association of bacterial forms (*Streptococcus, Staphylococcus, and Bacillus).*

The microbiological study results confirmed that bee families after the winter period have a predominant associative bacterial flora. This combination of bacterial forms includes coliform bacteria from genera such as *Escherichia* and *Salmonella* (fig. 7) along with an association of bacteria from genera *Streptococcus, Staphylococcus*, bacillary flora and some forms of fungi (fig. 8).

The results of microscopic investigations confirmed the presence of bacteria of the genera *E. coli* which represented over 50% of the number of all bacterial forms, followed by *Streptococcus* and *Staphylococcus* in a range of 30%, fungal flora 15%, and up to 5% forms of bacteria from the genera *Salmonella* spp.

**DISSCUTIONS**

One of the riskiest periods for the survival of bee families is the cold period of the year, known as the winter season. Beekeepers should fundamentally prepare for the winter of bee families. The bacterial flora through which the bee family passes during the winter period is decisive for their health.

Another equally important factor is the volume and quality of food (quality honey) for winter period. As a rule, because the winter period is often unpredictable in duration, the food reserve should include a surplus of 15-20% beyond the ordinary needs of a bee family. In addition, the composition of the microbiocenosis of bee families is the basic health indicator of the bee families during the winter period. For this reason, it would be highly recommended to take samples from the bee families before the wintering period. This would allow the analysis of the microbiological risks that may persist or become important factors affecting the viability of the bee nest during the winter period.

In the microbiological study carried out, it was demonstrated that the bacterial flora in bee families after the winter period is polymorphic, consisting of bacterial forms from genera, *Escherichia, Salmonella, Streptococcus and Staphylococcus* in combination with microscopic fungal forms. The results obtained demonstrate that a considerable percentage of the dead bees are the result of the presence of conditionally pathogenic bacterial forms that, under the action of some favorable factors, become pathogenic for some bee families. This is exemplified by a 25-30% increase in mortality compared to other bee families, where the intensity of the bacterial flora is much reduced. The data obtained suggest that periodic monitoring of bee families and the implementation of veterinary sanitary measures would minimize the incidence of risk factors favoring the increase in the percentage of mortality of honey bees during the winter period.

## CONCLUSIONS

1. Performing the bacteriological research in bee families after the winter period would allow us to determine the bacteriocenosis in bee families during the cold period of the year. This, in turn, enable us to intervene with some actions to reduce the risk factors for the occurrence of some bacterial or fungal diseases in bee families before and during the winter period.
2. The bacterial flora, consisting of bacillary, coccoid and fungal forms, constitutes a permanent risk of triggering some diseases in adult bees., It also presents an increased danger for the brood of bees that will appear during the active foraging period in the bee families.
3. Bacteriological monitoring of bee hives can highlight and minimize the risks of infectious diseases in bee families. It also helps in reducing or eliminating factors that might trigger infectious diseases in bees.

## CONFLICT OF INTEREST

There is no conflict of interest regarding the material presented in the paper, both between the authors and the material basis for the research.

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MEDICAL SCIENCES

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# CONTRIBUTION TO THE STUDY OF "SMOKING AND RADON" INTERACTION IN THE LUNG CANCER DEVELOPMENT IN THE REPUBLIC OF MOLDOVA

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***Keywords:*** *tobacco smoking, radon, lung cancer, cluster analysis.*

***Introduction.*** *The major risk factors for lung cancer include tobacco smoking and exposure to residential radon. A comprehensive study was conducted in the Republic of Moldova to explore the interaction of smoking and radon as triggering factors for lung cancer morbidity.*

***Material and methods.*** *The study used data on tobacco smoking prevalence from the national 2021 STEPS study, lung cancer morbidity data from 2012 to 2020, and radon measurements obtained through RADTRAK2 during 2018 and 2021. The data processing tools employed summary statistics and cluster analysis.*

***Results.*** *The distribution of high radon values, the number of smokers and the lung cancer morbidity is uneven throughout the country. The formation of a larger cluster combining all variables developed from the formation of two distinct clusters: 1. the incidence and prevalence of lung cancer, the total number of tobacco smokers, the number of male smokers, the number of female smokers, and the number of urban smokers, 2. the radon concentration and the number of rural smokers.*

***Conclusions.*** *The study results demonstrate the leading role of tobacco smoking on morbidity rate among adult population, regardless of gender across urban areas with a rather low radon concentration. At the same time, they indicate the cumulative effect of smoking and increased radon concentration in rural areas due to home construction features and lifestyle.*

***Cuvinte-cheie:*** *fumat de tutun, radon, cancer bronhopulmonar, analiză clusteriană.*

## CONTRIBUȚIE LA STUDIUL INTERACȚIUNII „FUMAT ȘI RADON” ÎN DEZVOLTAREA CANCERULUI BRONHOPULMONAR ÎN REPUBLICA MOLDOVA

***Introducere.*** *Expunerile la fumul de tutun și la radonul rezidențial reprezintă factorii de risc major în dezvoltarea cancerului bronhopulmonar A fost efectuat un studiu care a avut în vizor interacțiunea fumat & radon ca factor declanșator al morbidității cancerului bronhopulmonar în Republica Moldova.*

***Material si metode.*** *Au fost analizate datele privind prevalența fumatului din studiul național STEPS 2021, indicii vizând morbiditatea cancerului bronhopulmonar în 2012-2020 și rezultatele măsurătorilor concentrațiilor de radon obținute prin detectoare RADTRAK2 în 2018-2021. Instrumentele de prelucrare a datelor au inclus statistici rezumative și analiza clusteriană.*

***Rezultate.*** *Distribuția valorilor sporite de radon, a numărului de fumători și a morbidității cancerului bronhopulmonar a fost neuniformă pe întreg teritoriul țării. Formarea unui cluster mare, care combină toate variabilele, derivă prin formarea a două alte clustere: 1. incidența și prevalența cancerului bronhopulmonar, numărul total de fumători de tutun, numărul de bărbați fumători, numărul de femei fumătoare și numărul de fumători din mediul urban; 2. concentrația radonului și numărul de fumători din mediul rural.*

***Concluzii.*** *Rezultatele studiului demonstrează rolul principal al influenței fumatului asupra morbidității în rândul populației adulte, indiferent de sex, într-un mediu urban cu o concentrație diminuată de radon și, în același timp, indică asupra efectului cumulativ al fumatului și al valorilor sporite ale concentrației de radon în zonele rurale, din cauza caracteristicilor de construcție ale locuințelor și a stilului de viață.*

## INTRODUCTION

Lung cancer (LC) is the most common cancer worldwide, as well as the most common cause of cancer-related death in men (1). In 2020, it ranked second as the most common cancer in the Republic of Moldova with over 726 people being diseased in the same year, thus, accounting for 8.5% of all newly diagnosed cancers in both men and women (2). Moreover, lung cancer is the second leading cause of death among the malig-nant tumors in the country (3, 4). The major risk factor contributing to the occurrence of LC is tobacco smoke, responsible for approximately 80% to 90% of all cases (5). Furthermore, the gas radon ranks among the other significant risk factors (6).

Radon (222Rn or Rn in further) is a natural noble gas with no smell, color, or taste, produced through the radioactive decay of uranium in the Earth's crust. While outdoor concentrations of Rn are generally low, they can accumulate indoors, particularly in houses, where the general population faces the highest exposure. Radon, being 7.5 times heavier than air, tends to concentrate in cellars, basements, and ground floors of buildings. However, cases of radon detection can also occur on the upper floors of residential and public buildings due to the "candle" effect. This phenomenon results from pressure differences between warm and cold air, causing the gas to rise. In homes, schools, and offices, radon levels can vary significantly, ranging from 10 Bq/m$^3$ to over 10,000 Bq/m$^3$. People might unknowingly live and work here in conditions with dangerously high radon concentrations, posing a serious threat to their health. Even minimal inhalation of radon and its short-lived decay products, which deposit in respiratory tracts and emit α-particles, can be hazardous to human health. These particles can cause damage to target organs, particularly the bronchial epithelium and tissue at the junctions of the respiratory tracts (7). The lower the radon concentration is in a house, the lower the risk of lung cancer is, as there is no known threshold below which radon exposure carries no risk. The risk of lung cancer occurrence increases by 16% per 100 Bq/m$^3$ with continued exposure to even average levels of radon concentration. This dose-response relationship is linear, meaning the risk of lung cancer proportionally increases with rising radon exposure (8). According to the World Health Organization (WHO) estimates, radon causes lung cancer in 3% to 14% of all cases, depending on the average level of radon concentration in the country and the prevalence of smoking (9, 10, 11). Moreover, the risk of LC development is significantly higher in smokers compared to non-smokers (11 – 14).

In many European countries a lot of studies of the assessment of the lung cancer risk associated with residential radon exposure have been conducted over several decades (10, 11, 15 – 19). It is possible to increase the statistical power of research results by combining information from several studies, but this is very difficult to do based on the published information. Urban areas tend to show lower radon concentrations than rural ones because a large number of people live high above ground in multi-storey buildings and the bedrock under the urban localities is usually sedimentary. Moreover, urban areas generally have a higher prevalence of tobacco smoking among population (10). Similar studies using multivariable logistic regression analysis methods were conducted in China (20). These studies revealed that exposure to incense smoke at home may elevate the risk of lung cancer among smokers, and concurrent exposure to radon may further increase this risk. A parallel statistical analysis in a Korean study produced results indicating that both residential radon and cigarette smoking were associated with increased odds of lung cancer. The difference in odds ratios (ORs) based on radon exposure was much more pronounced in smokers than in non-smokers (21).

Considering that lung cancer ranks second among total cancer morbidity and is also the second leading cause of death from malignant tumors in the Republic of Moldova (4), with more than a quarter of the adult population exposed to smoking (22) and radon posing an increased risk factor for public health in residential premises (23, 24), the *purpose of this study* is to assess the interaction between tobacco smoking and residential radon exposure in relation to lung cancer morbidity in the Republic of Moldova over the last ten years. The *research hypothesis* states that the use of statistical methods is a key tool for identifying and assessing the most vulnerable population groups to the cumulative effects of tobacco smoking and radon exposure as risk factors for lung cancer in the country.

Since the radon exposure is the second cause of lung cancer after tobacco smoking, obtaining the reliable relationships of their interaction is an important component in the rationale for the development and implementation of measures to control, mitigate and prevent the negative effect of radon on public health in the country.

## MATERIAL AND METHODS

***Radon concentration data,*** measured in the air of various house types (n=2500) across rural and urban areas in the main zones of the Republic of Moldova (North, Center, and South), between 2018-2021 by the National Agency for Public Health of the Republic of Moldova, have been used (25). The measurements mainly have been taken on the ground floor, in bedrooms or in living rooms. Long-term passive detectors, RADTRAK2 were used for the measurements, with an exposure period of 90 days. In 51% of homes, the radon concentration exceeded the national/European standard level of 300 Bq/m$^3$. Given the considerable variability in radon concentrations during measurements due to various factors, it is customary to use its arithmetic mean value in the scientific research.

***Morbidity data*** on the incidence and prevalence of lung cancer per 100,000 of population (standardized index) disaggregated by gender, age and urban/rural residence in 2012-2020 have been provided by the Health Data Management Department of the National Agency for Public Health.

One of the important issues that emerged was the limited availability of data ***on number of smokers*** restricted to the whole country and only from a population study that had a limited number of interviewees, which poses several challenges when attempting to combine these data with regional radon measurement data and the lung cancer morbidity rates for the assessment. There-fore, a proportional extrapolation of the data on the number of tobacco smokers from the National Household Survey on non-communicable diseases (NCD) risk factor prevalence STEPS 2021 (total number of interviewees was 4097 people, age 18-69 y.o., both genders, urban/rural residence) has been carried out using the population data of the National Bureau of Statistics during this period (22, 26). The mathematical proportion between the total population number in each age group, gender and place of residence with the number of the population in the same categories by regions (rayons) has been calculated. Then, based on these proportional links, the number of smokers by region of the country has also been calculated. After that, a standardized number of smokers per 100,000 inhabitants has been calculated for further use in the study of *smoking x radon x lung cancer* associations (fig. 1).

***Methods.*** Proportional extrapolation calculations were conducted within a created database in MS Access 2010. The spatial distribution of variables

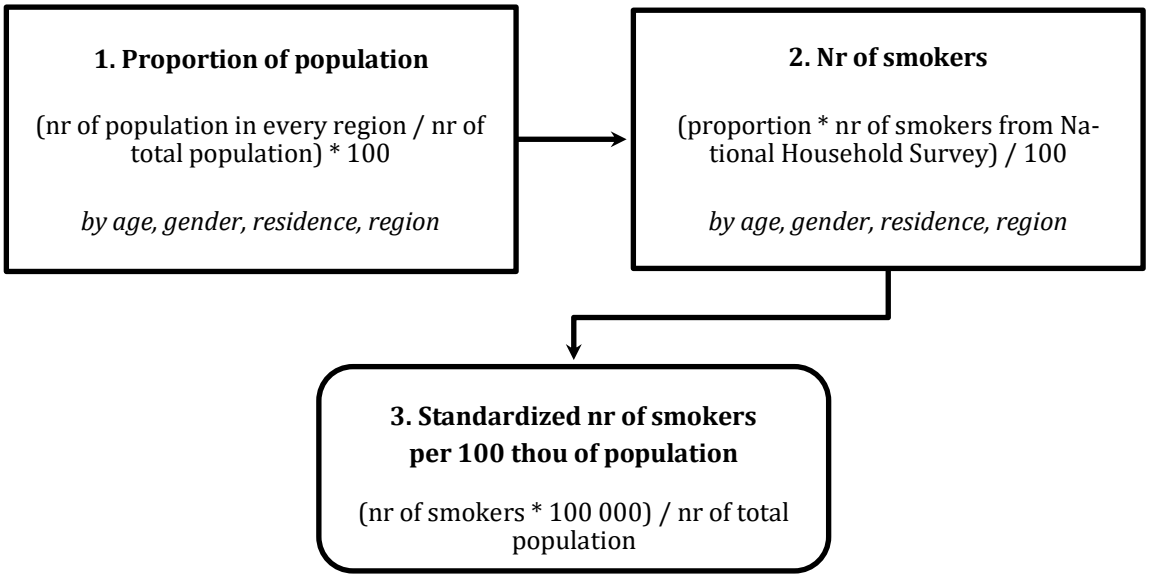


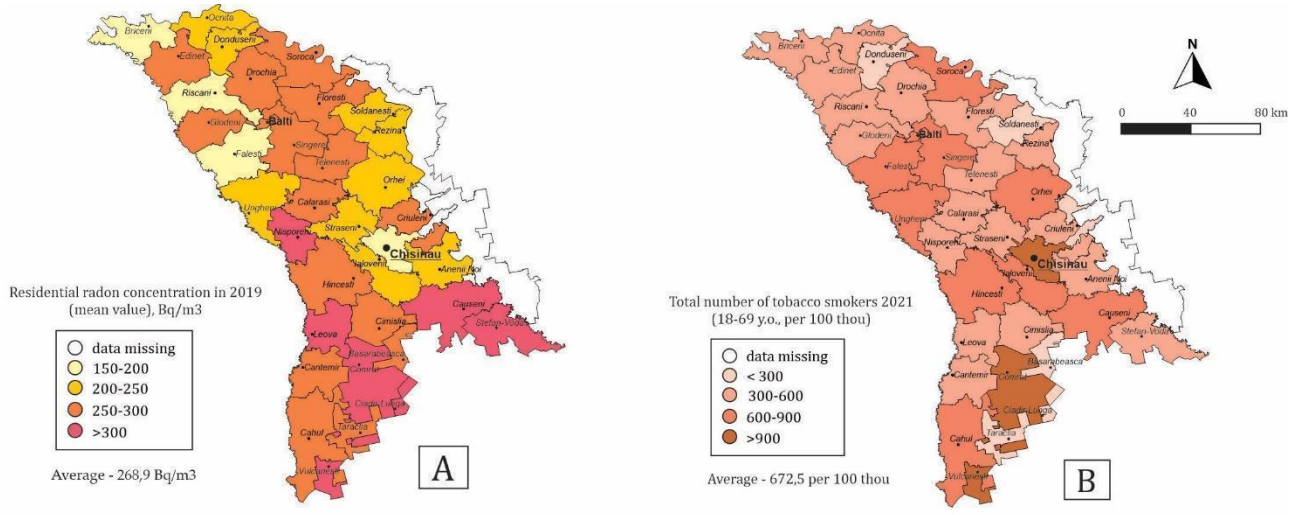

Figure 1. The proportional extrapolation algorithm used in the study.

as cartograms was performed using CorelDRAW 2020. Statistical analysis was performed using the specialized software Statgraphics Centurion XVIII, and the statistical toolkit included summary statistics of variables, such as the mean value, standard error, standard deviation, standardized kurtosis and asymmetry and cluster analysis. The use of statistical tools in such studies is quite widespread, being used to obtain reliable results within the medical and biomedical fields (11, 27).

## RESULTS

According to the 2021 National Household Survey on the Prevalence of NCD Risk Factors (STEPS) (22), in the Republic of Moldova, 29.9% of the population are current tobacco users, with the prevalence about seven times higher among men (52.0%) than among women (7.7%). Smoking tobacco is far more common among men than women (48.9% vs 6.3%), are while women in urban areas are almost four times more likely to be current smokers than women in rural areas (10.0% vs 2.4%). The data, obtained through regional extrapolation, enabled us to create a spatial distribution of tobacco smokers. The spatial distribution of tobacco smokers by rayons is shown in Figure 2, B. The highest number of smokers is recorded in the Chisinau municipality (more than 5700 per 100 thou), while many rayons in the center of country show a fairly high number of smokers (600-900 per 100 thou). In the Autonomous Territorial Unit of Gagauzia the number of smokers is also higher than the national average. Spatial distribution (fig. 2, A) of radon measurements results in dwellings throughout the country demonstrates that the rayons along the Dniester and Prut rivers are relatively safe in terms of the national radon level (radon concentration 300 Bq/m$^3$), ranging 150-200 Bq/m$^3$, while the northern, southern and southeastern rayons show radon concentrations over 250 Bq/m$^3$. Comparing the incidence and prevalence of lung cancer over a 10-year period (fig. 2, C and D) reveals that almost the same areas consistently indicate high values, particularly in the north, east, and southwest of the country.

The level of the lung cancer incidence (per 100,000) varies from year to year showing the highest rates observed in the North of the Republic of Moldova (fig.3). An analysis of lung cancer morbidity in recent years indicates that the highest incidence occurred in Donduseni rayon (50.1 per 100,000 in 2018), and the maximum prevalence was reported in Criuleni rayon (142.8 per 100,000 in 2017). Conversely, the lowest incidence and prevalence of lung cancer were registered in Cantemir rayon (8.0 per 100,000 in 2012) and Calarasi rayon (9.1 per 100,000 in 2017), respectively (28). Over the last three years, there has been a decrease in the LC incidence at the national level, and the 9-year linear trend demonstrates a slight decrease (it decreases by 0.14 per 100 thousand per year). On the other hand, lung cancer prevalence shows a modest increase, with a tendency of 0.3 per 100,000 per year. However, a slight decrease has been observed in the last three years, and the southern region of the country exceeded the central region in this parameter between 2018 and 2020. It is noteworthy that the maximum values of lung cancer prevalence were also recorded in the North of the country.



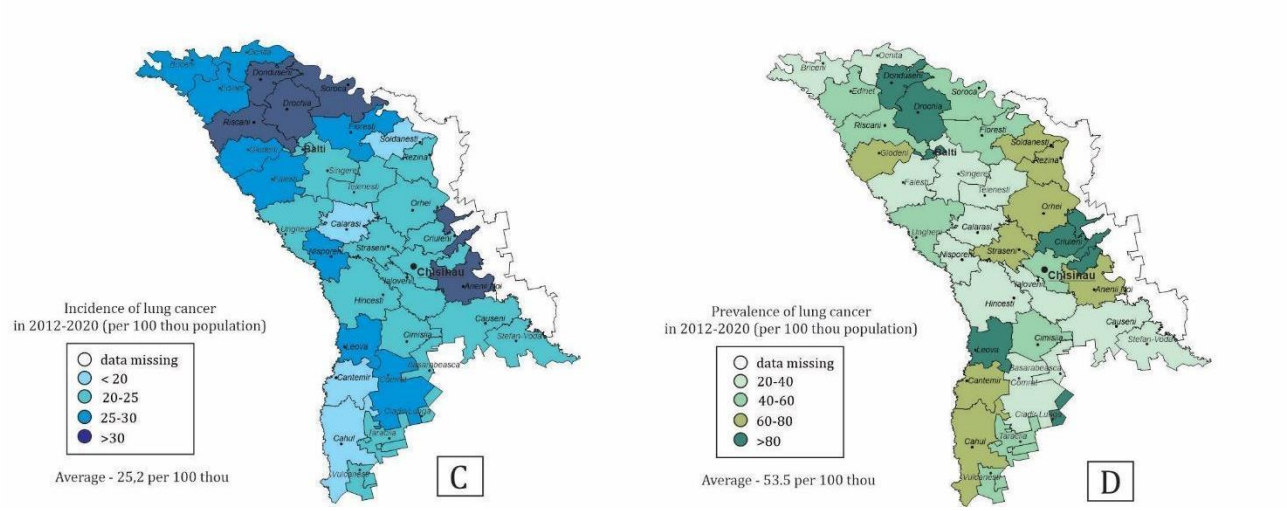



Figure 2. Cartograms of spatial distribution of radon concentration (A), total tobacco smokers (B), lung cancer incidence (C) and lung cancer prevalence (D) in the Republic of Moldova.

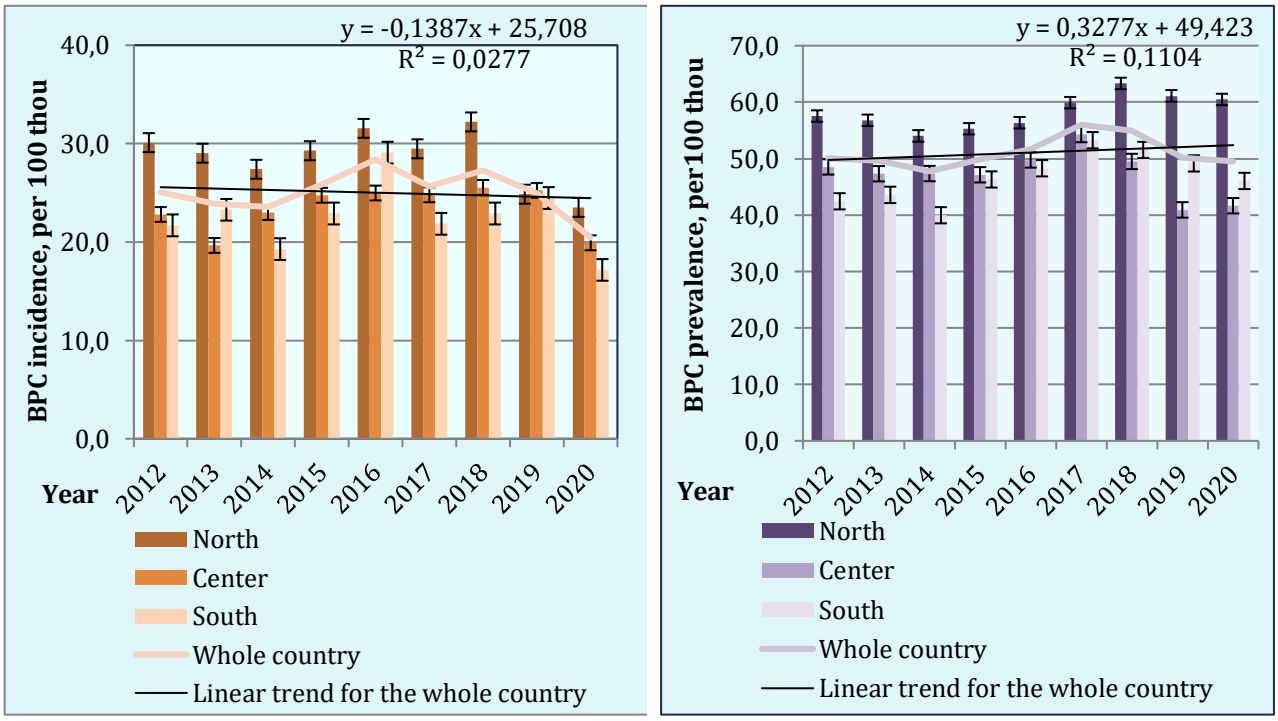



Figure 3. Incidence (left) and prevalence (right) of lung cancer (LC) in the Republic of Moldova (2012-2020) with linear trends and formulas *($R^2$ - approximation confidence value).*

The descriptive-statistical analysis of the incidence and prevalence of lung cancer in the Republic of Moldova during the last 9 years is presented in Table 1. The standard error which estimates how the value of the statistic criterion changes from one sample to another for the LC incidence was small (~1), and it was even smaller across the country (0.8).

The standard error of lung cancer prevalence also indicates relatively small changes in relation to the average value in the regions, but when considering the entire country, it was even smaller at 3.5. However, the standard deviation for lung cancer incidence suggests average variability in the data (σ>2), while the standard deviation for prevalence was large (σ>19).

Average variability was further confirmed by the coefficient of variation (CV), which shows the proportion of the average spread of a random variable to the average value of this variable.

For lung cancer incidence, this value was approximately 17%, with the smallest variation recorded in the northern part of the country. However, for lung cancer prevalence, the coefficient of variation was around 40%.

Table 1. Descriptive analysis of lung cancer morbidity, averaged over 2012-2020 (per 100 thousand).

| Variable | Incidence | | | | Prevalence | | | |
|---|---|---|---|---|---|---|---|---|
| | *North* | *Center* | *South* | *Whole country* | *North* | *Center* | *South* | *Whole country* |
| **Mean** | 28.6 | 23.4 | 22.5 | 25.0 | 58.3 | 47.4 | 46.7 | 51.1 |
| **Standard error** | 1.0 | 1.1 | 1.2 | 0.8 | 6.7 | 5.1 | 6.4 | 3.5 |
| **Standard deviation (σ)** | 3.34 | 3.98 | 3.6 | 4.48 | 23.20 | 18.55 | 19.3 | 20.61 |
| **Coefficient of variation (CV), %** | 11.7 | 17.0 | 16.2 | 17.9 | 39.8 | 39.1 | 41.3 | 40.4 |
| **Minimum** | 22.1 | 19.2 | 17.5 | 17.5 | 32.7 | 20.9 | 26.2 | 20.9 |
| **Maximum** | 33.1 | 33.6 | 29.5 | 33.6 | 103.8 | 92.5 | 80.4 | 103.8 |

As shown, a direct comparison and an attempt to explain the relationship between smoking and radon with lung cancer morbidity, given the variability of these variables cannot be effectively carried out. To find this relationship, a statistically correct method was needed. The search for an appropriate method for assessing these risk factors must precede their statistical description, enabling an understanding of the nature of the variables under study. Table 2 provides data from a standard set of descriptive statistics on mean radon concentration, number of smokers, and LC morbidity in the country.

The coefficient of variation was quite high for the number of smokers, with a difference between the smallest and largest standard deviations exceeding 3 to 1, indicating significant heterogeneity. Of a particular interest are the standardized asymmetry and a standardized kurtosis, which can be used to determine whether the samples come from normal distributions. Values outside the range of -2 to +2 for tobacco smokers indicate considerable deviations from normality. This highlights a pronounced non-normality in the distribution of data, thereby challenging the assumption that the data originate from normal distributions.

Table 2. The statistical characteristics of the variables studied in the Republic of Moldova.

| Variables | Statistic parameters | | | | | | |
|---|---|---|---|---|---|---|---|
| | *Average* | *Median* | *Standard error* | *Standard deviation (σ)* | *Coefficient variation (CV), %* | *Standardized asymmetry* | *Standardized kurtosis* |
| **Mean average Rn concentration, *Bq/m³*** | 261.8 | 262.8 | 9.62 | 56.1 | 21.4 | 1.51 | 1.73 |
| **Number of total tobacco smokers (per 100 thou)** | 685.3 | 525.0 | 156.14 | 910.4 | 132.8 | 12.89 | 36.52 |
| **Incidence of lung cancer (per 100 thou)** | 25.0 | 24.0 | 0.77 | 4.48 | 17.9 | 0.83 | 2.21 |
| **Prevalence of lung cancer (per 100 thou)** | 51.1 | 45.0 | 3.53 | 20.61 | 40.4 | -1.04 | 0.33 |

Checking the normality of the distribution of the variables using the Shapiro-Wilks and Kolmogorov-Smirnov tests as statistically principal tools for this purpose (tab. 3), revealed that only the total number of smokers deviates from a normal distribution (criterion $p \leq 0.05$) with a confidence level of 95%. Conversely, the remaining indicators exhibit a significance value of $\geq 0.05$, affirming that these variables conform to a normal distribution. This analysis shows the heterogeneity of the variables, requiring the use of appropriate tools to investigate their subsequent interactions.

Table 3. Parameters used to test the normality of the distribution of the studied variables.

| Variables | *p* - criterion Shapiro-Wilks | *p* - criterion Kolmogorov-Smirnov |
|---|---|---|
| **Mean average Rn concentration, *Bq/m³*** | 0.169 | 0.746 |
| **Number of total tobacco smokers (per 100 thou)** | 3.37173E-11 | 0.000198146 |
| **Incidence of lung cancer (per 100 thou)** | 0.176424 | 0.64719 |
| **Prevalence of lung cancer (per 100 thou)** | 0.0220654 | 0.509546 |

To select an appropriate estimator for capturing the heterogeneity of variables, a multivariate analysis was conducted using the calculation of various statistics. The most appropriate method for this task, cluster analysis (a multivariate method), was employed, and the results are presented in Figure 4. Cluster analysis can be used as a tool for qualitatively assessing data that differs in nature but presumably has mutual associations a priori. For example, in our case, radon and smoking are considered trigger factors for the occurrence of lung cancer.

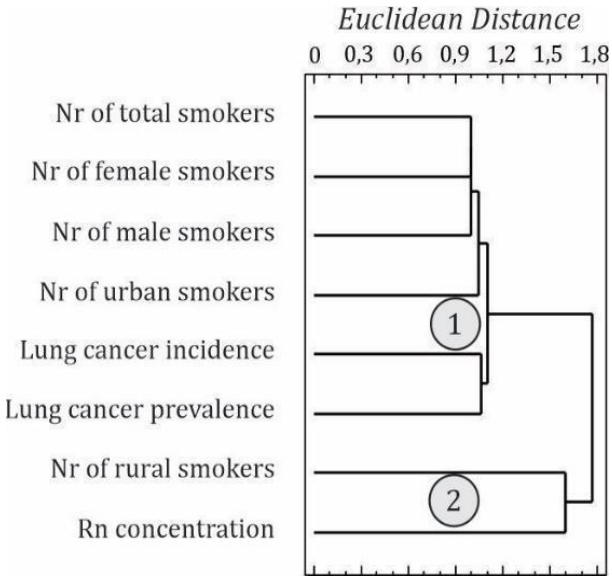



Figure 4. Dendrogram of the cluster analysis of the prevalence and incidence of lung cancer, the number of smokers and the concentration of radon in the Republic of Moldova.

A clustered multifactorial analysis was conducted to determine the Euclidean distances of the interactions among the studied parameters. The process began with the formation of a higher clus-ter (Euclidean Distance = 1.8), combining all variables, followed by the formation of two clusters with the smallest distances between variables, indicating their similarity: 1. The lung cancer incidence, the lung cancer prevalence, the number of total tobacco smokers, the number of male smokers, the number of female smokers and the number of urban smokers (Euclidean Distance = 1.1), 2. The radon concentration and the number of rural smokers (Euclidean Distance = 1.6).

## DISCUSSIONS

The exposure to the residential radon is suspected to be a risk factor for lung cancer development, in addition to tobacco smoking, the carcinogenic potential of which is firmly established by more than three decades of evidence (12, 16, 29, 30, 31). So far, few studies have focused on the joint effect of tobacco smoking and residential radon on the occurrence of lung cancer. Only in the last 10 years, such studies have become more widespread due to the large amount of accumulated data and increased attention from the scientific community and public health bodies. A vast majority of these studies are based on case-control methods (32), with only a few being purely statistical and using multivariable logistic regression (20, 21). For example, in Slovenia, three data sources were recently associated at the level of settlements: lung cancer patients, residents, and the radon map of Slovenia (33). Analyzed using spatial smoothing models with Bayesian hierarchical models, it was found that about 60 people develop lung cancer every year due to radon exposure in the living environment, representing 5% of all individuals who develop this disease. An analysis of relative risk by gender showed that in Slovenia, an increased relative risk of lung cancer occurs in areas with higher radon exposure, mainly in men rather than in women. However, the lack of quantitative data on smoking in the statistical study limits its comprehensiveness, as it does not provide a complete assessment of the synergistic effect of smoking and radon in the development of lung cancer.

A limited number of statistical studies is primarily explained by the availability and accessibility of

suitable data for such research across various categories, such as measuring radon concentration, tobacco smoking statistics, and lung cancer morbidity. Secondly, there are significant financial, human, and time costs associated with conducting such studies. While the results of case-control studies provide a closer understanding of the pathophysiological features of the *radon-smoking-cancer* interaction at the individual level, however, they have limitations in the regional and population aspects. Paradoxically, the statistical description of these associations at the regional and national levels serves more as a qualitative assessment than a quantitative one, which allows public health services to obtain an overall perspective on the public health problem, enabling them to adjust their policies to reduce the burden of diseases caused by tobacco smoking and natural ionizing radiation (6, 12).

Based on these considerations, this study attempts to statistically assess the association between lung cancer morbidity across the Republic of Moldova and both tobacco smoking and residential radon concentration. The distribution of high radon values, the number of smokers, and lung cancer morbidity is uneven throughout the country and does not always coincide, making it challenging to quantify the superposition of their interaction and the distribution of variables. Additionally, it is essential to provide a numerical expression of the number of tobacco smokers for statistical processing and analysis in this study, although the statistical extrapolation can be considered not as a quantitative but rather a qualitative assessment of the total number of smokers due to the uncertainties in the representativeness of the available data. In turn, this implies a qualitative assessment of the study results rather than a quantitative one. The use of statistical tools for the analysis of input data confirmed a heterogeneous variability of the variables and facilitated the selection of an appropriate assessment method. So far, cluster analysis of heterogeneous data has assisted in identifying arguments for the *smoking-radon-cancer* interaction. Formatted clusters, demonstrating the smallest Euclidean distances between risk factors and lung cancer morbidity, highlight the predominant role of tobacco smoking in the morbidity among the adult population, regardless of gender, in urban environments with relatively low radon concentrations. At the same time, these clusters indicate the interaction effect of smoking and increased radon concentration in rural areas, due to the construction features of houses and lifestyle (construction material, the presence of basements, living in one-story houses, etc.), aligning well with international studies (16, 21, 34).

## CONCLUSIONS

1. To conclude, tobacco smoking remains a major public health problem worldwide as a major carcinogenic risk factor for radon-induced lung cancer. The combined concern of radon and smoking interaction presents a challenge for scientific research in this area. This analysis reveals a radon and tobacco smoking in the incidence and prevalence of lung cancer, manifesting in area-specific characteristics, i.e. abiotic and geogenic factors. The use of cluster analysis has allowed us to qualitatively assess the interaction of these factors on a regional scale. The association between tobacco smoking, radon and lung cancer morbidity has been confirmed nationwide through statistical analysis tools.
2. The availability of reliable results of such an assessment is an unconditional basis for developing and adopting appropriate preventive measures at all levels of the public health system and the health system as a whole. It is only natural that costly remedial actions to reduce indoor radon exposure should be based on a scientific risk assessment. Thus, there is a need in providing the strategic guidance on the synergistic approach to radon and tobacco control. This leads us to an important policy statement: reducing tobacco smoking among the population is the most cost-effective strategy for reducing the public health burden of radon exposure.

## CONFLICT OF INTEREST

The authors declare no conflict of interests.

**FUNDING STATEMENT**

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**ETHICAL APPROVAL**

The study does not need to be approved by the Ethics Committee because the initial data refer to public statistical data from yearbooks and databases of the National Bureau of Statistics and Health Data Management Department of the National Agency for Public Health collected and processed routinely, and the data about number of smokers are taken from the STEPS 2021 population study, which is based on the documented personal consent of the interviewees, and the results of which are also public.

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# ASPECTS REGARDING BURNOUT SYNDROME IN HEALTHCARE WORKERS WITH SECONDARY EDUCATION IN THE REPUBLIC OF MOLDOVA, DURING THE PANDEMIC PERIOD

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***Keywords:*** *professional burnout syndrome, mid-level medical personnel, COVID-19 infection.*

***Introduction.*** *The COVID-19 pandemic has created favorable conditions for the onset and exacerbation of burnout syndrome. The aim of the research was to assess the degree of impact of burnout syndrome on mid-level medical staff during the pandemic period, as well as its determinants.*

***Material and methods.*** *The research was based on a selective descriptive, quantitative study conducted from November 2022 to March 2023. The quantitative component involved surveying a sample of 463 nurses and midwives involved in providing medical care to COVID-19 positive patients.*

***Results****. The results indicate that burnout syndrome during the pandemic period affected 100% of mid-level medical personnel who provided medical care to COVID-19 patients. The most pronounced dimension of burnout syndrome was psycho-emotional exhaustion, at 29.2%. Younger age and shorter work experience were more affected among mid-level medical personnel. Medical staff in republican, departmental, and municipal medical institutions were more affected than those in district institutions.*

***Conclusions.*** *Burnout syndrome was detected in all medical workers involved in healthcare delivery, predominantly manifesting itself through psycho-emotional exhaustion, with varying degrees of intensity among different categories of medical personnel.*

***Cuvinte-cheie:*** *sindromul de ardere profesională, personal medical mediu, infecția COVID-19.*

***ASPECTE PRIVIND AFECTAREA PRIN ARDERE PROFESIONALĂ A LUCRĂTORILOR MEDICALI CU STUDII MEDII DIN REPUBLICA MOLDOVA ÎN PERIOADA PANDEMICĂ***

***Introducere.*** *Pandemia COVID-19 a creat condiții propice pentru instalarea și accentuarea sindromului burnout. Scopul cercetării a fost de a evalua gradul de afectare prin sindromul de ardere profesională a personalului medical mediu în perioada pandemică, precum și factorii determinanți ai acestuia.*

***Material și metode****. Cercetarea prezentă a constat în studiul de tip selectiv descriptiv, cantitativ, efectuat în perioada noiembrie 2022 – martie 2023. Componenta cantitativă a implicat chestionarea unui eșantion de 463 de asistenți medicali și moașe implicate în acordarea asistenței medicale pacienților cu COVID-19.*

***Rezultate****. Rezultatele arată că sindromul de ardere profesională în perioada pandemică a afectat 100% din personalul medical mediu care a acordat asistență medicală pacienților cu COVID-19. Dimensiunea cea mai exprimată a sindromului burnout a fost epuizarea psiho-emoțională, în 29,2%. Cel mai afectat a fost personalul medical mediu de vârstă tânără și cu stagiu de muncă mic. Personalul medical din instituțiile medicale republicane, departamentale și municipale au fost mai afectați decât cei din instituțiile raionale.*

***Concluzii.*** *Sindromul de ardere profesională a fost depistat la toți lucrătorii medicali implicați în actul medical, exprimându-se, în special, prin epuizare psiho-emoțională, diversă după intensitate, în diferite categorii de personal medical.*

## INTRODUCTION

Burnout syndrome (BOS) is one of the many issues facing modern society, arising from one's professional life and work environment, with immediate consequences on professional activities as well as the health of those who perform that work (1).

Burnout syndrome was initially described specifically in relation to medical activity. In 1981, Maslach and Jackson proposed a multi-dimensional approach to burnout, defining it as "a syndrome of emotional exhaustion, depersonalization, and reduced personal accomplishment" (2). Emotional exhaustion represents a state of psycho-emotional fatigue resulting from mental overload, an inadequate perception of one's own emotions, and indifference. Reduced personal accomplishment signifies a tendency towards negative self-assessment of personal achievements, abilities, and success. Depersonalization is a symptom characterized by the loss of self-awareness due to psychological exhaustion, a disruption in relationships with others (1).

An essential stress factor for the population, especially for the healthcare system, has been the COVID-19 pandemic. To date, the virus has affected approximately 761 million people and continues to evolve, generating new strains and new victims. Humanity is approaching the tragic milestone of around 6.8 million deaths caused by COVID-19 infection on a global scale (3).

Although the pandemic lasted for approximately two years, the subject of burnout among healthcare professionals due to burnout syndrome has become very attractive to various authors. A meta-analysis and systematic review of 148 studies with a sample of 154,194 healthcare workers conducted in Asia from the beginning of the pandemic until March 15, 2021, found burnout syndrome in 68.3% of the respondents. The prevalence among nursing staff was 50% higher than among doctors (4).

The study of the impact of burnout on medical nurses through its three dimensions has sparked interest. Galvanis P. et al., in 2021, conducted a systematic review (following Cochrane criteria) of sixteen studies that included 18,935 nurses. According to this review, the global prevalence of emotional exhaustion was 34.1%; depersonalization – 12.6%, and reduced personal accomplishment was 15.2%. The main risk factors that increased burnout among nurses were young age, low social support, increased perceived threat of contracting the SARS-CoV-2 virus, extended working hours in quarantine areas, working in hospitals with inadequate and insufficient material and human resources, increased workload, and lower levels of training regarding COVID-19 (5).

Nurses are facing unprecedented damages due to the COVID-19 pandemic. A survey conducted by AMN Healthcare (a staffing agency in the USA) found that nurses surveyed experienced significantly elevated levels of stress, exhaustion, and other challenges, leading nearly 1 million nurses to consider the possibility of leaving the medical profession (6).

In the Republic of Moldova, the impact of burnout syndrome on mid-level medical staff was studied in 2015, where an impact of 60.6% was identified, in the absence of pandemic conditions (1).

The *aim of the research* is to assess the level of impact of burnout syndrome on mid-level medical workers in COVID-19 profile departments in the Republic of Moldova during the pandemic period, as well as to identify predisposing factors of burnout syndrome.

## MATERIAL AND METHODS

The research in question represents a cross-sectional, selective, descriptive study conducted through questionnaire surveys of a representative sample of 413 mid-level nurses (calculated from the total number of nurses practicing in the country – 15,811 individuals) who worked in COVID-19 departments during the pandemic, providing direct medical care to COVID-19 positive patients. The exclusion criterion was refusal to participate in the study. The questionnaire was developed for the purposes of the study, taking into account scientific standards and international study data. The questionnaire structure consists of 41 items divided into three parts, including: Socio-demographic data; Maslach Scale (*Burnout Inventory*) (7) with 25 items structured into 3 dimensions of BOS (emotional exhaustion, depersonalization, derealization); Opinions on BOS factors in COVID-19.

In distributing the questionnaires, consideration

was given to the level of the institution, geographical distribution, and willingness to participate in the study. To ensure the representativeness of the sample, only nurses who worked in COVID-19 departments and provided medical care to COVID-19 infected patients were selected. A total of 600 questionnaires were distributed across 9 medical institutions: one republican, one departmental, 3 municipal institutions, and 4 district institutions, including 3 level 2 institutions and one level 1 institution. The questionnaires were administered anonymously, being self-administered, and a total of 495 questionnaires were returned, of which 463 were validated. Data collection took place from November 2022 to March 2023, with an unrestricted timeframe for the observed pandemic period. Data analysis was conducted using Excel.

## RESULTS

Within the study, following the application of the Maslach Scale, it was found that 100% of the respondents were affected by burnout syndrome. The lowest recorded score was 27 points (the minimum score of the *Scale* being 25 points), with two such questionnaires, and the highest score was 112 points (the maximum score of the *Scale* being 125 points). The healthcare staff was affected by burnout syndrome differently (minimal, moderate, or high): the majority of respondents reported a moderate level of impact (58%); lightly affected individuals accounted for 26% of respondents; and 16% reported a high level of burnout (fig. 1).

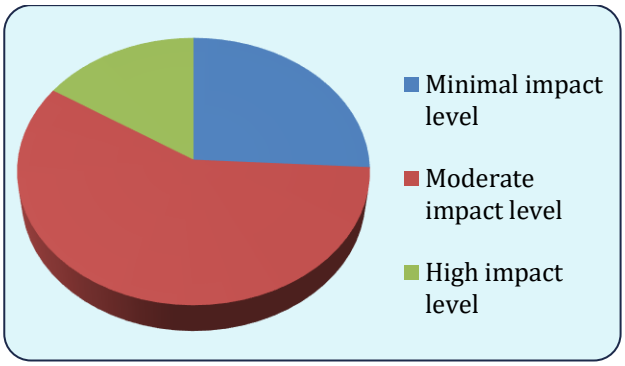


Figure 1. Impact of burnout syndrome on mid-level medical personnel.

With regard to the three characteristics of the syndrome – emotional exhaustion, depersonalization, and reduced personal accomplishment – a *high impact level* was reported by respondents as follows: 29.2% for emotional exhaustion, only 6.8% for depersonalization, and 7.8% for reduced personal accomplishment. The respondents' *moderate impact level* on the dimensions of BOS is as follows: 60.9% – reduced personal accom-plishment; 50.5% – emotional exhaustion; 22.4% – depersonalization. For the *low impact level*, the dimension of depersonalization was reported in the highest proportion (70.8%) (fig. 2).

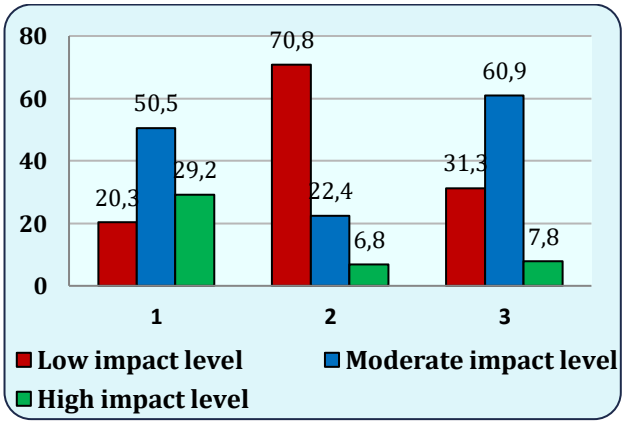


Figure 2. Impact on mid-level personnel through the dimensions of burnout syndrome (%).

***High-level burnout syndrome impact by categories.*** Given that the *high impact level* through burnout syndrome poses the greatest risks to the health of individuals and the quality of services they provide, our study aimed to investigate the connection between *high level* burnout syndrome impact and certain socio-demographic characteristics of the respondents, such as age, years of work experience, duration of work in COVID-19 departments, institutional level, isolation from loved ones, and experiencing COVID-19 infection.

Thus, a *high impact level* due to burnout syndrome was reported by respondents in the age group of up to 25 years (19.6%), while those least affected were respondents in the 55 years and older age group (12.1%). According to the presented data, younger respondents appear to be more vulnerable, while older respondents seem to be more resistant ($p<0.001$) (t-test Paired Two sample for Means in Excel). Regarding psycho-emotional exhaustion, a *higher impact level* is reported by mid-level medical personnel in the age group up to 25 years (34.8%), while a lower proportion (21%) is reported by those aged 55 years and older. Therefore, psycho-emotional exhaustion is inversely proportional to the age of the respondents ($p<0.001$).

Regarding work experience, it was determined that those with up to 5 years of experience are affected at a *high level* by burnout syndrome in 21.13% of cases, while those with over 30 years of experience are affected in only 13.3% of cases ($p<0.001$). The same trend continues for the component of psycho-emotional exhaustion of burnout syndrome: healthcare personnel with up to 5 years of experience are assessed as having a *high impact level* in 38% of cases, whereas those with over 30 years of experience are affected in only 15.5% of cases ($p<0.001$).

According to the obtained data, there is a correlation between the duration of work in COVID-19 departments and the level of burnout syndrome impact. Thus, individuals who worked in COVID-19 departments for up to 3 months experienced a *high level* of burnout in only 5.6% of cases, while those who worked for 12 months or more were *strongly affected* in 18.8% of cases ($p<0.001$). The level of psycho-emotional exhaustion is also dependent on the duration of work in COVID-19 departments, being directly proportional to the time spent in these sections. Therefore, among individuals who provided medical care to COVID-19 positive patients for up to 3 months, a *high impact level* was observed in only 11.1% of cases, whereas for those who worked for more than 12 months in COVID-19 departments, a *high level* of psycho-emotional exhaustion was observed in 33.2% of cases ($p<0.001$).

An aspect studied in the research was the level of impact on personnel depending on the type of institution. Correspondingly, a *high impact level* due to burnout syndrome was reported to a greater extent by mid-level medical personnel in departmental institutions (27%), presumably because COVID-19 patients were still being treated there at the time of the study. This was followed by a consecutive decrease in the proportion of those affected as follows: republican institutions (16.8%), municipal institutions (17.2%), and district institutions (10.5%) ($p<0.001$). *High level* psycho-emotional impact was reported by personnel in institutions located in the city of Chisinau, specifically at 43.2% in departmental institutions, 40.8% in municipal institutions, 39.6% in republican institutions, and 14% in district institutions. These differences could be explained by the complexity of cases and the duration of providing medical care to COVID-19 patients in the mentioned institutions ($p<0.001$).

Considering that many of the mid-level medical personnel who provided medical care to COVID-19 patients spent a significant amount of time in isolation, we aimed to study the influence of isolation on the level of burnout syndrome im-pact. Accordingly, we found that those who did not adhere to the isolation regimen were affected at a slightly higher, but not statistically significant, proportion of a high degree – 16.6% versus 15.4% ($p<0.001$) – with a slight upward trend for *psycho-emotional exhaustion*, which affected a greater proportion of those who adhered to the isolation regimen (31.6%) compared to those who did not adhere (26%) ($p<0.001$).

We also investigated the impact level due to burnout syndrome depending on experiencing COVID-19 infection. Among mid-level medical personnel who experienced COVID-19 infection, a *higher degree* of impact was assessed in a greater proportion than among medical personnel who did not experience the infection: 17.6% versus 11.3% ($p<0.001$). Accordingly, 31.8% of those who experienced COVID-19 infection reported an advanced *level* of psycho-emotional exhaustion, compared to 23.3% of those who were never infected with COVID-19 ($p<0.001$).

***Influencing factors related to burnout syndrome.*** In the study, the respondents' opinions regarding factors that could prevent or facilitate burnout syndrome were evaluated, placed in the multiple-choice questions of the questionnaire. According to the respondents' opinions, several factors could have contributed to reducing or preventing burnout during the COVID-19 pandemic, including: support from superiors and colleagues (19.9%); support from loved ones (19.2%); a suitable work schedule (18.8%); sufficient human resources (18.2%); adequate professional training (12%); and better information about the disease in question (11.2%) (fig. 3).

In the respondents' view, the most important factor that could have led to the onset of burnout syndrome is the increased risk of infection at 21%, followed by the difficulty of cases and the work environment at 18.7%, and the extended work schedule at 18.4%. Relationships with colleagues could have caused burnout syndrome in 11%, self-isolation in 10%, and the relationship with superiors and patients in less than 10% (fig. 4).

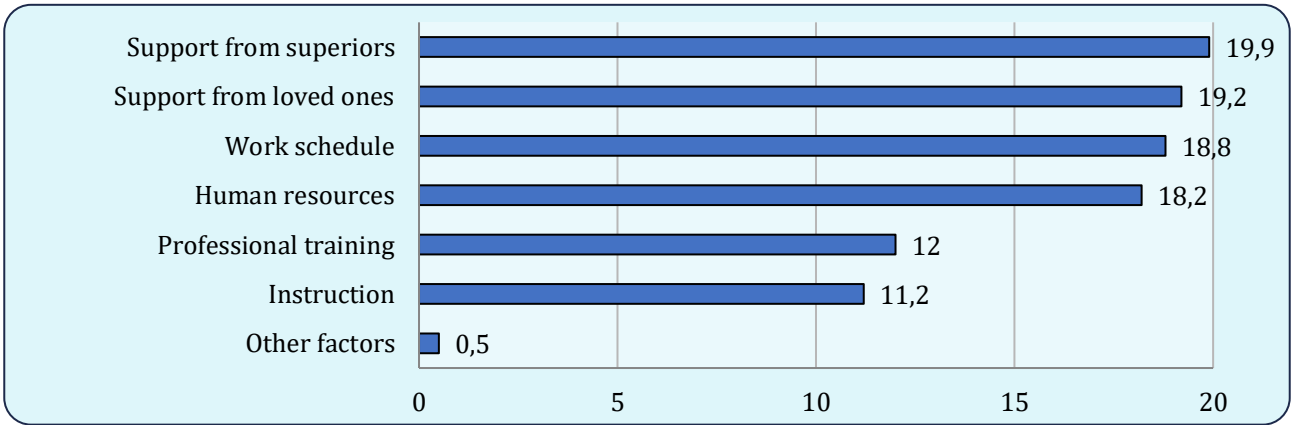


Figure 3. Factors that could have reduced the onset of burnout syndrome (%).

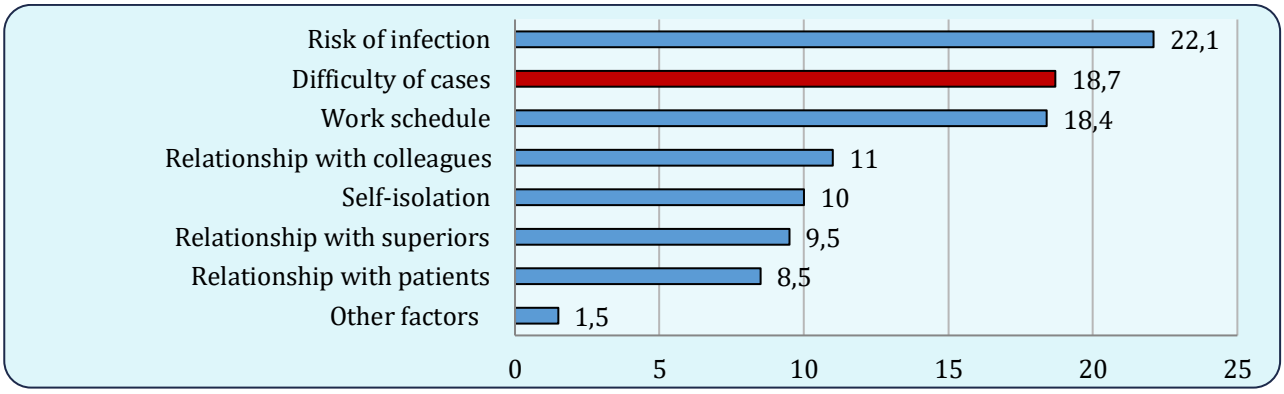


Figure 4. Factors that could have led to the onset of burnout syndrome (%).

To overcome burnout syndrome, 181 individuals reported that they needed external help. The main sources of requesting help were respondents' families in 125 cases, help from superiors was requested by 99 individuals, and help from colleagues was sought by 96 individuals.

## DISCUSSIONS

The results of the conducted study emphasize the influence of the COVID-19 pandemic as a predictive factor in the onset of burnout syndrome among mid-level medical personnel. The onset of BOS is a gradual process and occurs in the presence of persistent stress factors. During the pandemic, medical staff were constantly exposed to stress, which is why burnout syndrome was continuously increasing. Teo I. and the team of authors studied changes in medical personnel regarding stress, anxiety, and workplace exhaustion over six months of the pandemic and found that the number of individuals reporting workplace stress and exhaustion increased by approximately 1.0-1.2% monthly (8).

The 100% impact on mid-level medical personnel in the study could thus represent the cumulative effect of the two years of the pandemic (the study data were obtained in the final phase of the COVID-19 pandemic). Psycho-emotional exhaustion represents the dimension of burnout syndrome with the highest prevalence for *high impact level*, as reported by other studies, such as the one conducted by Galanis P. and the team of authors (5).

In the study, we found that a range of factors are associated with a *high level* of burnout, including: young age and short work experience, extended work duration in COVID-19 sections/units, exposure to COVID-19 infection, and self-isolation. Among the risk factors mentioned by respondents, the top three are: the risk of infection, the difficulty of treated cases, and extended working hours. Among the preventive factors for BOS were mentioned: family support, support from superiors, a balanced work schedule, and sufficient resources. Similar results have been obtained in other studies. Galanis P. points out that the main

risk factors that increased nurses' exhaustion included the following: young age, perceived threat of SARS-CoV-2 infection, extended working hours in quarantine zones, working in hospitals with inadequate and insufficient material and human resources, increased workload, and lower level of training regarding COVID-19 (5). In 2021, Zhang Y. reported the importance of age and work experience as triggering factors for burnout during the pandemic: participants of a younger age experience greater emotional exhaustion and fewer personal achievements, which is derived from a lack of experience, adaptation, and stress resilience. Burnout was observed in 78.5% of cases in the emotional exhaustion subscale (9). Teo I. mentions that long working hours were associated with a higher likelihood of developing burnout syndrome, while teamwork and a sense of appreciation at the workplace from superiors and colleagues were associated with lower chances of stress, anxiety, and workplace exhaustion (8). Thus, burnout syndrome manifested similarly in different countries.

## CONCLUSIONS

1. Burnout syndrome was reported by the surveyed mid-level medical staff at a rate of 100%, and the most impactful dimension of the syndrome was psycho-emotional exhaustion, affecting the mid-level staff at high (29.2%) and moderate (50.5%) levels.
2. The severity of BOS varies depending on a range of factors, including the young age of medical staff, short work experience, prolonged time spent in COVID-19 units, and the level of the institution, etc.
3. According to the respondents' opinion, an adequate work schedule and sufficient human resources can prevent burnout syndrome, while the fear of infection, the complexity of cases, and a stressful work environment, as well as extended working hours, can contribute to its onset.

## CONFLICT OF INTEREST

The authors do not have any conflicts of interest.

## FUNDING STATEMENT

The study was conducted as part of a master's research project within the School of Public Health Management.

## ETHICAL APPROVAL

The study does not present ethical risks and does not require examination and approval by a Research Ethics Committee.

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# KNOWLEDGE AND PRACTICES OF USING ANTIBIOTICS BY PATIENTS WITH ACUTE RESPIRATORY INFECTIONS

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***Keywords:*** *antibiotics, antibiotics in acute respiratory infections, antibiotics in primary health-care, self-medication, antibiotic resistance.*

***Introduction.*** *Excessive and inappropriate antibiotic (AB) consumption is associated with the development of antibiotic resistance (AR). Insufficient knowledge and irrational practices in the use of AB for acute respiratory infections (ARI) by patients contribute to the development of AR. Aim: to assess the knowledge and practices of AB use in patients with ARI in order to identify educational needs.*

***Material and methods.*** *The study included 393 patients with ARI. The standardized Happy Audit-2 questionnaire was applied. Complaints, the frequency of ARI occurrence, administered AB treatment, AB acquisition method, AB compliance, knowledge about AB, and AB resistance were assessed.*

***Results.*** *AB were used by 40.3% of the patients. Antibiotic treatment was recommended by a doctor in 87.6% of cases, taken independently by 5.6%, recommended by a pharmacist in 5.0%, and recommended by relatives in 1.9% of cases. AB were obtained without a prescription in 6.3% of cases, and household reserves were used in 20.8% of cases. Patients were compliant with antibiotic treatment in 89.7% of cases but 10.3% of respondents did not adhere to the prescribed duration/dosage. 26.7% of respondents believed that AB were effective against viral infections, and 13.8% believed they were harmless to the human body. In 24.1% of cases, respondents were unaware of the adverse effects of AB, including the development of bacterial resistance in 29.7% of cases.*

***Conclusions.*** *Insufficient knowledge about the effects of antibiotics and irrational usage practices, including self-medication, emphasize the need for strengthening awareness and education measures for potential antibiotic users.*

***Cuvinte-cheie:*** *antibiotice, antibiotice în infecții respiratorii acute, antibiotice în asistența medicală primară, automedicația, rezistența la antibiotice.*

***CUNOȘTINȚE ȘI PRACTICI DE UTILIZARE A ANTIBIOTICELOR DE CĂTRE PACIENȚII CU INFECȚII RESPIRATORII ACUTE***

***Introducere.*** *Consumul excesiv și inadecvat de antibiotice (AB) este asociat cu dezvoltarea rezistenței la antibiotice (RAB). Cunoștințele insuficiente și practicile irațională de utilizare AB în infecții respiratorii acute (IRA) de către pacienți contribuie la dezvoltarea RAB. Scopul lucrării este de a evalua cunoștințele și practicile de utilizare a AB la pacienții cu IRA, în scopul identificării necesităților educaționale.*

***Material și metode.*** *Studiul a inclus 393 de pacienți cu IRA. S-a aplicat chestionarul standardizat Happy Audit-2. Au fost evaluate acuzele, frecvența suportării IRA, tratamentul AB aplicat, modul achiziționării AB, complianța la AB, cunoștințele despre AB și a rezistenței la AB.*

***Rezultate.*** *S-au tratat cu AB – 40,3% de pacienți. Tratamentul AB a fost recomandat de medic în 87,6% de cazuri, luat de sine stătător – de 5,6%, recomandat de farmacist – 5,0%, la recomandarea rudelor – 1,9% de cazuri. S-au procurat AB fără rețetă în 6,3% de cazuri, s-au utilizat AB din rezervele casnice în 20,8% de cazuri. Au fost complianți la tratamentul AB –89,7%, dar nu au respectat durata/doza – 10,3% de respondenți. Au considerat că AB sunt eficace în infecțiile virale – 26,7% și inofensive pentru organismul uman – 13,8% de respondenți. În 24,1% de cazuri, respondenții nu cunoșteau despre efectele adverse ale AB, inclusiv despre dezvoltarea rezistenței bacteriene în 29,7% de cazuri.*

***Concluzii.*** *Cunoștințele insuficiente despre efectul AB și a practicelor irațională de utilizare, practicarea automedicației impune necesitatea fortificării măsurilor de conștientizare și educare a potențialilor utilizatori de AB.*

## INTRODUCTION

Incorrect use of antibacterial preparations is recognized as a major cause of antibiotic resistance development (1, 2). The determinants of irrational antibiotic use relate to knowledge about rational use and the phenomenon of bacterial resistance in the general population (3). Incorrect behaviors and concepts regarding antibiotic use are observed in both developed and developing countries, where the general popular-tion, for the most part, has insufficient knowledge about antibiotics and a mistaken perception of their impact on bacterial resistance development (4). Over the decades, there has been no significant trend towards reducing irrational use and improving antibiotic use behaviors (5).

Self-medication with AB is widespread and varies in different communities, often associated with their inappropriate consumption (6). On one hand, self-medication can offer some benefits to individuals and healthcare systems by saving patient and physician time and reducing work absenteeism. On the other hand, self-medication is a practice that contributes to the increase in bacterial resistance (7).

Antibiotic resistance can be reduced through the systematic amplification of knowledge about rational use and the associated risks of irrational use of AB (8).

*Aim*: the conducted study assessed the knowledge and practices of antibiotic use by patients with acute respiratory infections (ARIs) in Chisinau, with the aim of identifying the needs for appropriate educational measures.

## MATERIAL AND METHODS

Within the study, 393 patients from Chisinau, originating from various public medical-sanitary institutions (IMSP), who displayed signs of ARI at the time of seeking medical assistance, were included. Patients were selected by their own family physicians (FP), and after informed consent was obtained, they were enrolled in the study. The doctors who recruited the patients participated in the study's component on antibiotic use practices in ARI and also provided their consent to participate, which was prepared for FP. The standardized Happy Audit-2 questionnaire for patients with ARI, proposed by the Baltic countries' collaboration network (BARN) (9), was administered. Recruited patients were assisted in completing the questionnaire by their family doctors. The study period spanned from September 2016 to March 2018. Inclusion criteria: age >18 years, resident of Chisinau, patients with signs of ARI at the time of visiting the family physician (FP), frequent ARI (more than 3 episodes in the previous year), absence of mental disorders. Exclusion criteria: age <18 years, absence of ARI at the time of recruitment, residents from other localities, fewer than 3 episodes of cold in the previous year, presence of mental disorders. Analyzed indicators: age, gender, education, frequency of ARI suffered in the previous year, main complaints at the time of seeking medical help, day of illness, seeking information from other sources before visiting the FP, time since the last cold, method of treatment with or without AB administration, access to the FP's consultation during the cold, trust in the FP for postponing AB use, adherence to the AB dosage and duration, procurement of AB based on prescription, and knowledge about the effect of antibiotics on bacteria, viruses, and the development of bacterial resistance.

## RESULTS

The studied sample included: women – 71.3%, men – 28.7%; average age – 34.2±18.1 years; with higher education – 50.4%, secondary education – 44.2%, incomplete secondary education – 5.4% of respondents.

In the section on reported symptoms leading to seeking medical attention, the following predominated: cough – 81.7% (321/393), sore throat – 74.3% (292/393), general weakness – 66.7% (262/393), runny nose – 62.6% (246/393), headache – 53.9% (212/393), fever – 52.2% (217/393). Less frequent were: loss of appetite – 32.3% (127/393), chest pain – 21.1%, sleep disorders – 21.6%, pain in paranasal regions – 10.2%, ear pain – 10.7%, others – 2.5% (tab. 1).

Access to primary healthcare in case of ARI constituted 44.2% (173/391), while 55.8% (218/391) self-treated. The time of seeking primary care was as follows: within the first 3 days of illness – 76.3% (113/148) of patients; on the 4th-6th day of illness – 23.0% (34/148), after 6 days – 0.7% (1/148). Sources of information before- consulting -the- family -doctor- were: the in-

ternet – 36.8% (42/114), another doctor – 31.6% (36/114), a relative/neighbour – 25.4% (29/114), medical books – 6.1% (7/114) of patients (fig. 1). In 12.9% (50/387) of cases, patients encountered difficulties in scheduling appointments with their family doctor. In cases where patients could not get an appointment for consultation, they resorted to the following practices: self-medication – 34.3% (135/393), consulting another doctor – 40.2% (158/393), consulting a pharmacist – 25.7% (101/393) (fig. 2).

Table 1. Common clinical signs of patients with ARI at the time of seeking medical attention.

| Clinical signs | Number of cases (total 393) | Frequency of clinical signs in patients with ARI (%) |
|---|---|---|
| **Cough** | 321 | 81.7% |
| **Sore throat** | 292 | 74.3% |
| **General weakness** | 262 | 66.7% |
| **Runny nose** | 246 | 62.6% |
| **Headache** | 212 | 53.9% |
| **Fever** | 217 | 52.2% |
| **Loss of appetite** | 127 | 32.3% |
| **Sleep disorders** | 85 | 21.6% |
| **Chest pain** | 87 | 21.1% |
| **Ear pain** | 42 | 10.7% |
| **Pain in paranasal regions** | 40 | 10.2% |
| **Others** | 10 | 2.5% |

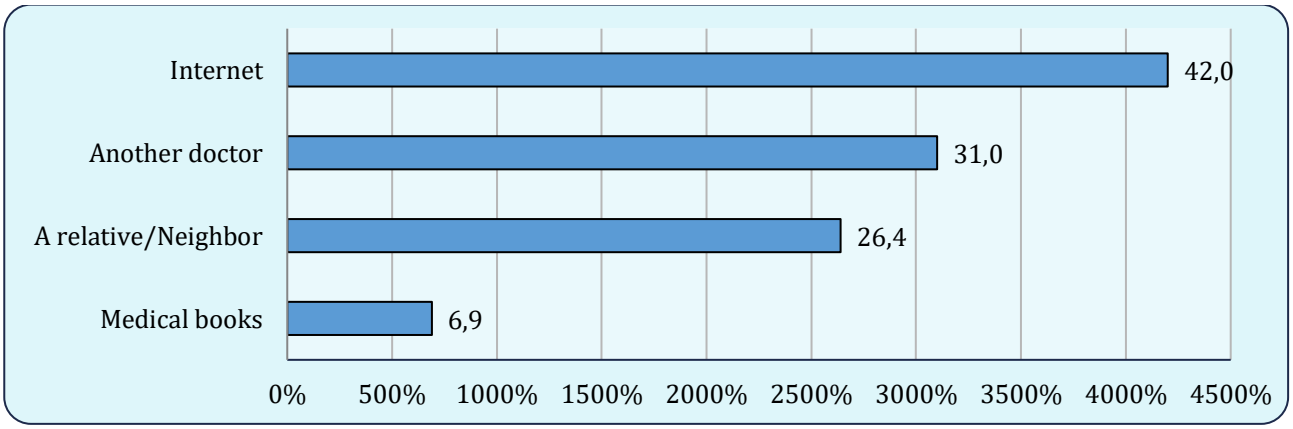


Figure 1. Sources of information for patients with ARI before consulting the family doctor

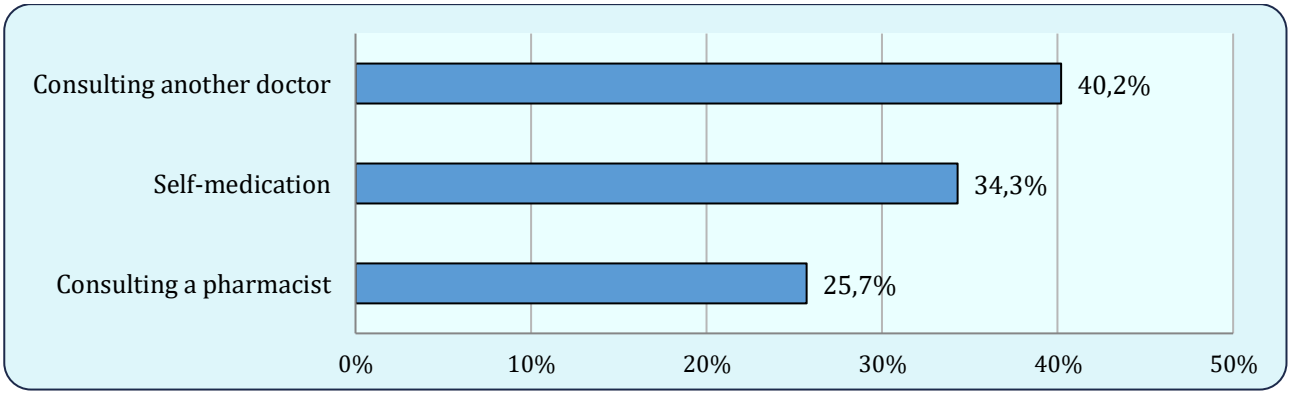


Figure 2. Practices of patients with ARI in the case of unsuccessful scheduling of a consultation with the family doctor

Out of a total of 389 respondents, 70.7% (275/389) consulted their family doctor before starting antibiotic treatment, while 19.5% (76/389) did not consult. Additionally, 9.8% (38/389) of respondents who frequently experienced respiratory infections occasionally consulted their family doctor regarding this decision (fig. 3).

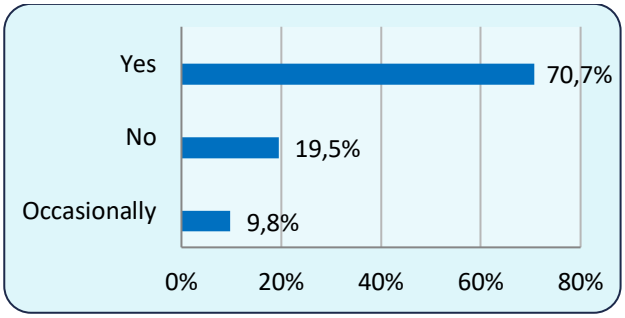


Figure 3. Proportion of patients with ARI who consult a doctor before antibiotic treatment

Out of a total of 388 cases, 79.4% (308/388) had an acute respiratory infection (ARI) in the last 6 months. Among these, 13.1% (51/388) had an ARI two to three weeks ago, 27.8% (108/388) had it one to two months ago, and 31.4% (122/388) had it three to four months ago. Acute respiratory infections older than 6 months were reported by 20.6% (80/388) of respondents. Antibiotic treatment was recommended by a doctor in 87.6% (141/161) of cases. In other situations, respondents resorted to self-medication with antibiotics: 5.6% (9/161) of them took antibiotics based on previous medical recommendations, 5.0% (8/161) received suggestions from a pharmacist, and in 1.9% (3/161) of cases, self-medication was influenced by relatives (fig. 4).

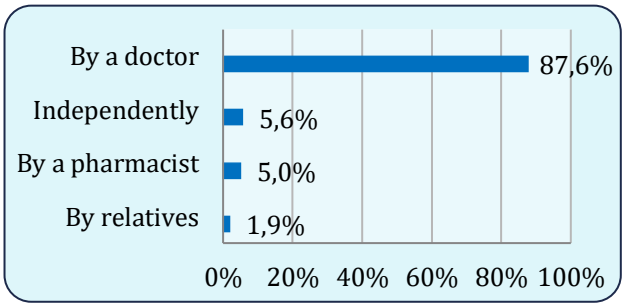


Figure 4. Sources of antibiotic recommendations in ARI

The antibiotics administered were purchased with a prescription in 87.9% (335/381) of cases, but in 6.3% (24/381) of cases, they were obtained without a prescription, and 5.8% (22/381) of respondents mentioned that they sometimes procure antibiotics with a prescription (fig. 5).

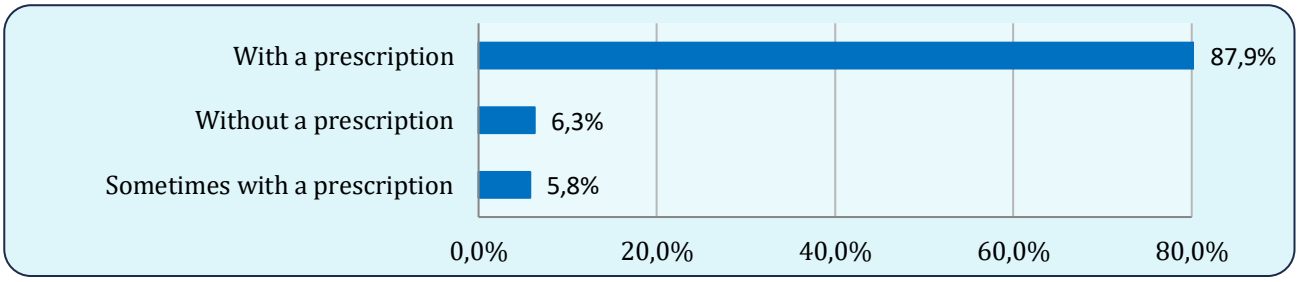


Figure 5. Ways of acquiring AB in ARI

Among patients who took antibiotics without a prescription, 20.8% (81/389) indicated that these came from household reserves. They started taking antibiotics in the first 3 days of illness – 18.5% (15/81) of respondents, while 81.5% (66/81) began administration after 3 days from the onset of acute respiratory infection.

Patient trust in the family doctor influences the choice of antibiotic treatment for ARI. In 84.1% (328/390) of cases, respondents mentioned that they trust their family doctor when they postpone antibiotic treatment, while 6.2% (24/390) do not trust, and 9.7% (38/390) have doubts. In 31.5% (124/393) of cases, patients requested laboratory tests before deciding to start antibiotic treatment. Among patients who took antibiotics, 89.7% (349/389) followed the doctor's recommenddations, while 10.3% (40/389) did not follow them.

Regarding patients' knowledge about ARI, the study identified the following findings: 26.7% (104/389) of respondents believed that antibiotics act on viruses, while 28.3% (110/389) did not provide a clear answer on this aspect. Additionally, 62.1% (242/390) of respondents considered antibiotics to be harmless to the human body, while 24.1% (94/390) were unsure about this.

Regarding the development of bacterial resistance to antibiotics, 29.7% (116/390) of respondents had no knowledge about this aspect. Moreover, 76.8% (289/388) of respondents believed that antibiotics reduce the duration of acute respiratory infections (tab. 2).

## DISCUSSIONS

Self-medication is a behavioral practice observed especially in low- and middle-income countries (10, 11). Self-medication with antibiotics in the case of acute respiratory infections involves using these medications without well-founded medical recommendations (6, 12).

Numerous studies have identified self-medication as a contributing factor to the emergence of antibiotic resistance (6, 13). According to the literature, respiratory conditions such as sore throat (34.0%), common cold with fever (47.0%), and cough (40.0%), along with the belief that antibiotics can reduce the duration of acute respiratory infections (6), and the ease with which they can be obtained without a prescription, contribute to the prevalence of self-medication with antibiotics (14). Our study found that patients exhibited symptoms such as cough (81.7%), sore throat (74.3%), runny nose (62.6%), fever (52.2%), and the belief that antibiotics reduce the duration of the illness (76.8%). Additionally, the difficulty of getting an appointment with the family doctor (12.9%) was a likely reason for antibiotic administration (40.3%), of which 12.5% represented cases of self-medication with antibiotics. The data obtained in our study show that the rate of self-medication with antibiotics is three times lower compared to the data from a meta-analysis of 34 studies conducted in low- and middle-income countries (12) but three times higher compared to developed countries (15). The COVID-19 pandemic likely had an impact on antibiotic self-medication practices in the Republic of Moldova as in other countries. Recent studies have shown a reduction in the prevalence of this phenomenon, ranging from 20.8% to 45.8% among the general population, compared to the pre-COVID-19 period when prevalence ranged from 19% to 82% (16). According to research conducted by Zheng Y in 2023, during the pandemic, antibiotics were the most commonly mentioned drugs used in self-medication, often left over from previous treatments or purchased from unreliable sources. Self-medication with antibiotics in the context of COVID-19 was driven by concerns about infection and limited access to medical services (17).

According to the meta-analysis, the main source of antibiotics obtained without a prescription in low- and middle-income countries was pharmacies (65.5%)(18). In our study, the percentage of antibiotics procured without a prescription from pharmacies was 5.4 times lower (12.1%). Additionally, the habit of keeping antibiotics at home, including from unfinished batches and even after the expiration date, promotes the practice of self-medication (19). According to studies, 50.0% of antibiotics obtained without a prescription come from household reserves (12, 19, 20). This could result from the over-dispen-sing of antibiotics or the lack of adherence to treatment by patients with acute respiratory infections (13). In our study, we observed that the use of antibiotics from household reserves was 2.4 times lower (20.8%) than reported in the meta-analysis (12). However, this practice raises questions about the reasons for storing antibiotics at home, and these reasons require detailed evaluation.

Low educational level and age have been identified in studies as factors associated with antibiotic self-medication, especially in developing countries (13, 16). According to a study by Faten Alhomoud in 2017 (21), the prevalence of self-medication was higher among middle-aged individuals (40-59 years), while a study conducted by Torres, Chibi et al. in 2021 showed a higher prevalence among young and middle-aged adults (18-40 years) (14). In our own study, we observed that self-medication was more common among middle-aged individuals (40-59 years), with a proportion of 52.6% for those with higher education and 47.4% for those with secondary education, while respondents with incomplete secondary education did not mention this habit. Therefore, the level of education may be one of the determinants of antibiotic self-medication. During the COVID-19 pandemic, factors associated with self-medication, such as gender, age, education, marital status, and the level of concern about COVID-19, were also identified (17).

Self-medication appears as a favorable alternative in regions without access to qualified medical care when potential benefits are considered to outweigh associated risks (10). The patients in our study were from urban areas and had good access to primary healthcare (PHC), received

without difficulty in 80.9% of cases. However, 12.9% of respondents faced difficulties in getting an appointment for a family doctor consultation. These difficulties likely contributed to the practice of self-medication with antibiotics, based on previous medical recommendations, in 5.0% of cases. Our study highlighted that the majority of respondents had limited knowledge about the effects of antibiotics on the human body and microorganisms. Specifically, over half of the respondents (55.0%) did not know that antibiotics are ineffective in treating viral infections, while more than a third of respondents (37.9%) were not aware of the harmful effects of antibiotics on the human body. However, the majority of respondents (76.8%) were confident that antibiotics reduce the duration of acute respiratory infections Similar data have been observed in other countries, such as those in Europe, Asia, North America, and Australia, where over 53.9% of interviewed individuals believed that antibiotics are useful in treating viral infections (3).

This study has several limitations. One of them is the geographical coverage, as the research was conducted exclusively in urban areas, within the Public Health Institutes of Chisinau municipality, although the sample was designed to represent the entire Republic of Moldova. Another limitation arises from the differences between patients with ARI in urban and rural environments. It's also worth noting that the data were collected before the COVID-19 pandemic, which could have influenced antibiotic usage practices. Importantly, the study was conducted during the implementtation of the Ministry of Health's order regarding the prescription-only release of antibiotics (22).

## CONCLUSIONS

1. Our study identified a low level of knowledge among patients with ARI regarding antibiotics and their effects. To address these gaps and improve attitudes towards antibiotic use, it is necessary to strengthen educational measures aimed at potential users.
2. The practices of using antibiotics from household reserves highlight issues related to the quantity of prescribed/dispensed antibiotics or the level of patient compliance. Purchasing antibiotics without a prescription from pharmacies underscores the need to strengthen control measures for enforcing current legislation regarding the prescription only release of antibiotics.

## CONFLICT OF INTEREST

In conducting this study, we had no conflicts of interest.

## ETHICAL APPROVAL

The study was approved by the Ethics Committee of the *Nicolae Testemitanu* State University of Medicine and Pharmacy (approval number 56 from 12.06.2015).

## ACKNOWLEDGEMENT

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# PUBLIC HEALTH PROBLEM OF RESISTANT BACTERIA IN LOW AND MIDDLE-INCOME COUNTRIES, FOLLOWING THE EXAMPLE OF MOLDOVA

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***Keywords:*** *antimicrobial resistance, bacteria, LMICs, susceptibility testing, bacteriophages, water treatment.*

***Introduction.*** *Antimicrobial resistance is an important public health concern. This phenomenon has become an environmental problem, due to the spread of resistant microorganisms in water. This problem is now more visible in Low-and Middle-Income Countries, where it increases the social burden. One of the newest methods to fight antimicrobial-resistant bacteria is the use of strain-specific bacteriophages.* ***Material and methods.*** *The bacterial strains were obtained from inpatients and identified using VITEK 2 Compact system and culture. The disk diffusion method was used to determine the resistance profiles, which were then analyzed using EUCAST methodology. The presence of resistance mechanisms was checked by phenotypic testing. For research purposes, 31 bacterial strains were selected.* ***Results.*** *The strains of K. pneumoniae, P. aeruginosa, Acinetobacter spp., S. aureus, E. coli, and Enterococcus spp. were identified. The resistance profile of the isolates revealed: 61.5% of K. pneumoniae isolates were pan-drug-resistant, while 23,1% were only susceptible to Carbapenems. E. coli strains were extensively drug-resistant, 71.4% of P. aeruginosa and 75% of Acinetobacter spp. were pan-drug-resistant bacteria. The susceptibility profile of S. aureus strains showed that 3/4 were resistant to Cephalosporins and Fluoroquinolones.* ***Conclusions.*** *The study identified all six highly virulent and antibiotic-resistant bacterial pathogens in low and middle-income countries and Moldovan hospitals. The analysis conducted in the study could serve as an argument for using bacteriophages in water treatment as a cost-effective method to combat antimicrobial resistance.*

***Cuvinte-cheie:*** *rezistența la antimicrobiene, bacterii, țări cu venituri mici și medii, teste de sensibilitate, bacteriofagi, tratarea apei.*

***PROBLEMA BACTERIILOR REZISTENTE PENTRU SĂNĂTATEA PUBLICĂ ÎN ȚĂRILE CU VENITURI MICI ȘI MEDII DUPĂ EXEMPLUL MOLDOVEI***

***Introducere.*** *Rezistența la antimicrobiene reprezintă un subiect important pentru sănătatea publică. Fenomenul în cauză a devenit o problemă de mediu, fiind cauzat de răspândirea microorganismelor rezistente în apă. Aces fapt este mai vizibil în țările cu venituri mici și mijlocii, unde crește povara socială. Una dintre cele mai noi metode de combatere a bacteriilor rezistente la antimicrobiene este utilizarea bacteriofagilor specifici tulpinii.* ***Material și metode.*** *Tulpinile de bacterii au fost obținute de la pacienții internați și au fost identificate cu ajutorul sistemului VITEK 2 Compact și prin metoda clasică. Metoda discdifuzimetrică a fost aplicată pentru a determina profilurile de rezistență, care au fost apoi analizate folosind metodologia EUCAST. Prezența mecanismelor de rezistență a fost verificată prin teste fenotipice. Pentru cercetare au fost selectate 31 de tulpini bacteriene.* ***Rezultate.*** *Au fost identificate tulpinile de K. pneumoniae, P. aeruginosa, Acinetobacter spp., S. aureus, E. coli și Enterococcus spp. Profilul de rezistență al izolatelor a relevat: 61,5% din izolatele de K. pneumoniae au fost pan-rezistente, iar 23,1% au fost sensibile doar la carbapeneme. Tulpinile de E. coli au demonstrat reziztență extinsă, 71,4% din P. aeruginosa și 75% din Acinetobacter spp. erau bacterii pan-rezizstente. Profilul de sensibilitate al tulpinilor de S. aureus a arătat că 3/4 erau rezistente la cefalosporine și fluorochinolone.* ***Concluzii.*** *Studiul a identificat toți cei șase agenți patogeni bacterieni, extrem de virulenți și rezistenți la antibiotice, în țările cu venituri mici și medii și în spitalele din Republica Moldova. Analiza efectuată în cadrul studiului poate servi drept argument pentru utilizarea bacteriofagilor în tratarea apei ca metodă rentabilă de combatere a rezistenței la antimicrobiene.*

**ABBREVIATIONS:** *AMR* – Antimicrobial Resistance, *ARB* – Antimicrobial Resistant bacteria, *ESBL* – extended-spectrum beta-lactamases, *EUCAST* – European Committee on Antimicrobial Susceptibility Testing, *LMICs* – low- and middle-income countries, *MDR* – multidrug resistant, *PDR* – pan-drug-resistant, *WHO* – World Health Organization *XDR* – extensively drug resistant.

## INTRODUCTION

In 1928, when Alexander Fleming discovered Penicillin – "the saving drug of the 20th century" – the glorious history of medicine began. Over the subsequent 60 years, the 13 classes of antibiotics that we still use to treat bacterial infections were discovered (1). Considering that the last 40 years have seen a multitude of epidemics and pandemics in which strictly human bacterial pathogens caused 44% of cases, contemporary medicine can only keep pace by using antimicrobials in treatment and prophylaxis, involving pre- and post-surgery antimicrobial and post-chemotherapy prophylaxis (2, 3, 4). Antibiotics have so far saved thousands of lives worldwide, but according to the laws of nature and ecosystems – everything must be in balance and living organisms must be constantly evolving. Thus, as early as 1940, the first enzyme that allowed *E. coli* strains to destroy penicillin was discovered (1, 5, 6). Since then, the phenomenon of antimicrobial resistance (AMR) has gained momentum, becoming a significant public health problem (5, 6, 7).

It should be noted that antimicrobial-resistant pathogens not only cause an increased number of deaths (mortality caused by multi-drug resistant (MDR) *P. aeruginosa* reaches up to 61% of cases, and pan-drug resistant (PDR) *K. pneumoniae* – maximum 71%) and disability, but also additional costs for hospitalization, treatment, and recovery, which cannot be accurately calculated (5, 8, 9). Specialists from various countries have concluded that the economic status of a country significantly influences the impact of AMR on the population, largely due to investment size in surveillance systems for antimicrobial resistance of microorganisms, but also to the presence and quality of alternative resources that can be used to fight infections (10). Some studies also list the private healthcare system as a determinant of AMR, citing the patient's substantial influence on antibiotic prescriptions due to commercial motivations (11).

Moreover, it is necessary to realize that humans are part of the ecosystem and are constantly influenced by other components of the ecosystem, especially as anthropogenic influence on the environment is unquestionable. It cannot be denied that people use chemicals, including antibiotics, in agriculture, fish farming, and animal husbandry to obtain richer harvests or better food-producing animals to compete on the market (12, 13, 14). Farms and animal husbandry are important sources of antimicrobial-resistant pathogens and an important component that ensures the continuous AMR cycle in the environment, due to animal care and handling processes including treatment, hygiene, and slaughter (14 - 17). However, by far the most important source of resistant microorganisms possessing genes for enzyme production (ESBLs – extended-spectrum beta-lactamases – or carbapenemases) is the sick human (whether or not admitted to a healthcare facility), whose contaminated biological products are discharged into water or released into the environment after minimal treatment, thus maintaining the AMR cycle (18 - 21). The limited number of methods used to combat AMR, combined with the preference of LMIC patients (and others) towards self-medication, are factors that make it difficult to align with the 2015 World Health Assembly Global Action Plan on AMR goals, particularly goals 4 and 5 (2, 3, 22, 23, 37). The overuse of antibiotics in LMICs, which has increased by 65% over the last decade, resulting in the emergence of multi-drug resistant (MDR) and extensively drug-resistant (XDR) superbugs, calls for innovative measures to combat AMR at the level of every component of the ecosystem (5, 7, 14, 19, 24). The European Union also emphasizes the role of Gram-negative bacteria in the etiology of infections with antimicrobial-resistant bacteria, which supports the results of World Health Organization (WHO) reviews concerning AMR (25). New antimicrobials and combinations are being sought and developed to combat MDR *Enterobacteriaceae* (e.g. Meropenem-Vaborbactam), but a cost-effective and less time consuming measure would be the use of bacteriophages on their own or in combination with usual antibiotics for per-

sonalized patient treatment, wastewater decontamination, farm animals, and crop plants (5, 8, 13, 23).

*This article aims to* identify and characterize potential targets for phages in bacteria circulating in the environment, originating from patients treated in medical institutions. These bacteria release biological fluids into the environment after minimal treatment. The focus lies on exploring the application of bacteriophages in LMICs as a cost-effective alternative to antibiotics and a method for water treatment, as they are readily available in nature and capable of development. This research gains significance, especially after WHO listed pathogens in 2017 that urgently demand new antibiotics to combat infections. Notably, ESKAPE pathogens account for over 70% of deaths attributed to antimicrobial resistance (AMR) (17, 26).

## MATERIAL AND METHODS

### *Obtaining isolates*

The isolates under investigation were sourced from patients admitted to *Timofei Mosneaga* Republican Clinical Hospital in Chisinau, Republic of Moldova. Substrates were collected only after meeting specific criteria: (I) the patient was over 18 years of age; (II) the patient consented to the use of the biological material for research; (III) the clinical picture of bacterial infection was established, and (IV) patients were admitted during the second and third quarters of 2022. Out of the total of isolates obtained, 31 strains met 4 criteria: (a) sourced from patients, (b) identifiable, (c) exhibited multi-resistance to antimicrobials, and (d) showed suspicion of a resistance mechanism. These were randomly selected and are described within this study.

### *Strain identification*

The classical method – culture – was used to purify the isolates. Identification down to genus and species was done using the automated method – Vitek 2 Compact (BioMérieux, France). The standard protocol followed these steps: (I) Preparation of a bacterial suspension in sterile 0.45% NaCl saline solution using 18-24-hour fresh cultures in 3 ml polystyrene tubes; (II) Ensuring the suspension's turbidity was approximately 0.5 McFarland (±0.05 McFarland) with DensiCheck; (III) Bringing the ID cards to room temperature and placing them in transfer tube cassettes, which were then placed in the bacterial suspension tubes; (IV) Placing the cards within the cassette in a vacuum and initiating the card filling cycle; (V) Transferring the filled card cassette to the analyzer to obtain results after several hours.

### *Determination of isolate susceptibility*

The resistance profile of the strains was determined using the disk diffusion method, with result interpretation following the EUCAST ver. 2022 standard. Mueller-Hinton solid medium and inoculum from a fresh 24h±6 h culture with a turbidity of 0.5 McFarland were used for this test. The procedure entailed: (a) Preparing the inoculum using sterile NaCl saline in 3 ml tubes and 3-5 colonies from a fresh culture; (b) Inoculating Petri dishes with Mueller-Hinton Agar medium using a swab; (c) Placing antibiotic-impregnated discs on the Petri dishes based on the species; (d) Incubating the Petri dishes with antibiograms at 37°C±1°C; (e) Reading and interpreting the results the following day according to EUCAST 2022 standards.

### *Determination of resistance mechanisms occurrence*

Screening tests for ESBL, double diffusion test (double disc method), Combo test, and phenotypic tests were used to detect ESBL enzymes: class A – KPC, class B – MBL (VIM, NDM, IMP), class C – AmpC, class D – OXA-48, OXA-23. Tests for other resistance mechanisms were also used: colorimetric tests for the detection of carbapenemases – PACE Normand Poirel; immunochromatographic tests for detection of enzymes such as OXA-23 – *Acinetobacter* spp., OXA-48, and MBL for enterobacteria and *Pseudomonas aeruginosa*.

### *Statistical analysis*

The proportion of strains among the total number of isolates, the prevalence of resistant bacterial strains within specific species, and the proportion of strains exhibiting phenotypically expressed resistance mechanisms were determined using the relative statistical indicator – proportion. These calculations were performed following the formulas:

$$\frac{No.of\ strains\ of\ particular\ species}{Total\ no.of\ bacterial\ strains} \text{x}100\ \% \qquad \text{(a)}$$

$$\frac{No.of\ bacterial\ strains\ of\ the\ species\ \ resistant\ to\ Antibiotic\ clas}{Total\ no.of\ strains\ of\ the\ species} \text{ x } 100\% \quad \text{(b)}$$

$$\frac{No.of\ bacterial\ strains\ of\ the\ \ species\ exposing\ a\ resistance\ mechanism}{Total\ number\ of\ strains\ of\ the\ species} \text{ x } 100\ \% \quad \text{(c)}$$

***Bibliosemantic method***

An online search was conducted in the PubMed and SCOPUS databases using the keywords: *antimicrobial resistance, bacteria, LMICs, susceptibility testing, bacteriophages, and water treatment.* This search yielded a total of 660 papers, from which 215 duplicates were removed. Articles that did not meet the specified criteria were also excluded:

- Written before 2012 (105 articles were excluded)
- Written in English (60 articles were excluded)
- Referring to ESKAPE pathogens in human health (65 articles were excluded)
- Not open access source (79 articles excluded)
- Not full-text type (99 papers excluded)

The remaining papers (n=37) were used as sources for the Introduction and the Discussion sections of this paper. Priority was given to articles detailing the status of ESKAPE pathogens in other LMICs globally, as well as in the three largest economies from distinct regions of the world: Japan (Asia), Germany (Europe), and the USA (Americas), for comparative analysis.

**RESULTS**

In the process of selecting strains for research, different biosubstrates were sampled. The proportion of biosubstrates was as follows: 8 isolates from blood and urine, 4 samples from pharyngeal swabs and wound content. Six samples were obtained from different biosubstrates: sputum, bronchoalveolar lavage, and feces. The isolated species were: *E. coli, K. pneumoniae, S. aureus, E. faecium, P. aeruginosa,* and *Acinetobacter* spp. (fig.1).

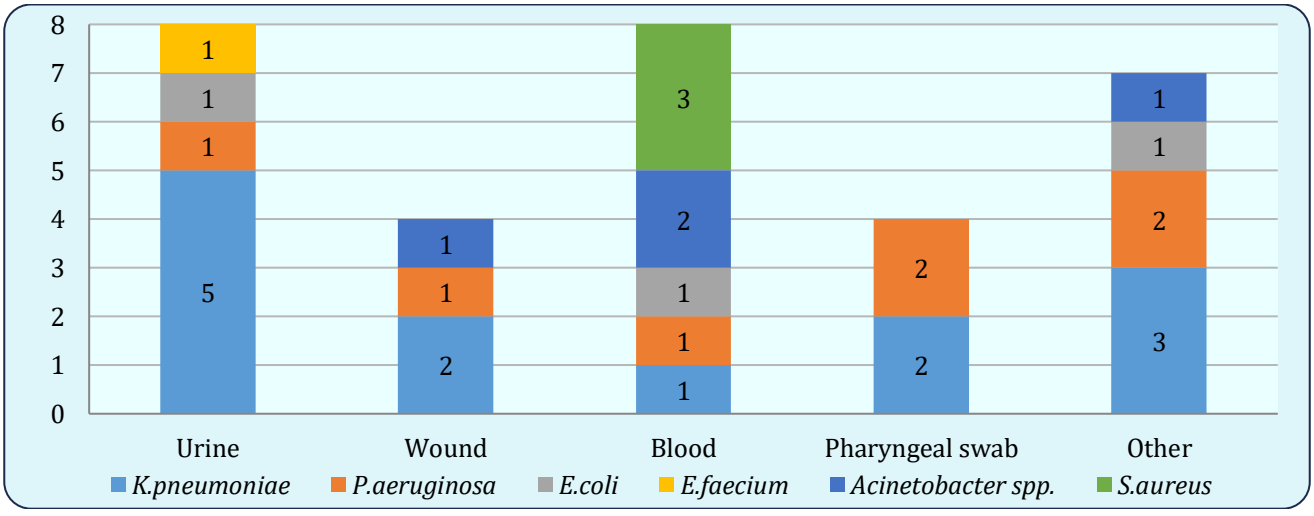


Figure 1. Biosubstrates investigated and bacterial species identified in each biosubstrate, absolute numbers.

Out of the 31 strains selected for research, 41.93% were *K. pneumoniae,* 22.58% were identified as *P. aeruginosa*, followed by *E. coli* and *Acinetobacter* spp. with 9.68% and 12.9%, respectively. *S. aureus* and *Enterococcus* spp. were identified in 9.68% and 3.23% of cases, respectively. Subsequently, the resistance profile for each isolate was determined and then grouped by species. Thus, 8 out of 13 isolates (61.5%) of *K. pneumoniae* were found to be PDR micro-organisms (non-susceptible to all commercially available antimicrobial agents), and 3 others (23.07%) were XDR strains, being susceptible only to Carbapenems. *E. coli* isolates (n=3) showed distinct

susceptibility patterns with selective susceptibility to agents of each class. It should be noted that for ord. Enterobacterales susceptibility to Penicillins, Cephalosporins, Carbapenems, Fluoroquin olones, and Aminoglycosides was tested, with a total of 16 antibiotics. Susceptibility testing of *P. aeruginosa* isolates included the same 5 classes of antibiotics (7 antibiotics) and the results are included in Table 1. The antibiotics tested from each class were: Piperacillin-tazobactam from Penicillins; Ceftazidime and Cefepime from Cephalosporins; Imipenem and Meropenem from Carbapenems; Ciprofloxacin from Fluoroquinolones and Amikacin from Aminoglycosides, respectively.

Table 1. Results of susceptibility testing of *P. aeruginosa* isolates.

| Isolate | Penicillins | Cephalosporins | Aminoglycosides | Fluoroquinolones | Carbapenems |
|---|---|---|---|---|---|
| **Isolate no. 13** | R | R | R | R | R |
| **Isolate no. 16** | R | R | R | R | R |
| **Isolate no. 17** | R | I | R | R | I |
| **Isolate no. 18** | I | I | S | I | I |
| **Isolate no. 22** | R | R | R | R | R |
| **Isolate no. 25** | R | R | R | R | R |
| **Isolate no. 29** | R | R | R | R | R |

*Note*: R – resistant; S – susceptible; I – intermediate (susceptible, increased exposure)

*The interpretations in the table mean that the results obtained were the same for all tested antimicrobials belonging to the same class.

The statistics showed that 71.4% of the isolates were PDR strains and one isolate (14.28%) showed characteristics of an XDR strain. Of all isolates, only one was susceptible to aminoglycoside antibiotics.

The 4 isolates identified as *Acinetobacter* spp. were tested for susceptibility to 6 antimicrobials categorized into 3 groups: Aminoglycosides, Fluoroquinolones, and Carbapenems. The results showed that 3 out of 4 isolates are PDR strains, and the fourth is susceptible only to Aminoglycosides (Tobramycin, Gentamycin, and Amikacin). The same testing procedure was done for *S. aureus* strains (tab. 2). Susceptibility of *S. aureus* strains was tested for: Cefoxitin (Cephalosporins), Ciprofloxacin, Ofloxacin, Norfloxacin (Fluroquinolone); Vancomycin (Glycopeptides and lipoglycopeptides), and Linezolid (Oxazolidinones).

Table 2. Resistance profile of *S. aureus* strains, n=4.

| Isolate | Cephalosporins | Fluoroquinolones | Glycopeptides | Oxazolidinones |
|---|---|---|---|---|
| **Isolate no. 8** | R | I | S | S |
| **Isolate no.18** | R | R | S | S |
| **Isolate no.19** | R | R | S | S |
| **Isolate no.30** | R | R | S | S |

Note: R – resistant; S – susceptible; I – intermediate (susceptible, increased exposure)

*The interpretations in the table mean that the results obtained were the same for all tested antimicrobials belonging to the same class.

The resistance profile of *S. aureus* strains showed that they were 100% susceptible to Glycopeptides and Oxazolidones. They were also 100% resistant to Cephalosporins and 3 out of 4 isolates were also resistant to Fluoroquinolones. The only isolate of the genus Enterococcus (*E. faecium*) was susceptible to Vancomycin and Linezolid and was resistant to Ampicillin.

Testing for the presence of resistance mechanisms revealed that among the 13 *K. pneumoniae* isolates, one exhibited the ESBL mechanism, while eight showed the presence of carbapene-

mases. In all cases, the OXA-48 type enzyme (subtype NDM) was detected in 62.5% of these instances (confirmed by immunochromatographic tests). In the other cases, although initially the strains were suspected of resistance mechanisms, this was not confirmed phenotypically. In the case of *E. coli,* 2 out of 3 isolates were ESBL-producing strains, the third being negative for both tested mechanisms. In the case of the 7 *P. aeruginosa* strains, testing for the presence of resistance mechanisms gave the following results: 71.42% (n=5) tested negative for carbapenemases, and in the case of the other 2 isolates, the result was positive, with one isolate producing NDM and the second, VIM enzymes. Finally, the double diffusion test and immunochromatographic tests of *Acinetobacter* spp. showed that 2 isolates (50%) were OXA-23 enzyme-producing strains, and the remaining were not.

## DISCUSSIONS

Throughout our research on AMR, with a focus on infection etiology and the status of circulating strains (MDR, XDR, PDR), we have consistently found that antimicrobial resistance is a global problem, but the level of understanding and depth of approach varies from country to country and region to region. Consequently, we were able to compare our results with those obtained in individual country studies as well as regional and global studies. Inoue K. et al. concluded that mortality from infections with ESBL-producing microorganisms is higher compared to the rest of the ARB (22). The study by Silvester R. et al. showed that *K. pneumoniae* in all LMICs tends to exhibit ESBL resistance mechanisms, CP as well as combined genotypes, and these cause a greater burden on the healthcare system, especially as there are cases where healthy people are reservoirs of enzyme-producing bacteria, but also because they are the most frequently isolated bacteria encountered in hospital settings (10, 17, 20). Our study aligns with these findings, as Moldova is an LMIC, and AMR, as a multidimensional process, is a significant concern for the healthcare system.

However, many studies focus on various aspects of AMR occurrence and "frequency" in the health system, society, and the environment. Three studies conducted in Cameroon, Morocco, and Vietnam showed *Enterobacteriaceae* as the most frequently isolated microorganisms, followed by *S. aureus*, as we determined, but Camara N. and Haeeb A. determined an opposite situation in Tanzania and Saudi Arabia (2, 9, 27, 28, 29). When analyzing isolates according to the source (biosubstrate), studies focusing on uropathogens showed that *E. coli* is the most frequent uropathogen followed by *K. pneumoniae* (in Ethiopia and USA), compared to 2 studies from Romania showing 2 distinct situations - one study showed a similar situation as in Ethiopia, and the second one showed a distinct situation – with *P. aeruginosa* on the first place similar to the one in Mexico (14, 25, 30 31, 32). Still, none of them delivered results similar to the ones in this paper.

Finally, the comparative analysis of ESKAPE pathogen resistance profiles in different countries and regions is equally diverse. For *E. coli* isolates, studies in Tanzania showed the highest rate of resistance to Penicillins and Aminoglycosides, which correlates with the results of similar studies in Greece, Romania and Mexico and is similar to the results obtained in this paper (2, 31, 32, 33). However, there are also studies showing higher rates of resistance to other classes of antimicrobials – Fluoroquinolones and Cephalosporins (9, 32). Regarding *K. pneumoniae*, this study had similar results to those from Mexico and Tanzania, where the isolates had the second highest proportion of MDR profiles, and Morocco, where in addition to MDR status an alarming rate of carbapenemase-producing strains was detected (2, 29, 32). The results obtained on carbapenemase production by ord. Enterobacterales are in contrast to those obtained by researchers in Vietnam (LMIC) and Germany (9, 34). *P. aeruginosa*, another important microorganism in the etiology of infectious diseases, is one with a high proportion of PDR strains in this study, which is however very rarely found in other regions and countries, such as China, Greece, EU (33, 34, 35). The situation in *Acinetobacter* spp. is not much different in the same regions.

If we look at the Gram-negative microorganisms as a whole, part of the ESKAPE group, the trend of MDR strains (either XDR or PDR) circulation is constantly increasing, even reaching 100% in some LMICs. This phenomenon will inevitably create huge treatment costs, potentially amounting to tens of millions of dollars, as estimated by Australian epidemiologists (26, 29, 32, 36), calculated for a one-year period in Australia. LMICs do not have sufficient financial resources to treat pa-

tients and combat the long-term effects of reduced work capacities of the ill population, as well as the environmental damage and impacts on other economic sectors. To this end, both our study and the analyzed research focused on the description of pathogens of the ESKAPE group, the latter being potential targets for treatment with specific bacteriophages after their unintentional discharge into surface waters or sewage systems. Given the ubiquitous presence of bacteriophages in the environment and their ease of development, their use is much more economically efficient for water treatment and for slowing down the spread of AMR in the environment, and consequently, for minimizing the danger that this phenomenon poses for the human population.

## CONCLUSIONS

1. AMR is an ongoing and significant issue that can lead to serious consequences. The data and analysis from this study demonstrate that AMR is a prevalent concern in healthcare institutions, irrespective of regional variations and circumstances.
2. To comprehend the AMR characteristics within the country, it is imperative to conduct an initial analysis on a small scale. This will facilitate the development of a comprehensive overview that can be compared with the regional situation.
3. This study revealed the concerning presence of AMR among ESKAPE pathogens in Moldovan hospitals, with limited treatment options available. This necessitates an urgent response and the exploration of innovative solutions.
4. This study stands as a cornerstone for the development of optimal solutions to combat AMR in the country, as it includes the analysis of potential cost-effective treatment targets.
5. Given the country's economic conditions, research and interventions aimed at characterizing the AMR phenomenon and identifying/developing bacteriophage targets for water treatment will serve as a crucial starting point to mitigate the impact of AMR. This is particularly significant considering that phages are present in the environment where resistant bacteria from hospitals ultimately contribute to the cycle of the AMR phenomenon.

## CONFLICT OF INTEREST

The authors declare no conflict of interest in relation to the study and this paper.

## ACKNOWLEDGEMENT

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## ETHICAL APPROVAL

The research protocol was positively approved by: (i) the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy no. 7 issued on 09.01.2022 and (ii) the National Committee for Clinical Trial Ethics of the Ministry of Health of Republic of Moldova No. 1245 issued on 26.01.2022. The informed consent of every patient was obtained prior to sample collection procedure.

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# EVALUATION OF COSTS RELATED TO ANTIMICROBIAL RESISTANCE OF PRIORITY GRAM-NEGATIVE BACILLI

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***Keywords:*** *economic impact, antimicrobial resistance, Gram-negative bacilli, invasive infections, burden of AMR.*

***Introduction.*** *Antimicrobial Resistance (AMR) is one of the most serious threats to global public health, causing over 700 thousand deaths annually. In addition to the social burden, AMR generates high medical costs, and estimating these at the hospital level has been the subject of research in many international studies.*

***Material and methods****. A comprehensive study was conducted, encompassing 172 cases of invasive infections recorded in patients hospitalized at the "Timofei Mosneaga" Republican Clinical Hospital, from which strains of antimicrobial-resistant E. coli, K. pneumoniae, A. baumannii, and P. aeruginosa were isolated in the years 2019-2021. Based on data regarding the duration of hospitalization, treatment costs, and expenses for investigations, indicators such as average cost, the median, minimum and maximum values of treatment, including the cost of antibiotics and laboratory analyses, were calculated.*

***Results.*** *The economic impact indicators of AMR at the hospital level include: the duration of hospitalization, the total costs of a treated case, the costs of antibiotics, and the costs of laboratory analyses. In the structure of microorganisms isolated from the patients included in the study, K. pneumoniae predominates, accounting for 44.2%, and the total treatment cost for this organism was the highest at 202731.5 dollars. The maximum duration of hospitalization was recorded in patients from whom A. baumannii was isolated (27.7 days).*

***Conclusions.*** *The resistance of strains isolated from patients has directly contributed to the extension of the hospitalization period. The resistance of A. baumannii and K. pneumoniae species has predominantly led to the increase in the economic burden of AMR.*

***Cuvinte-cheie:*** *impact economic, rezistență la antimicrobiene, bacili Gram-negativi, infecții invazive, povara RAM.*

***EVALUAREA COSTURILOR AFERENTE REZISTENȚEI LA ANTIMICROBIENE A BACILILOR GRAM-NEGATIVI PRIORITARI***

***Introducere.*** *Rezistența la antimicrobiene (RAM) reprezintă una dintre cele mai grave amenințări la adresa sănătății publice la nivel global, determinând anual peste 700 mii de decese. Pe lângă povara socială, RAM generează costuri medicale înalte, iar estimarea acestora la nivel de spital a constituit obiectul de cercetare a multor studii internaționale.*

***Material și metode.*** *A fost realizat un studiu integral, care cuprinde 172 cazuri de infecții invazive, înregistrate la pacienții internați în Spitalul Clinic Republican „Timofei Moșneaga", de la care au fost izolate tulpini de E. coli, K. pneumoniae, A. baumannii și P. aeruginosa rezistente antimicrobiene, în anii 2019-2021. În baza datelor privind durata spitalizării, costurile tratamentului și cheltuielile pentru investigații, au fost calculați indicatorii: costul mediu, mediana, valorile minime și maxime ale tratamentului, inclusiv costul antibioticelor și al analizelor de laborator.*

***Rezultate.*** *Indicatorii impactului economic al RAM la nivel de spital sunt: durata spitalizării, costurile totale ale unui caz tratat, ale antibioticelor și ale analizelor de laborator. În structura microorganismelor izolate de la pacienții incluși în studiu, predomină K. pneumoniae, cu o pondere de 44,2%, pentru care și costul total al tratamentului a fost maxim – 202731.5 dolari. Durata de spitalizare maximă s-a înregistrat la pacienții de la care s-a izolat A. baumannii (27,7 zile).*

***Concluzii.*** *Rezistența tulpinilor izolate de la pacienți a contribuit în mod direct la extinderea duratei de spitalizare. Rezistența speciilor A. baumannii și K. pneumoniae au determinat în cea mai mare măsură creșterea poverii economice a RAM.*

## INTRODUCTION

The burden caused by microorganisms resistant to antimicrobial agents is a very heavy one for all of humanity, and especially a daily challenge for doctors in intensive care units. In these units, multidrug-resistant Gram-negative bacteria are responsible for 45-70% of cases of ventilator-associated pneumonia, 20-30% of cases of bloodstream infections, and urinary tract infections associated with catheters (1).

Currently, there is a significant increase in the number of infectious diseases worldwide. The species *Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Escherichia coli* are the most common pathogens involved in the etiology of infectious diseases. In 2017, they were included in the global surveillance list developed by the World Health Organization (WHO) (2).

Significant depleted stocks of antimicrobial agents, the rise of complex cases of infections with resistant microorganisms both in hospital and community settings have conditioned the urgent need for the development of new, effective antimicrobial agents against resistant microorganisms (2).

Antibiotic resistance in Gram-negative bacilli involved in major infectious pathology significantly increases healthcare costs, the severity of infections, as well as death rates. According to studies, annually there are over 700 thousand deaths due to bacterial resistance, with the potential for a rapid increase in this figure to 10 million deaths in the next 30 years, accompanied by a cumulative cost of 100 trillion dollars, in the absence of effective measures for prevention and combating this phenomenon (3, 4).

The World Bank has estimated that without preventive and control measures, the global economic damages caused by infections from antimicrobial-resistant microorganisms could lead to a financial crisis equivalent to that of 2008-2009 by the year 2050. Additionally, by 2050, this phenomenon could annually decrease the global gross domestic product (GDP) by 1.1%, with its deficit exceeding 1 trillion dollars annually, starting as early as 2030 (5).

According to a study conducted by Nelson et al. (2017) in a hospital in the USA, the annual cost for treating patients with healthcare-associated infections (HAI) caused by *A. baumannii* was $39,787.0, including $74,306.0 for HAI treatment and $62,396.0 for community-acquired infections caused by the same species (6).

Thus, combating AMR is globally recognized as one of the priorities in public health due to its social impact and the economic harm it inflicts on the healthcare system. Measures for preventing and controlling this phenomenon should be based on the results of monitoring resistance to antimicrobial agents (1, 2).

Monitoring the consumption of antimicrobials among the population, both in the community and in hospital institutions, is a first step in the fight against the spread of antimicrobial resistance (AMR). These data will contribute to forming an overall picture of the prescription, dispensing, and use of antimicrobial agents (2).

According to research in the Republic of Moldova, Gram-negative bacilli isolated from patients with invasive infections also exhibit elevated resistance rates. It has been estimated that the highest resistance rates for strains of E. coli and K. pneumoniae were recorded for the penicillin group (73.9% and 91.8%, respectively), followed by the third-generation cephalosporin group with 56.5% and 94.1%, respectively, of resistant strains. For *P. aeruginosa* strains, there is alarming resistance to aminoglycosides (81.8%), as well as to last-resort drugs – carbapenems (72.7%). Strains of A. baumannii show resistance of over 90% of the isolated strains practically to all groups of antimicrobial agents (1, 5).

An important method for assessing the economic impact of AMR is evaluating the treatment costs at the hospital unit level, which has its own financial resources. These resources are directed towards the most rational use of the available budget, aiming to achieve efficiency both in terms of treatment and economically (6).

Numerous international studies highlight the main indicators for measuring the economic impact of treating patients with infections caused by resistant microorganisms within the hospital setting. These studies include a detailed analysis of the economic burden caused by AMR, reflected in the medical costs or the duration of hospitalization for patients (6, 7).

The study was conducted to assess the costs associated with the treatment of patients with infections caused by Gram-negative bacilli resistant to antimicrobials, who were hospitalized at the Republican Clinical Hospital during the years 2019-2021.

## MATERIAL AND METHODS

According to the Regulation on the National System for Epidemiological Surveillance of Antimicrobial Resistance (SSERAM) approved by Order of the Ministry of Health, Labour and Social Protection (MSMPS) 711/2018, within the National System for Epidemiological Surveillance of Antimicrobial Resistance (SSERAM), strains of clinically significant Gram-negative bacilli, including *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Acinetobacter* spp., are reported in blood and cerebrospinal fluid (CSF).

A comprehensive study was conducted, encompassing all cases of invasive infections caused by strains of *E. coli, K. pneumoniae, P. aeruginosa*, and *A. baumannii* with resistance mechanisms, recorded during the period 2019-2021. Thus, 172 cases of invasive infections were studied during that period.

For the execution of this study, the records accompanying the strains of Gram-negative bacilli isolated in the Microbiological Laboratory of the *Timofei Mosneaga* Republican Clinical Hospital, which has been part of the National AMR Surveillance Network since 2018, were examined and sent to the national reference laboratory for AMR to confirm the resistance mechanisms. Additionally, the medical records of the hospitalized patients from whom these strains were isolated during the period 2019-2021 were also examined.

Excluded from the study were:

- patients with invasive infections from whom strains of *Escherichia coli* have been isolated from blood and/or CSF, resistant to one or more drugs from the beta-lactam group;
- patients with invasive infections from whom strains of *Klebsiella pneumoniae* have been isolated from blood and/or CSF, resistant to one or more drugs from the beta-lactam group;
- patients with invasive infections from whom strains of *Acinetobacter baumannii* have been isolated from blood and/or CSF, resistant to one or more drugs from the beta-lactam group;
- patients with invasive infections from whom strains of *Pseudomonas aeruginosa* have been isolated from blood and/or CSF, resistant to one or more drugs from the beta-lactam group;

Excluded from the study were:

- patients from whom strains of Gram-positive microorganisms were isolated;
- patients from whom strains of Gram-negative bacilli sensitive to all groups of antimicrobial agents were isolated;
- patients from whom strains of Gram-negative bacilli, not included in the global surveillance list, were isolated;
- patients from whom strains of Gram-negative bacilli were isolated from biosubstrates other than blood and cerebrospinal fluid.

The collected patient data, based on the personal records in the Archive of the *Timofei Mosneaga* Republican Clinical Hospital, focused on the duration of hospitalization, the total cost of treatment within the hospital, the cost of antimicrobial agents used for treatment, and the cost of laboratory analyses for each treated case.

A database was created in Excel, organizing the obtained information, and subsequent calculation of respective statistical indices, such as mean values, the median, minimum, and maximum values for the costs of each parameter. The indicators were calculated cumulatively for all three years and individually for each isolated resistant pathogen.

The normality of the data distribution was tested using the Shapiro-Wilk and d'Agostino-Pearson methods. To determine the statistical significance of the observed differences, the non-parametric Kruskal-Wallis test was conducted. When obtaining a p-value less than 0.05, the null hypothesis was rejected, concluding that there is a statistically significant difference between the observed variables.

## RESULTS

The processing of the respective data collected within the *Timofei Moşneaga* Republican Clinical Hospital (RCH) allowed for the evaluation of cases of infections with resistant microorganisms concerning the costs associated with AMR. Figure 1 presents data on the general characteristics of resistant pathogens isolated from 172 patients hospitalized during the period from 2019 to 2021.

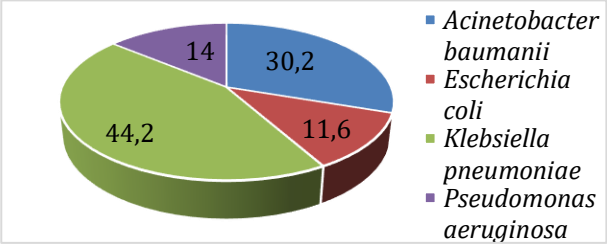


Figure 1. The cumulative percentage share (%) of resistant pathogens isolated from blood and CSF from patients hospitalized in the RCH during the years 2019-2021.

Based on the data presented in figure 1, it is observed that the majority of Gram-negative bacterial infection cases reported by the RCH during the period from 2019 to 2021 are caused by *K. pneumoniae* – 44.2±7.6%, followed by *A. baumannii* with a share of 30.2±7.0%, *P. aeruginosa* with 14.0±5.2%, and *E. coli* with 11.6±4.8%.

As a result of the analysis, no statistically significant differences (p>0.05) were determined regarding the duration of hospitalization based on the isolated Gram-negative bacterial species. The median value of this indicator varied from 19 days (min=4, max=98 days) for *P. aeruginosa* to 24 days (min=3, max=66 days) for *A. baumannii* (tab. 1).

The total costs for the treatment of a patient during hospitalization included: expenses for antimicrobial agents used, expenses for other types of medications besides antimicrobial agents, expenses for clinical and paraclinical investigations, as well as expenses for laboratory investigations.

Based on the costs recorded for the patients included in the study, the median cost of a case of infection with the isolated resistant microorganism was calculated using the SPSS software. Thus, it was found that the total treatment cost, estimated through the Kruskal-Wallis test, was higher for patients affected by *A. baumannii* strains compared to *E. coli* (p=0.0004) and *P. aeruginosa* (p=0.0123), while the observed difference compared to *K. pneumoniae* was not statistically significant (p=0.0571). Similarly, the difference in treatment costs for patients with *E. coli* compared to those from whom *P. aeruginosa* was isolated was not significant (p=0.2385).

The median value of the total cost varied from 1,328 thousand dollars (min=0,100 thousand dollars; max=12,109 thousand dollars) for *E. coli* to 2,841 thousand dollars (0,406-13,187 thousand dollars, respectively) for *A. baumannii* (tab. 2).

Table 1. The duration of hospitalization for patients with infections caused by antimicrobial-resistant microorganisms, years 2019-2021 (days).

| Bacterium | Duration of hospitalization | | | | |
|---|---|---|---|---|---|
| | *n* | mean (SD) | the median | min | max |
| ***Acinetobacter baumannii*** | 1441 | 26.9 (14.9) | 24 | 3 | 66 |
| ***Escherichia coli*** | 458 | 23.2 (24.5) | 20 | 1 | 91 |
| ***Klebsiella pneumoniae*** | 2446 | 26.5 (18.2) | 23 | 1 | 118 |
| ***Pseudomonas aeruginosa*** | 614 | 25.6 (21.3) | 19 | 4 | 98 |

Table 2. The total costs incurred by the hospital for the treatment of cases of infections with resistant microorganisms isolated from patients during the period 2019-2021 (thousands of dollars).

| Bacterium | Cost of treatment | | | | |
|---|---|---|---|---|---|
| | *n* | mean | median | min | max |
| ***Acinetobacter baumannii*** | 165.435 | 3.181 | 2.841 | 0.406 | 13.187 |
| ***Escherichia coli*** | 396.430 | 1.972 | 1.328 | 0.100 | 12.109 |
| ***Klebsiella pneumoniae*** | 202.732 | 2.668 | 2.291 | 0.191 | 10.997 |
| ***Pseudomonas aeruginosa*** | 50.761 | 2.115 | 1.679 | 0.525 | 6.613 |

In addition to the total treatment costs, the cost specifically for antimicrobial agents was also estimated, using the Kruskal-Wallis test.

The antimicrobial agents used for the treatment of patients included ciprofloxacin, levofloxacin, vancomycin, ceftazidime, meropenem,

ertapenem, imipenem, erythromycin, amoxicillin, amoxicillin clavulanic-acid, ceftriaxone, cefoperazon, cefoperazon-sulbactam, ceftazidim, ceftriaxon, cefuroxim, piperacillin-tazobactam, colistin, metronidazole, gentamicin, amikacin. The doses and duration of administration varied depending on the patient.

The highest expenses for antimicrobial agents used for a case of infection were recorded in patients with infections caused by *A. baumannii*, compared to infections caused by *E. coli* (p=0.0008) and *K. pneumoniae* (p=0.0003). However, the difference in the median cost between the treatment of infections caused by *A. baumannii* and those caused by *P. aeruginosa* was not statistically significant (p=0.0505).

The median cost value for antimicrobial agents varied from 0,112 thousand dollars (min=0.001 thousand dollars; max=1,002 thousand dollars) for *E. coli* infections to 0,453 thousand dollars (0,008-1,720 thousand dollars, respectively) for *A. baumannii* infections (tab. 3).

The data obtained for the indicators calculated in the SPSS statistical software, based on the expenses for laboratory investigations, using the same Kruskal-Wallis test, indicate higher median values for infections caused by *A. baumannii* with 0,661 thousand dollars (min=0,194 thousand dollars; max=2,625 thousand dollars). The lowest median values were recorded for investigations of infections caused by *E. coli* – 0,445 thousand dollars (min=0,056 thousand dollars; max=3,522 thousand dollars). As a result of the analysis, no statistically significant differences (p>0.05) were determined regarding the costs of laboratory analyses based on the isolated agent (tab. 4).

Table 3. The cost of antimicrobial agents used in cases of infections caused by resistant bacteria during the period 2019-2021 (thousands of dollars).

| Bacterium | Cost of antibiotics | | | | |
|---|---|---|---|---|---|
| | ***n* (%)** | **mean** | **the median** | **min** | **max** |
| ***Acinetobacter baumannii*** | 26.022 (15.7) | 0.500 | 0.453 | 0.008 | 1.720 |
| ***Escherichia coli*** | 4.445 (1.1) | 0.222 | 0.112 | 0.001 | 1.002 |
| ***Klebsiella pneumoniae*** | 27.377 (13.5) | 0.360 | 0.193 | 0.009 | 3.298 |
| ***Pseudomonas aeruginosa*** | 8.793 (17.3) | 0.366 | 0.280 | 0.031 | 1.772 |

Table 4. The costs related to expenses for laboratory analyses conducted on patients during hospitalization, 2019-2021 (thousands of dollars).

| Bacterium | Cost of investigations | | | | |
|---|---|---|---|---|---|
| | ***n* (%)** | **mean (SD)** | **the median** | **min** | **max** |
| ***Acinetobacter baumannii*** | 41.213 (24.9) | 0.793 | 0.661 | 0.194 | 2.625 |
| ***Escherichia coli*** | 13.559 (34.4) | 0.678 | 0.445 | 0.056 | 3.522 |
| ***Klebsiella pneumoniae*** | 58.374 (28.8) | 0.775 | 11.128 | 0.065 | 3.560 |
| ***Pseudomonas aeruginosa*** | 14.111 (27.8) | 0.588 | 8.9905 | 0.106 | 1.274 |

The extensive number of studies aiming to quantify the costs associated with AMR at the hospital level either focuses on the overall hospital expenditures or on department-specific expenses. These studies concentrate on indicators such as the duration of hospitalization, antibiotic costs, laboratory analysis costs, total costs per treated case, which are also the focal points of the present study (8 - 11).

The Gram-negative bacilli included in the research were isolated from blood samples taken from patients with invasive infections, similar to the studies conducted by Dos Santos et al. and Lashari et al. These patients were in critical condition and admitted to intensive care units (12, 13).

In the etiological spectrum of invasive infections, the species *K. pneumoniae* predominates with a percentage of 44.2%, followed by *A. baumannii* with 30.2%. This finding is in line with studies conducted by Lashari et al. and Nelson et al. (11, 13).

A study conducted in 10 hospitals in Korea estimated that the hospitalization duration of patients who contracted an infection with resistant *A. baumannii* was the highest compared to patients from whom other Gram-negative microorganisms were isolated (14). Similarly, in the current research, it was found that the hospitalization duration was longer for patients with infections caused by *A. baumannii.*

A shorter duration of hospitalization was observed for patients with infections caused by *E. coli*, as found by Hernandez-Pastor et al. in a study based on the PINC AI Healthcare US database. This reduced duration of hospitalization was also recorded in that particular study (15).

The majority of the analyzed studies included patients from whom strains of Gram-negative bacilli resistant to antimicrobial agents were isolated, a criterion that the present study also focused on, evaluating the costs associated with these bacilli during the patient's hospitalization (16, 17, 18).

Another calculated indicator for assessing the economic impact determined by AMR was the total cost of treatment during a patient's hospitalization. This indicator had maximum values for patients from whom antimicrobial-resistant *A. baumannii* was isolated, followed by patients with infections caused by resistant *K. pneumoniae*. Similar findings were also observed in the studies conducted by Lashari et al., Huang et al., and Wilson et al. (8, 13, 19).

In the case of costs for antibiotics used in treatment, as well as expenses for laboratory investigations, patients who contracted infections with *A. baumannii* are at the forefront with the highest expenditures, results similar to those observed in the research. After *A. baumannii*, the highest antibiotic costs were recorded for patients from whom *P. aeruginosa* was isolated, a finding also noted by Blanchette et al. and Zhen et al (7, 20).

Values dissimilar to the studies by Tabak et al., Zhen et al., and Kim et al. were determined for the costs of laboratory investigations, which placed *P. aeruginosa* in the second position, and the current research found higher values for this indicator for *K. pneumoniae* after *A. baumannii* (7, 14, 21).

The duration of hospitalization, as well as all treatment-related costs for patients with infections caused by resistant pathogens, have consistently been higher for patients with infections caused by antimicrobial-resistant *A. baumannii* (11, 14).

The study conducted by us has the following limitations:

- it is based on data collected over a period of 3 years. To conduct a more comprehensive investigation, data over a longer period, adjusted for annual inflation, is needed;
- lack of a control group to perform comparative calculations of hospitalization duration, treatment cost, and investigations for patients with infections caused by resistant and sensitive microorganisms, respectively, to determine incremental cost;
- patients were not separated into those with community-acquired infections and those with healthcare-associated infections;
- 47.7% of the patients died during hospitalization, which influenced the estimated costs for the treatment of patients with infections caused by antimicrobial-resistant Gram-negative bacilli.

Taking these aspects into account, further research in this field could develop the estimation of the economic impact of AMR and perform more complex calculations.

## CONCUSIONS

The application of the methodology for estimating the economic impact of AMR based on the data collected during the period 2019-2021 has revealed the following:

1. In the etiological structure of infections caused by resistant microorganisms, the species *K. pneumoniae* predominates with a share of 44.2%.
2. The hospitalization period for patients with infections caused by antimicrobial-resistant *A. baumannii* proved to be the longest, with the median value of infection cases being 24 days.
3. The highest costs were recorded for the treatment of patients with infections caused by resistant *A. baumannii*, with the median cost of treatment being 2,841 dollars.

4. The median cost of antibiotics for an infection case was higher for the treatment of patients with infections caused by resistant *A. Baumannii* – 0,453 dollars. Similarly, the median value of laboratory analysis costs was highest for infections caused by this pathogen.

Based on the above, it is observed that antimicrobial resistance in K. pneumoniae and A. baumannii species largely contributes to the increased economic burden of AMR. Therefore, it is imperative to pay attention and allocate sufficient resources to preventive and control measures for infections caused by these bacteria.

Following the conducted research on assessing the costs related to antimicrobial resistance of priority Gram-negative bacilli, it can be concluded that as a logical next step, comparative studies on diseases caused by other microbial agents would be welcomed.

## CONFLICT OF INTEREST

No author reported any conflicts of interest.

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## ETHICAL APPROVAL

Favorable opinion of the Research Ethics Committee of the Nicolae Testemițanu State University of Medicine and Pharmacy, No. 1, dated September 27, 2022.

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## BOOK REVIEWS AND PRESENTATIONS – REVUES DE LIVRES ET PRÉSENTATIONS


# Recenzie la monografia *„Metode instrumentale în cercetarea şi analiza medicamentelor”*, autoare dr. şt. farm., conf. univ. Livia UNCU

Monografia „Metode instrumentale în cercetarea şi analiza medicamentelor”, reprezintă o abordare detaliată şi specializată în domeniul analizei medicamentelor. Această temă este importantă în contextul dezvoltării rapide a cercetării în domeniul farmaceutic şi a necesităţii de a asigura calitatea, eficacitatea şi siguranţa medicamentelor. Actualitatea subiectului este relevantă în contextul în care noi medicamente sunt dezvoltate constant, iar reglementările privind analiza şi testarea acestora devin tot mai stricte. Metodele instrumentale moderne reprezintă un aspect esenţial în asigurarea calităţii medicamentelor, oferind sensibilitate sporită în detectarea şi caracterizarea substanţelor active.

Pornind de la bazele utilizării metodelor instrumentale de analiză în capitolul 1, autoarea prezintă reperele metodologice ale analizei şi principiile de bază ale proiectării unei analize, caracterizează funcţiile de bază ale instrumentelor analitice. O abordare reuşită a procesului de pregătire a probelor pentru analiză este redată în următorul capitol, prin caracterizarea detaliată a metodelor de prelucrare a mostrelor, eşantionare, diverse tehnici de purificare. De menţionat, că majoritatea publicaţiilor dedicate metodelor de analiză sunt axate pe abordarea teoretică a tehnicilor instrumentale şi nu se referă la aspecte specifice ale procesului de măsurare, comparare a rezultatelor sau la importanţa interferenţelor în analiză şi modalităţile de evitare a acestora. În acest aspect, prezenta momografie asigură o prezentare complexă a domeniului.

Importanţa metodelor instrumentale moderne în analiza medicamentelor rezidă în capacitatea lor de a oferi rezultate precise, repetabile şi rapide. Autoarea caracterizează toate tehnicile avansate utilizate în analiza şi cercetarea medicamentelor, cum ar fi de exemplu, spectroscopia de masă, cromatografia de lichide de înaltă performanţă. Fiecare metodă este redată prin prisma avantajelor, dezavantajelor, domeniilor şi posibilităţilor de utilizare, cu accent pe analiza farmaceutică.

Autoarea nu se opreşte doar la descrierea metodelor analitice, merge mai departe şi în ultimul capitol abordează aspecte privind asigurarea performanţei metodelor analitice, prin prisma importanţei prelucrării statistice a rezultatelor analizelor şi, în special, a procesului de validare. În plus, abordarea subiectului de aplicaţii specifice ale metodelor instrumentale, îi oferă monografiei un grad sporit de originalitate. Monografia se încheie cu o scurtă incursiune în bazele reglementării metodelor analitice, şi cu prezentarea perspectivelor în dezvoltărea tehnicilor instrumentale.

Această monografie este un suport valoros pentru studenţi, care vor beneficia prin dobândirea unor cunoştinţe solide despre tehnologiile de ultimă oră, iar cercetătorii şi specialiştii în domeniul medicamentului vor avea acces la o resursă valoroasă de informaţii pentru a-şi actualiza cunoştinţele şi pentru a fi la curent cu cele mai recente inovaţii. Este o lucrare fundamentală, cu un conţinut bogat şi variat, cu o redactare ştiinţifică riguroasă a materialului, care este expus clar şi accesibil.

Consider că monografia propusă abordează o temă extrem de actuală şi importantă în domeniul farmaceutic, prin furnizarea de informaţii detaliate asupra metodelor instrumentale cu aplicaţie în cercetarea şi analiza medicamentelor, contribuind astfel la dezvoltarea cunoaşterii în acest domeniu de mare importanţă.

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Decan Facultatea de Farmacie,
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## REQUIREMENTS FOR AUTHORS

**Rules of drafting**
The manuscript (written in English and French) should be in accordance with the guidelines published in: *Uniform Requirements for Manuscripts Submitted to Biomedical Journal (1994) Lancet 1996, 348, V2; 1-4* (www.icmje.org). The manuscripts should be written in font Cambria, size 11 points, spaced at 1.0, fully justified alignment, fields 2 cm on all sides. All pages must be numbered consecutively (in the right bottom corner) and continuously. Abbreviations should be explained at first occurrence in the text and should not be excessively used. The manuscripts must not exceed the number of words (without the title, affiliation, abstract and references): review articles – 4,500 words; research articles – 3,000 words; expert opinions – 2,500 words; case presentation – 1,700 words; experimental and clinical notes – 1,300 words; book reviews and presentations – 2,000 words; teaching articles – 4,000 words. The volume of tables and figures should not exceed ⅓ from the volume of the manuscript. The journal reserves the right to make any other formatting changes. Rejected manuscripts are not returned.

**All manuscripts submitted for publication should be accompanied by two abstracts: in the language of origin of the article and English.**

**Title and authors**
The title should be as short as possible (maximum – 120 signs with spaces), relevant for the manuscript content. The names of the authors should be written in full: name, surname (*e.g.:* Jon JONES). Affiliation should include: Department/Unit/Chair, University/Hospital, City, Country of each author. Beneath the affiliation, the author`s details and contact information – e-mail address (*e.g.:* corresponding author: Jon Jones, e-mail: jon.jones@gmail.com).

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The manuscript should comprise the following subheadings (capitalized):

- **SUMMARY**
- **INTRODUCTION** (will reflect the topicality and the general presentation of the problem studied, purpose and hypothesis of the study)
- **MATERIAL AND METHODS**
- **RESULTS**
- **DISCUSSIONS**
- **CONCLUSIONS**
- **CONFLICT OF INTERESTS**
- **ACKNOWLEDGEMENT** (optional)
- **ETHICAL APPROVAL** (specify the presence or absence of a positive opinion from the ethics committee: no, date, institution ad informed consent)
- **REFERENCES**

The **summary** should contain 1,600 signs with spaces:

- **Introduction**
- **Material and methods**
- **Results**
- **Conclusions**
- **Key words:** 3-5 words

The summary should not include tables, charts, and bibliographic notes; information not included in the article.

**Figures.** The text included in figures should be written in font Cambria, 10 point. Each figure should be accompanied by a heading and legend. They should be numbered with Arabic numerals and placed in parentheses (*e.g.:* fig. 1). Both the title (*e.g.* Figure 1) and legend are centred, below the figure.

**Tables.** The text included in tables should be written in font Cambria, 10 point. Each table should be accompanied by a heading. Tables should be inserted into the text and adjusted to the width of the page. The tables are numbered in Arabic numerals and mentioned in body text in parentheses (*e.g.* tab. 1). The title of the table is centred on the top of the table (*e.g.* Table 1).

**References** are numbered in the order they appear in the paper. The reference sources are cited at the end of the article by using AMA style and will include only the references cited within the text (the reference is numbered within round parentheses). The in-text citations that appear more than once are numbered si-milarly as in the first citation. The number of references should not exceed 50 sources. The scientific authors are responsible for the accuracy of their writings. The reference list should include only those references that have been consulted by the authors of the manuscript. The elements of the reference sources are written exactly in accordance with the requirements.

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**Structura manuscrisului**

Manuscrisul va cuprinde următoarele subtitluri (scrise cu majuscule):

- **REZUMAT** (vezi cerințele mai jos)
- **INTRODUCERE** (se va reflecta actualitatea și prezentarea generală a problemei studiate, scopul și ipoteza studiului)
- **MATERIAL ȘI METODE**
- **REZULTATE**
- **DISCUȚII**
- **CONCLUZII**
- **CONFLICT DE INTERESE**
- **MULȚUMIRI ȘI FINANȚARE** (optional)
- **APROBAREA ETICĂ** (se va specifica prezența sau lipsa avizului pozitiv de la comitetul de etică: nr, data, instituția și acordul informat)
- **REFERINȚE**

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- **Material și metode**
- **Rezultate**
- **Concluzii**
- **Cuvinte cheie**: 3-5 cuvinte

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**Tous les manuscrits à publier doivent être accompagnés par deux résumés: dans la langue originale et en anglais.**

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Le titre doit être le plus court que possible (maximum – 120 signes avec espaces), éloquent pour le contenu du manuscrit. Les noms des auteurs seront écrits complets: prénom, nom (*ex:* Albert LEBRUN). Quant à l'affiliation, on devra indiquer: Section/ Département/Chaire, Université/Hôpital, Ville, Pays – pour chaque auteur. Les données de l'auteur correspondant et les coordonnées – adresse e-mail (*ex:* auteur correspondant: Albert Lebrun, e-mail: albert.lebrun@gmail.com*)* seront obligatoires ci-dessous.

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Le manuscrit comprendra les sous-titres suivants (avec lettres majuscules):

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- **INTRODUCTION** (reflétera l'actualité et la présentation générale du problème étudié, le but et l'hypothèse de l'étude)
- **METHODES**
- **RESULTATS**
- **DISCUSSIONS**
- **CONCLUSIONS**
- **CONFLIT D'INTERETS**
- **REMERCIEMENTS ET FINANCEMENT**
- **APPROBATION ÉTHIQUE** (préciser la présence ou l'absence d'avis favorable du comité d'éthique: no, date, institution et consentement éclairé)
- **REFERENCES**

Le **résumé** contiendra 1600 signes avec espaces:

- **Introduction**
- **Méthodes**
- **Résultats**
- **Conclusions**
- **Mots clés**: 3-5mots.

Le résumé ne comprendra pas des tableaux, graphiques et des notes bibliographiques; des informations non présentées dans l'étude.

**Figures.** Le texte inclus dans les figures doit être écrit avec police Cambria, taille 10 points. Chaque figure doit être accompagné par un titre et une légende. Ceux-ci seront numérotés avec des chiffres arabes et mentionnés dans le texte entre parenthèses (*ex:* fig. 1). Le titre (*ex:* Figure 1) et la légende de la figure doivent être centrés, au-dessous de la figure.

**Tableaux.** Le texte inclus dans les tableaux doit être écrit avec police Cambria, taille 10 points. Chaque tableau doit être accompagné par un titre. Les tableaux seront numérotés avec des chiffres arabes, mentionnés dans le texte entre parenthèses (*ex:* tab. 1), et seront insérés dans le texte, sans dépasser la largeur d'une page. Le titre du tableau sera placé au-dessus du tableau, centré (*ex:* Tableau 1).

Les **références** doivent être numérotées dans l'ordre où elles apparaissent dans le texte. La citation de la source de référence sera de style *AMA*, placée à la fin de l'article et n'inclura que des références citées dans le texte (mentionnant le numéro de référence entre parenthèses rondes). Si la même référence est citée plusieurs fois, elle sera transmise dans le texte avec le même numéro que celui de la première citation. Le nombre total de références ne dépassera pas 50 sources. La responsabilité pour l'exactitude des données est à la charge de l'auteur. Il faut indiquer dans le manuscrit seulement les références vraiment consultées par les auteurs. Les composants des sources de référence doivent être rédigés strictement selon les exigences.

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Подготовка рукописи (разработанной на английском и французском языках) будет осуществляться в соответствии с инструкциями, опубликованными в: *Uniform Requirements for Manuscripts Submitted to Biomedical Journals (1994) Lancet 1996, 348, V2; 1-4* (www.icmje.org). Авторы должны использовать шрифт Cambria, размер 11 точек, с интервалом 1,0, выравнивание по ширине, поля 2 см со всех сторон. Все страницы должны быть пронуме-рованы последовательно (в правом нижнем углу) и включать непрерывную нумерацию страниц. Сокращения должны быть объяснены при первом появлении в тексте и не должны использоваться чрезмерно. Объем рукописей не должен превышать (без названия, принадлеж-ности, резюме и литературы): для обзорных статей/рефератов – 4500 слов; для научных ста-тей – 3000 слов; для экспертных заключений – 2500 слов; для презентации случаев из клини-ческой/лабораторной практики – 1700 слов; для экспериментальных и клинических заметок – 1300 слов; для рецензий и презентаций книг – 2000 слов; для учебных статей – 4000 слов. Объем таблиц и рисунков не должен превышать ⅓ от объема рукописи. Журнал оставляет за собой право вносить любые другие изменения форматирования. Отклоненные рукописи не возвращаются.

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Рукопись будет включать в себя следующие подзаголовки (они должны быть заглавными):

- **РЕЗЮМЕ** (см. требования ниже)
- **ВВЕДЕНИЕ** (будет отражать актуаль-ность и общее представление изучаемой проблемы, цель и гипотезу исследования)
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- **Материалы и методы**
- **Результаты**
- **Выводы**
- **Ключевые слова:** 3-5 слов

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**Рисунки** (графики, диаграммы). Текст, включенный в рисунки, должен быть написан в Cambria, размер 10 пунктов. Каждый рисунок должен сопровождаться заголовком и описанием. Название (*например:* Рисунок 1) и описание рисунка должны быть вписаны по центру, в низу рисунка. Они должны быть пронумерованы арабскими цифрами и указаны в тексте в скобках (*например:* рис. 1).

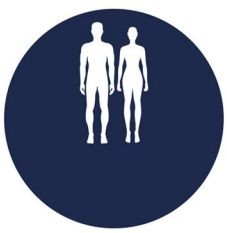
**Таблицы.** Текст, включенный в таблицы, должен быть написан в Cambria, размер 10 пунктов. Каждая таблица должна сопровождаться заголовком. Они должны вставляться в текст, не превышая ширину страницы. Должны быть пронумерованы арабскими цифрами и указаны в тексте в скобках (*например:* таб. 1). Название таблицы должно располагаться над таблицей в центре (*например:* Таблица 1).

**Литература.** Источники должны быть пронумерованы в порядке их появления в тексте. Ссылки на источники должны быть в стиле *AMA*, помещены в конце статьи и включать только источники, цитируемые в тексте (упоминание номера источника в круглых скобках). Если один и тот же источник цитируется несколько раз, он будет передан в тексте с тем же номером, что и первый раз. Общее количество источников не должно превышать 50. Ответственность за точ-ность данных лежит на авторе. Будут цитиро-ваться только те источники, с которыми ознакомились авторы рукописи. Компоненты справочных источников должны быть написаны строго в соответствии с требованиями.

Дополнительная информация на: http://journal.ohrm.bba.md/index.php/journal-ohrm-bba-md/editing_guidelines

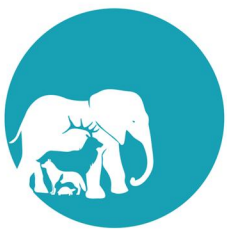
# The *One Health* concept

**Human health**



The WHO defined health in 1946 as "a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity", with the later addition of "the capacity to lead a socially and economically productive life".

**Animal health**



The OIE defines animal welfare in 2008: an animal is in good condition if it is healthy, enjoys comfort, is well fed, is safe, is able to display its innate (natural) behavior and does not suffer from unpleasant conditions such as pain, fear and stress.

**Plant and environmental health**

Environmental health refers to those aspects of human health that include the quality of life determined by physical, biological, socio-economic and psycho-social factors in the environment. The interrelationships of people with the environment concern medicine, when an ecological system is in a state of equilibrium, the health of the population prevails.

Globally, the *One Health* concept is a worldwide strategy to expand interdisciplinary collaborations and communications in all aspects related to the health care of humans, domestic animals or wildlife, which can no longer be approached separately, but only jointly.

*One Health* addresses not only human and animal disease concerns, but also issues related to lifestyle, diet, exercise, the impact of different types of human-animal relationships, and environmental exposures that can affect both populations. In order to achieve the expected effects, it is also necessary to educate the population to make them aware of the risk factors and benefits of prevention, as well as communication and understanding between patients and healthcare providers.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