„NICOLAE TESTEMITANU" STATE UNIVERSITY OF MEDICINE AND PHARMACY OF THE REPUBLIC OF MOLDOVA

FACULTY OF RESIDENCY
DEPARTMENT OF PNEUMOPHTHISIOLOGY

Evelina LESNIC, Alina MALIC

INTRODUCTION IN TUBERCULOSIS

Methodical recommendations for practical work, seminars, and individual work

CHISINAU
2022

„NICOLAE TESTEMITANU" STATE UNIVERSITY OF MEDICINE AND PHARMACY OF THE REPUBLIC OF MOLDOVA

FACULTY OF RESIDENCY
DEPARTMENT OF PNEUMOPHTHISIOLOGY

Evelina LESNIC, Alina MALIC

INTRODUCTION IN TUBERCULOSIS

Methodical recommendations for practical work, seminars, and individual work

CHISINAU
Centrul Editorial-Poligrafic *Medicina*
2022

CZU: 616.24-002.5
L 52

Recommended for publication by the Central Scientific Methodological Commission on Internal Medicine of the „Nicolae Testemitanu" State University of Medicine and Pharmacy of the Republic of Moldova, Minutes Nr.3 of 07.06.2021.

Authors:

Evelina Lesnic, Ph.D., associated professor,
Alina Malic, Ph.D., asistent professor.

Peer reviewers:

Ustian Aurelia, Ph.D., associated professor,
Djugostran Valeriu, Ph.D., professor.

În redacția autorului.

Compiterized layout: *Feodosia Caprari*

DESCRIEREA CIP A CAMEREI NAŢIONALE A CĂRŢII DIN REPUBLICA MOLDOVA

Lesnic, Evelina.

Introduction in tuberculosis: Methodical recommendations for practical work, seminars, and individual work / Evelina Lesnic, Alina Malic; „Nicolae Testemitanu" State University of Medicine and Pharmacy of the Republic of Moldova, Faculty of Residency, Department of Pneumophthisiology. – Chișinău: CEP *Medicina*, 2022. – 73 p.: fig., tab.

Bibliogr.: p. 72-73 (24 tit.). – În red. aut. – 50 ex.

ISBN 978-9975-82-265-7.

616.24-002.5
L 52

ISBN 978-9975-82-265-7

TABLE OF CONTENTS

Authors' note

The methodical recommendations for practical works, seminars and individual work were developed according to the actual guide on the methodological scientific publications elaborated by the ,,Nicolae Testemitanu" State University of Medicine and Pharmacy. This methodological guidance paper was developed to present the information used in the teaching-learning-evaluation process described in the curriculum of the discipline of pneumophthisiology and indicates how to transfer knowledge by applying the procedures established in the analytical program of the discipline.

The target group is composed of students attending university studies in the specialty of pneumophthisiology. The proposed theme represents the introduction notions in phthisiology written in the context of the international recommendations. The contents of the methodological recommendations include the basic definitions used in pneumophthisiology, epidemiological indicators established at the national and international levels, the basic notions of etiology, transmission, and pathogenesis of the mycobacterial infection in humans. We confirm that the content corresponds to the curriculum of the discipline pneumophthisiology, includes the introduction to the specialty, and reflects the theoretical and practical knowledge that students have to possess at the end of the course.

As theoretical support for the proposed methodological recommendations the national clinical standards, the international recommendations, and the scientific-methodological papers published by international and Moldovan scientists with an international profile were selected. Different teaching methods and didactic procedures that had been integrated into the academic process of the specialty pneumophthisiology were used as well.

The professional skills acquired as a result of the study of the proposed methodological recommendations will include epistemological, investigative, communicative, and metacognitive skills. The compul-

sory compartments included in the proposed methodological recommendations are the editorial page, approval page, introductory note, overall aim and specific objectives, informative material, as well as learning methodology according to the curriculum of the specialty pneumophthisiology, self-assessment tests, and bibliographic references.

Authors

12,

Abbreviation List

AFB – Acid-fast bacilli
AIDS – Acquired immunodeficiency syndrome
BACTEC – Liquide culture media
DOT – Directly Observed Treatment
DOTS – Directly Observed Treatment Short Course
DST – Drug susceptibility testing
E – Ethambutol
H – Isoniazid
HIV – Human immune deficiency virus
IFP – Institute of Phthisiopneumology
LJ – Lowenstein-Jensen media
MBT – Mycobacterium tuberculosis
MDR - TB – Multidrug-resistant tuberculosis
MH – Ministry of Health
NTCP – National Tuberculosis Control Program
PHC – Primary health care
R – Rifampicin
S – Streptomycin
TB – Tuberculosis
XDR TB – Extensively drug-resistant tuberculosis
WHO – World Health Organisation
Z – Pyrazinamide

INTRODUCTION

The methodical recommendations for practical work, seminars, and individual work were developed according to the actual guide on the methodological scientific publications elaborated by the „Nicolae Testemitanu" State University of Medicine and Pharmacy. This methodological guidance paper was developed to present the information used in the teaching-learning-evaluation process described in the curriculum of the discipline of pneumophthisiology, the rules, and the learning requirements for the practical applicability of the provided knowledge. In each chapter, the theoretical framework is exposed, which starts with the overall aim and the specific objectives, as well as the duration of the activity. The objectives correspond to those identified in the academic curriculum in pneumophthisiology. These are reflected in the materials and methods necessary for performing the practical work. For the students' self-assessment the questions/topics that have to be addressed for the individual preparation based on the presented information are exposed. The sources are indicated in the bibliography for the independent training.

The themes of the proposed methodical recommendations are:

- History of tuberculosis;
- Epidemiological indices of tuberculosis in the Republic of Moldova and worldwide;
- Etiology of tuberculosis. The ways of transmission of the tuberculosis infection and the sources of the infection;
- Pathogenesis of tuberculosis.
- The overall aim: Development of the specific competencies in the field of the history of tuberculosis, etiology, and pathogenesis of tuberculosis.

Objectives:

- To define tuberculosis and identify the scientists and researchers who contributed to the development of the specialty;

- To know how to calculate and define the epidemiological indicators, and characterize the epidemiological situation of tuberculosis.;
- To study the etiology of tuberculosis, characteristics of the pathogen, the routes of transmission, and the sources of the infection;
- To learn the general characteristics of latent tuberculosis infection and the differentiation from active tuberculosis.
- To enumerate the risk factors for the development of tuberculosis and to identify the high-risk groups in the current epidemiological context.

The duration of the study for the introductory course is one academic hour and for the associated practical activity, five academic hours are allocated.

Materials and academic methodology

In the academic methodical process, a wide range of methods and learning procedures for increasing the effectiveness of acquiring knowledge and practical skills was used. The induction in the specialty and the training are performed through an interactive study of the introductory course in pneumophthisiology. The students are asked to attend a compulsory lecture lasting for one academic hour. The academic process continues in a practice room. The students' presence at the practical work is compulsory. The student's absence from practical work implies the non-admission to the exam. The students' activities in the practical lessons involve interactive participation, interactive conversation with the university lecturer, assistant professor, or associated professor with explaining and demonstrating the acquired information.

The training methods include the main methodologies specific to the interference between the fields of medicine and pedagogy recommended by the module „Psychopedagogy for Teachers" developed by the Department of Management and Psychology. The application of the problem-based learning method (PBL) and the clinical case-based reasoning method (CBCR) was paid particular attention. The case study

was used to develop clinical judgment as a fundamental teaching tool in continuous medical education. The strategic approach to teaching, knowledge acquisition, and evaluation was considered by using the new electronic platforms: Google Meet, Zoom, and Moodle.

The formative assessment is systematically performed during the practical lessons. It involves different methods: oral and written ones, tests, and clinical situations with the physical presence or virtually. During the practical lessons, the students are evaluated based on the evaluation test, clinical activity, assessment of practical knowledge, and the case study.

The following questions are included in the final control test in the provided methodological recommendations, as well as in the exam cards:

1. Short history of tuberculosis. The scientists, researchers who contributed to the development of pneumophthisiology. The main policy and guidelines surrounding the fight against tuberculosis.
2. Epidemiology of tuberculosis. The epidemiological indicators of tuberculosis in the Republic of Moldova and worldwide.
3. Etiology of tuberculosis. Genus Mycobacterium. Classification of the etiologic agent. The biochemical structure of the etiologic agent. Microscopical morphology. Microbiological characteristics. Natural resistance against the physical and chemical agents.
4. Transmission of the tuberculosis infection. Sources of infection. Routes of transmission of the infection into the human body.
5. Pathogenesis and morphopathology of tuberculosis infection.
6. The body's response against tuberculosis infection. Experimental tuberculosis. The Koch phenomenon. Mechanism of the immune response of the organism against tuberculosis infection. The body's reaction to tuberculin injection. Particularities of delayed hypersensitivity response against the tuberculosis infection.
7. Evolution of the tuberculosis infection. The role of the environmental factors in the evolution of tuberculosis infection. The cycle of tuberculosis infection in humans. Differences between latent tuberculosis infection and active tuberculosis.

The students' self-evaluation consists of exposing the information according to the questions that need to be addressed for individual training based on the acquired information. An important component of the students' self-evaluation is to answer the tests, as well as the clinical case study exposed at the end of the methodological recommendations.

CHAPTER 1. HISTORY OF TUBERCULOSIS

The practical lesson aims to create specific skills in the field of tuberculosis history and the specialty of Phthisiopneumology, to learn about the scientists who contributed to the fight against tuberculosis and the international and national policies implemented for the control of tuberculosis.

The specific objectives are:

- To acquire knowledge about the history of tuberculosis and recent developments in pneumophthisiology.
- To learn the international and national policies implemented in the control of tuberculosis.

The type of activity is theoretical and involves the study of the information presented and the documents on legislative interventions in the field of the fight against tuberculosis.

The duration of the study for the introductory course is one academic hour and the associated practical activity will take place over five academic hours.

Theoretical framework

Tuberculosis is an infectious and contagious disease caused by the Mycobacterium tuberculosis complex, which includes the species: Mycobacterium tuberculosis hominis, Mycobacterium africanum, Mycobacterium bovis, Mycobacterium microtti. Tuberculosis can affect any organ or part of the body, but the most frequent localization is in the lungs.

Tuberculosis is a disease of the human community known for more than 100.000 years. Archaeological vestiges of the ancient age were identified in spinal cord injuries (Pott disease) and pulmonary tuberculous lesions. A 500.000-year old fossil of Homo erectus discovered in Turkey was identified with tuberculosis of the bones. The first name of the disease was given by Hippocrates calling it „phthisis" and was described as a severe disease with fatal outcomes. In the Old Testament, epidemic disease was described as „schachepheth" being attributed to

tuberculosis. Aristotle (384-322 BC) described scrofuloderma as tuberculosis of the cervical lymph nodes with cutaneous fistulization. In the medieval age, the English kings were curing scrofuloderma patients (fistualized tuberculosis of the cervical lymph nodes) by „royal touch". The current definition of tuberculosis was attributed to Johann Schonlein (in 1834) due to the multiple tubercles that were found in the lungs of a sick person.

The industrial revolution at the end of the 17th century and the first decades of the 18th century contributed to the increase of the tuberculosis epidemiological indices in Western Europe. The disease was called the „white plague" because it was associated with urban overcrowding and poor hygienic and sanitary conditions. Tuberculosis was also known as a romantic disease. The painters immortalized tuberculosis patients in a slow agony that usually led to inevitable death. Tuberculosis was so much romanticized in the XVIIIth century that Alexandre Dumas fils wrote, „It was fashionable to suffer from lungs; everyone was coughing, were consumed, especially the poets, it was normal to spit blood after any emotion, and what sensationally was to die before 30 years old".

History demonstrates that tuberculosis does not discriminate and thousands of personalities were sick:

- Kings and politicians - Cardinal Richelieu (1585-1642), Mohammed Ali Jinnah (1876-1948), Nelson Mandella (1918-2013), etc.
- Writers: Alexander Pope (1688-1744), Johann Wolfgang von Goethe (1749-1832), Friedrich Schiller (1759-1805), Emily Brontë (1818-1848), Fyodor Dostoevsky (1821-1881), Anton Chekhov (1860-1904)), David Lawrence (1885-1930), George Orwell (1903-1950), Iulia Hasdeu (1869-1888), Honoré de Balzac (1799-1850), etc.
- Musicians: Luigi Boccherini (1743-1805), Niccolò Paganini (1782-1840), Igor Stravinsky (1882-1971), Frédéric Chopin (1810-1849), etc.
- Scientists: Alexander Graham Bell (1847-1922), Dmitri Mendeleyev (1834-1907), etc.

Sandro Botticelli's picture *The Birth of Venus (Nascita di Venere)* created in 1484 is a representation of the goddess Venus that is traditionally associated with his great platonic love Simonetta Vespucci who died of tuberculosis at the age of 23 years.

Scientists which contributed to the development of the specialty

The first description has been attributed to Hippocrates (460-370 BC), who declared that the disease has a genetic transmission and defined it as „phthisis", which means in Greek „consumption". Sonanus (2nd century BC) was the first who described the clinical signs of phthisis. Girolamo Francastoro (1478-1553) established the contagious feature of the disease. Andreas Vesalius (16th century) described cavities in the lungs of sick patients. Franz de le Boë de Amsterdam (1614-1672) known as Franciscus Sylvius described the clinical symptomatology and little nodules observed at the dissection of the bodies and defined them as tuberculous. In the 17th centur,y the first notification/registration of patients with tuberculosis started. Percivall Pott (1762) was the first who described tuberculosis of the vertebral column and the disease was called „Morbus Pott" (Pott disease). Antonio Cocchi (1695-1758) was the first who described the treatment of patients with tuberculosis through „hospitalization" in specialized institutions called „sanatoriums" provided with big rooms, ventilation and exposure to the natural sunshine. René-Théophile-Hyacinthe Laennec (1781-1826) defined the clinical symptomatology of patients with tuberculosis according to the clinical forms of the disease and the perceived sounds through the „stethoscope", which is considered till nowadays his invention. By the side, René Théophile Laennec died of lung tuberculosis and skin tuberculosis that he obtained through direct inoculation from the dissected patients. Thomas Willis (1621-1675) was the first who described miliary tuberculosis. Karl Ernst Ranke (1870-1926) differentiated the evolution of the tuberculosis infection and defined the Ranke complex. Jean-Antoine Villemin (1865) provided evidence that tuberculosis is an infectious disease by inoculating rabbits with the material from infected humans. He demonstrated that the inoculation of rabbits with the sam-

ples collected from patients with tuberculosis develops a similar form of the disease in the rabbits. Johann Schönlein (1839) was the scientist who named the disease tuberculosis and argued its name by multiple nodules identified in the lungs of patients with tuberculosis.

Rudolf Virchow (1821-1902) described the microscopic aspect of tuberculous inflammation and the cellular components of tuberculous granuloma. On 24 March 1882, Robert Koch communicated to the Physiological Society of Berlin the discovery of the etiologic agent. He described it as rod-shaped bacilli stained in alcohol and methylene blue, agglomerated in groups, localized intra- or extracellular, and called them the Tuberklevirus. In 1883 the etiologic agent was renamed Mycobacterium tuberculosis. In 1912 Mycobacterium tuberculosis received its second name Koch's bacillus. 24 March was proclaimed World Tuberculosis Day on the proposal of the Internation Union Against Tuberculosis in 1999.

The microscopic assessment of samples collected from the patients uses Ziehl-Neelsen staining invented by bacteriologist Franz Ziehl (1859-1926) and pathologist Friedrich Neelsen (1854-1898). It is specific staining thath uses carbol fucsin, acid, alcohol and methylene blue. The development of cultural media was Koch's innovation. He modified the procedures described by Tyndall through solidification of the cow or sheep serum by repeated heating up to 58 ^{o}C in tubes for obtaining a higher surface for inoculation of the samples collected from sick people. R. Koch formulated 3 postulates, which permitted the diagnosis of tuberculosis:

1. the etiologic agent should be identified in the affected tissue or the normal tissue;
2. the etiologic agent should be obtained by growing on the culture media;
3. the inoculation of the specimens collected from patients (sputum, pus, affected tissue) to animals (rats, rabbits, and cats) produces the same lesions as in the sick people and the death of the animals occurred in 8 weeks.

In 1890 Robert Koch extracted tuberculin from the mycobacterial cultures. He injected subcutaneously into the animals the material extracted from cultures of the virulent strains of mycobacteria. The injections were followed by the development of subcutaneous nodules, which in two weeks were ulcerating and eliminating the caseous masses. After a new inoculation which was performed for more than 8 weeks, the reactions were less perceived and were attributed to type IV immune reaction, also known as delayed hypersensitivity. This body response was described for the first time by Robert Koch in 1891 and was named the Koch phenomenon. For his discoveries, Robert Koch was awarded the Nobel Prize in 1905.

The extraction of tuberculin by Léon Charles Albert Calmette (1863-1933) from the mycobacterial cultures started the era of tuberculosis treatment by tuberculization, which failed. However, the injection of tuberculin in a large number of persons permitted the identification of persons with a positive reaction. As consequence, the diagnostic criteria for tuberculosis infection were established. In 1907 Clemens von Pirquet extracted tuberculin and defined the types of reactions of the scarified skin after applying the tuberculin extract. The first description of the skin reaction after the intradermal administration of tuberculin was performed by Charles Mantoux (1910). Calmette and Camille Guérin cultivated a particular strain of Mycobacterium bovis 200 times from 1908 to 1921. Due to the great number of growings, the strain lost its virulence but maintained its immunogenicity, the BCG vaccine (Bacillus Calmette-Guérin) being obtained. Thereby, the onset of BCG vaccination started which actually is the major measure having decreased the tuberculosis prevalence worldwide and remains the most extensive vaccination in childhood. In 1895 Wilhelm Conrad Roentgen (1845-1923) discovered the X-rays and performed the first chest X-rays on patients with tuberculosis. In 1901 he was awarded the Nobel Prize in Physics.

The fight against tuberculosis before the antibiotic era was performed through rest, good food, sanatorial hospitalization, and surgical procedures for pulmonary collapse (artificial pneumothorax, thoraco-

plasty, and pulmonary resection). In 1943 streptomycin was discovered by Albert Schatz, coordinated by Selman Waksman. He also discovered the antimycobacterial activity of streptomycin. Selman Waksman was awarded the Nobel Prize in 1952. The antibiotic treatment started with the use of streptomycin. However, in a short time after the onset of streptomycin, the rapid development of treatment failure was established. After the first use of streptomycin, the combined treatment with para-aminosalicylic acid started to be implemented in 1952. Pyrazinamide was discovered by McKenzie in 1949, but the antimycobacterial activity was demonstrated by Kushner. Isoniazid was developed by the Hoffman-La Roche laboratories and the Squibb Institute for Medical Research of the medical Bayer laboratories. Ethambutol was developed by the Lederle laboratories. Rifampicin was the last drug of the first-line antimycobacterial agents discovered in 1972 and used nowadays in the treatment of tuberculosis.

Although more than 70 years have passed since the discovery of streptomycin, the first antimycobacterial drug, the therapeutic regimen has no new agents and the development of new drugs for the treatment of tuberculosis remains a challenge for scientists worldwide.

Policies on the fight against tuberculosis in the actual epidemiological context

Tuberculosis affects the most vulnerable populations, which usually are belonging to high-risk groups. In 1993, the World Health Organization (WHO) declared tuberculosis a global emergency. In 2000 the Republic of Moldova approved the first National Program for Control and Prophylaxis of Tuberculosis, which led to the national implementation of the Directly Observed Treatment Short Course Chemotherapy (DOTS). The Strategy was recommended by the WHO and its implementation started through a pilot project that included Chisinau city and was extended to the national scale in 2005. Actually, the actions are performed to reduce the burden of tuberculosis on the public

health of the Republic of Moldova. The activities are regulated by the National Control of Tuberculosis Program (PNCT) established for the 2016-2020 years (Government Decision no.1160 from 20.10.2016) created according to the recommendations of the Health Protection law no. 411-XIII of 28.03 1995, law no. 10-XVI of 3.2.2009 regarding the public health supervision by the state, the law no.153-XVI of 4.7.2008 about the control and prophylaxis of tuberculosis, and the WHO's recommended End TB Strategy for 2015-2035 years.

The objectives of the NTCP for 2016-2020 consist of:

1. to ensure universal access to early diagnosis of all forms of tuberculosis, at least 85% of the estimated cases with rifampicin-resistant tuberculosis or multidrug-resistant tuberculosis by the end of 2020.
2. to ensure universal access of the patients to treatment through a patient-centered approach, achieving at least 85% success rate confirmed in new cases with bacteriologically confirmed tuberculosis and at least 75% success rate confirmed in new cases with confirmed rifampicin-resistant tuberculosis or multidrug-resistant tuberculosis.
3. to perform complementary activities with other national health programs for reducing the burden of TB/HIV co-infection to 5%.
4. to ensure tuberculosis prevention and maintain the BCG vaccination rate of at least 95% in all newborns.
5. to strengthen the capacities of the health system to ensure effective control of tuberculosis.
6. to develop and implement new methods and innovative interventions in tuberculosis control.
7. to strengthen the involvement of community and civil society organizations in tuberculosis control through the patient-centered approach.

The Sixty-seventh World Health Assembly held on 21.05.2014 adopted the global End TB Strategy and targets for tuberculosis pre-

vention, care, and control after 2015 for the achievement of Millenium Development Goal 6 (to combat HIV/AIDS, malaria and other diseases) and related tuberculosis targets defined in the End TB Strategy. The vision constitutes to raise, a world free of tuberculosis", zero deaths, disease, and suffering due to tuberculosis. The overall goal is to end the global tuberculosis epidemic.

The milestones of the End TB Strategy are the reduction of the number of deaths in 2015 by 35% compared with 2020 by 75% in 2025 compared with 2015. The targets included in the Sustainable Development Goals are the reduction of the number of deaths in 2030 by 90% compared with 2015 and those included in the End TB Strategy to reduce the number of deaths by 95% in 2035 compared with 2015. According to the incidence of tuberculosis, the milestones were: the reduction of incidence by 20% in 2020 compared with 2015 and the reduction of the incidence by 50% in 2025 compared with 2015. The targets included in the Sustainable Development Goals were: the reduction of the incidence by 80% in 2030 compared with 2015 and the targets included in the End TB Strategy was the reduction of the incidence by 90% in 2035 compared with 2015. As for indicators, the milestones for incidence rate should achieve less than 85/100.000 population in 2020 and less than 55 per 100 000 populations in 2025. The targets included in the Sustainable Development Goals for incidence were less than 20 per 100.000 population in 2030 and less than 10 per 100.000 population in 2035. A key milestone is a 75% reduction of tuberculosis deaths by 2025, compared with 2015. This requires reducing the global tuberculosis incidence from an average of 2% per year in 2015 to 10% per year by 2025. Secondly, the rate of case fatality needs to decline from a projected 15% in 2015 to 6,4% by 2025. It can be achieved by universal access to existing tools of diagnosis and social-economical developpment.

In order to sustain the progress beyond 2025 and achieve by 2035 a reduction of tuberculosis deaths by 25% and a reduction of the incidence by 90% from 110 per 100.000 to less than 10 per 100.000 population, will be an additional tool available by 2025. Other new tools im-

plemented by 2025 will be investments in research and development. The figure below shows the decline of tuberculosis incidence due to improvements in the diagnostic tools combined with the universal health coverage and social protection implemented starting in 2015 and the additional impact of the new tools by 2025. The milestone that no families affected by tuberculosis will have catastrophic costs involves minimizing the direct medical costs such as fees for consultation, hospitalization, testing, and treatment, as well the direct non-medical costs – transport or loss of income during the sickness. It requires the governments to ensure the full access of the patients to the social protection that covers or compensates the non-medical costs and loss of financial income.

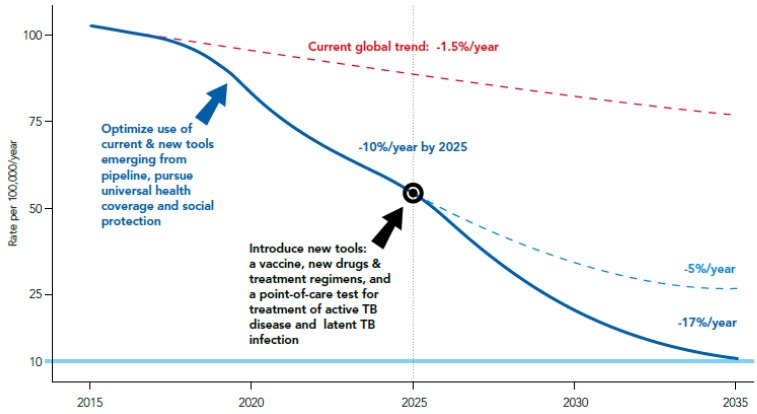

Figure 1. Projected acceleration in the decline of global tuberculosis levels

The principles of the End TB Strategy are:

1. Government stewardship and accountability with monitoring and evaluation;
2. Strong coalition with civil society organizations and communities;
3. Protection and promotion of human rights, ethics, and equity;
4. Adaption of the Strategy and targets at the country level, with global collaboration.

The first principle, „*government stewardship and accountability"* includes the responsibilities that should be shared at the local, provincial and central levels. The success of the Strategy depends on the execution of responsibilities by the governments in collaboration with all stakeholders: providing the direct in the health system, collecting the data for improvement and prevention of tuberculosis cases, exerting influence through regulations.

The second principle, *„strong coalition with civil society organizations"* represents a strong part of the solution to reach the targets of the Strategy. Community representatives and civil society should be enabled to engage more people in the program planning, designs, service delivery, monitoring, information, education, support of patients and their families, research, and advocacy.

The principle, *„protection, and promotion of human rights, ethics, and equity"* is based on the implementation of the policies and strategy in the frame of the national tuberculosis program that should address human rights, ethics, and equity. Access to high-quality case management is a major right to health. As well, affected people and communities could engage themselves in the promotion of human rights and implementation of all pillars and components of the strategy in the key affected populations. The major issue is preventing the conflict between the public interest in preventing the transmission of the disease and the patient's right to refuse the treatment, also the discrimination and stigmatization of the affected patients, the issue of low adherence to treatment, the care to be offered when the treatment options are exhausted.

The fourth principle, *„adaption of the strategy to the country level"* involves changing the activities to the diverse country settings. The interventions will be prioritized based on the local contest, needs and capacities. In a globalized world, the disease can be spread by international travel and trade. The close collaboration among countries adderssing the specific aspects of the disease epidemiology is important in the identification of the key affected populations and development of the guidance on different components of the strategy that could be implemented.

Pillars and components of the End TB Strategy

Pillar 1. Integrated, patient-centered care, and prevention.

Components that are defined as the key actions are:

A. Early diagnosis of tuberculosis including universal access to drug-susceptibility testing and systematic screening of contacts and high-risk groups.

B. Treatment of all people with tuberculosis including drug-resistant tuberculosis and patients support.

C. Collaborative TB/HIV actions and management of the comorbidities.

D. Preventive treatment of persons at high risk and vaccination against tuberculosis.

Were presented some explanations about the pillars and the components of the Strategy:

A. To ensure the early detection of tuberculosis means to fortify the universal access to early diagnosis of tuberculosis using new molecular tests. Even the sputum-smear microscopy is a low-cost tool, its low sensitivity diminishes the effectiveness of the case detection. As a result, the health care services miss tuberculosis patients or identify them in an advanced stage. The chest X-ray screening is a useful tool for diagnosis of the bacteriologically negative tuberculosis, extrapulmonary tuberculosis, and tuberculosis in children.

B. Early diagnosis and treatment onset of drug-resistant tuberculosis is a challenge in most of the regions of the world due to the limited bacteriological capacities of the national laboratories. Implementation of the new diagnostic tools, such as molecular genetic testing will permit diagnosing tuberculosis in less advanced forms, will reduce mortality, and will improve the success rate.

C. The collaborative activities between tuberculosis and HIV infection specialized institutions will involve the expansion of the TB/HIV programs. So, all HIV-infected persons should receive antiretroviral treatment. All tuberculosis patients should be screened for HIV. All patients with TB/HIV co-infection should receive cotrimoxazole preventive treatment.

Tuberculosis and HIV care should be integrated into the services for maternal and child health. Management of tuberculosis comorbidities such as diabetes mellitus, undernutrition, silicosis, smoking, alcohol drinking, drug use, and associated immune-compromising disorders and treatments is important to reduce the poor treatment outcomes. Patients with tuberculosis should be screened for other diseases especially lung diseases, as well.

D. Preventive treatment of persons at a high risk involves the latent tuberculosis infection screening by tuberculin skin test or interferon-gamma release assay and isoniazid preventive therapy in people living with HIV and children who are in contact with patients with tuberculosis. BCG vaccination continues to be performed systematically in all high tuberculosis prevalence countries because it prevents disseminated disease, such as meningitis and milliary tuberculosis.

The second pillar is attributed, *„to bold policies and implement supportive systems"*. The activities which should be performed are:

A. Political commitment with adequate resources for tuberculosis care and prevention;
B. Engagement of communities, civil society organizations, and public and private care providers;
C. Universal health coverage policy, and regulatory frameworks for case notification, vital registration, quality, and rational use of medicine and infection control.
D. Social protection, poverty alleviation, and actions on other determinants of tuberculosis.

Were exposed following explanations about the pillars and components:

A. Political support and commitment to tuberculosis care and prevention is required for achieving high effectiveness. It will involve adequate provision with financial and human resources support.
B. Engagement of the communities and civil society is the most powerful response to achieve de Strategy's targets. Involvement of the community society will increase the detection of the sick persons, referring them for diagnosis, providing support during

the treatment and diminishing the stigmatization and discrimination. Civil society organizations have competencies that include reaching out to high-risk groups, mobilizing communities, informing and warning the population, creating the demand for care, and addressing social determinants of the tuberculosis epidemics.

C. Universal health coverage is defined as „the situation when all people are able to use high-quality health services and not suffering financial hardship paying for them". For tuberculosis patients it implies a) expanding the access to high-quality services recommended in the Strategy; b) expanding coverage of the costs for consultations, testing, treatment, follow-up tests; c) expanding access to services of vulnerable groups.
D. Social protection and poverty alleviation include expanding the poverty reduction strategies, improving living and working conditions, reducing food insecurity, addressing the health issues of the migrants, involving civil stakeholders, preventing direct risk factors for tuberculosis such as smoking, alcohol drinking, drug use, promote healthy diets, and adequate care of the comorbidities.

The third pillar is the, *„intensified research and innovation"* which involves such actions as:

A. Discovery, development, and rapid uptake of new tools, interventions, and strategies;
B. Research to optimize the implementation and the impact of promoting innovations.

Synthesis of the information from the first chapter

- Tuberculosis is an infectious disease known since antiquity.
- Hippocrates described the disease as a severe consumption with a lethal end, calling it „phthisis".
- On March 24, 1882, Robert Koch communicated to the Berlin Society of Physiology the discovery of the etiological agent, calling it tuberclevirus, and in 1911 was awarded with the Nobel Prize.

- Tuberculosis was declared a global emergency in 1993, and the first WHO-recommended tuberculosis control strategy called DOTS (Directly Observed Treatment Short Course Chemotherapy) has been implemented in Moldova since 2001.
- The current Strategy in controlling tuberculosis is End TB and aims to reduce the death rate from tuberculosis by 95% and the incidence by 90% between 2015 and 2035, ensuring that no family will be burdened by catastrophic expenses due to tuberculosis.
- The era of anti-tuberculosis therapy started with the discovery of the antimycobacterial properties of streptomycin and combined regimens with other first-line antituberculosis drugs have been established, valid until now.

CHAPTER II. EPIDEMIOLOGICAL SITUATION OF TUBERCULOSIS IN THE REPUBLIC OF MOLDOVA AND WORLDWIDE

The aim of the practical lesson is to create specific competencies in the field of epidemiological indicators of tuberculosis at the global and national level with the identification of the causes of the aggravation of the epidemiological situation.

At the application level, the purpose of this topic was to calculate the epidemiological indicators of tuberculosis and to identify the risk factors.

The objectives are:

- To analyze and to evaluate the epidemiological indicators of tuberculosis recorded worldwide and at the national level.
- To study the methods for calculation of the epidemiological indicators of tuberculosis.
- To identify the causes that determined the evolution of the epidemiological situation of tuberculosis.

The type of practical activity is based on the calculation of epidemiological indicators of tuberculosis.

The duration of the study for the introductory course is one academic hour and the associated practical activity will take place over a period of five academic hours.

Theoretical framework

The control of tuberculosis is a public health problem. The main epidemiological indicators which evaluate, compare and initiate epidemiological actions are incidence (the number the new cases reported per 100.000 population), prevalence (the total number of patients reported per 100.000 population), mortality (number of deaths reported per 100.000 population), the incidence of resistant to rifampicin tuberculosis (RR-TB) or multidrug-resistant tuberculosis (reported per 100.000 population), treatment success rate (%), the rate of the tuberculosis patients

tested for HIV (%), the rate of the TB/HIV co-infected cases among tuberculosis patients (%). For the epidemiological indicators the following definitions have been established:

- Global incidence (reported per 100.000 population) is the total number of new and recurrent/relapsed cases of tuberculosis notified in the reference period reported per 100.000 population.
- Mortality (per 100.000 population) is the total number of deaths as all-cause-mortality before completing the anti-tuberculosis treatment notified in the reference period per 100.000 population.
- The prevalence of tuberculosis (per 100.000 population) is the total number of tuberculosis cases (including new cases, recurrences/relapsed cases, recovered after loss to follow-up and after therapeutic failure) notified in the reference period per 100.000 population.
- The incidence of new cases aged between 0 and 17 years 11 months and 29 days (per 100.000 population) represents the total number of new cases of tuberculosis in patients aged between 0-17 years 11 months and 29 days notified during the reference period per 100.000 population.
- The incidence of the resistant to rifampicin or multidrug-resistant tuberculosis among the new and relapsed cases represents the total number of cases resistant to rifampicin (RR-TB) or multidrug-resistant tuberculosis among new and relapsed cases recorded during the reference period per 100.000 population.
- The rate of the resistance to rifampicin (RR-TB) or multidrug-resistant tuberculosis cases among previously treated cases is the proportion between the number of previously treated cases established resistant to rifampicin or multidrug-resistant tuberculosis cases reported to the total number of the rifampicin or multidrug-resistant tuberculosis during the reference period of time multiplied with 100. It is reported in %.
- The treatment success rate of the new cases with bacteriologically confirmed pulmonary tuberculosis represents the proportion between

the total number of new cases with susceptible tuberculosis confirmed bacteriologically at the onset of treatment notified 12-15 months ago and which successfully completed antituberculous treatment reported to the total number of bacteriologically confirmed pulmonary tuberculosis cases who completed the treatment. It is reported in %.

- The treatment success rate of the new cases with bacteriologically confirmed resistance to rifampicin (RR-TB) or multidrug-resistant tuberculosis pulmonary tuberculosis represents the proportion of the total number of new cases with resistance to rifampicin (RR-TB) or multidrug-resistant tuberculosis confirmed bacteriologically at the onset of treatment notified 21-24 months ago and which successfully completed antituberculous treatment reported to the total number of bacteriologically confirmed resistant to rifampicin or multidrug-resistant tuberculosis pulmonary tuberculosis cases who completed the treatment. It is reported in %.
- The rate of tuberculosis patients tested for HIV infection is the total number of tuberculosis patients tested for HIV infection and notified over the reference period, reported at the total number of patients with tuberculosis multiplied by 100. It is reported in %.
- The rate of HIV co-infected among tuberculosis cases is the total number of tuberculosis patients with HIV co-infection reported at the total number of tuberculosis patients reported during the reference period multiplied by 100. It is reported in %.

Statistical indicators for the evaluation of the National Tuberculosis Control Program

The evolution of the epidemiological indicators of tuberculosis in the Republic of Moldova reflects the socio-economic situation of the country. During the period of time between 1970 and 1990, the major screening way was active detection through the compulsory chest X-ray examination of the entire adult population and tuberculin skin testing of all children (aged less than 0-17 years 11 months, and 29 days). The ac-

tive screening contributed to the early detection of limited forms of tuberculosis, which ensured a low disease prevalence, a stable control at the national level with a reported global incidence of 39 per 100.000 population in 1993. A quietly stable epidemiological situation was demonstrated by the mortality indicator, which showed the lowest level of 4.6 per 100.000 population in 1990. The onset of the political crisis and the failure of the standardized management of the tuberculosis specialized service contributed to the increase of the number of tuberculosis patients, which reached a 3-4 times higher value in 2005 than in 1990. According to the recommendation of the WHO the Republic of Moldova started implementing the first global control strategy of tuberculosis defined Directly Observed Treatment Short Course Chemotherapy (DOTS). Special attention was given to the equipment of the reference microbiological laboratories with high-performance tools and the passive detection of symptomatic patients using microbiological methods. As a consequence, epidemiological indicators increased 3 times, and the maximum value was established in 2005. The next period was the national implementation of the STOP TB Strategy, which was started in 2008 and contributed to the improvement of the epidemiological situation and the regression of the indicators. Starting in 2014 in the Republic of Moldova was implemented the molecular method GeneXpert MTB/Rif. The assay is used for the assessment of all suspects for tuberculosis and for identification of the mutation of the rpoB gene responsible for rifampicin resistance. As a result, the detection of drug-$_{\text{resistant}}$ tuberculosis increased and the Republic of Moldova was placed on the list of countries with the highest burden of TB-MDR. In 2015, the WHO adopted the End TB Strategy, whose implementation was initiated in the Republic of Moldova in 2016. The assessment of the tuberculosis suspects through the modern microbiological methods, the directly observed treatment, the use of the new drugs in the treatment of drug-resistant tuberculosis resulted in a significant reduction of the epidemiological indicators. The evolution of the global incidence is represented in *Figure 1.*

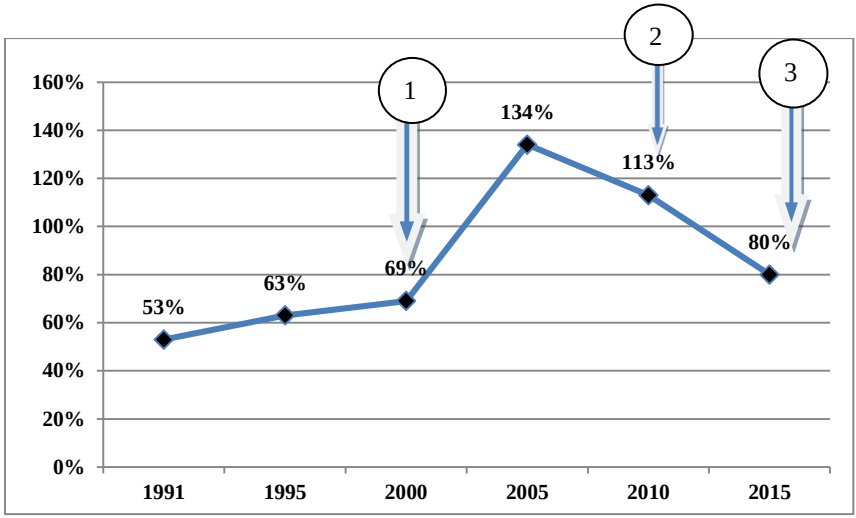

Fig. 1. Tuberculosis incidence in the Republic of Moldova between 1991-2015 (per 100.000 population).

Note: *1* – implementation of the DOTS Strategy in 2000; *2* – implementation of the STOP TB Strategy in 2008, *3* – implementation of the End TB strategy in 2016.

The evolution of the mortality followed the evolution of the incidence. Between 1991 and 2005, mortality increased four times, which was connected with a high incidence. Despite the improvement of the microbiological laboratory infrastructure, the impact on the decrease in mortality between 2000 and 2010 has not been established. Implementation of the GeneXpert MTB/Rif for the investigation of the new cases, the use of the new anti-TB drugs, and the supportive measures of the patients contributed to an important reduction of the mortality rate. The evolution of mortality in the Republic of Moldova is shown in *Figure 2.*

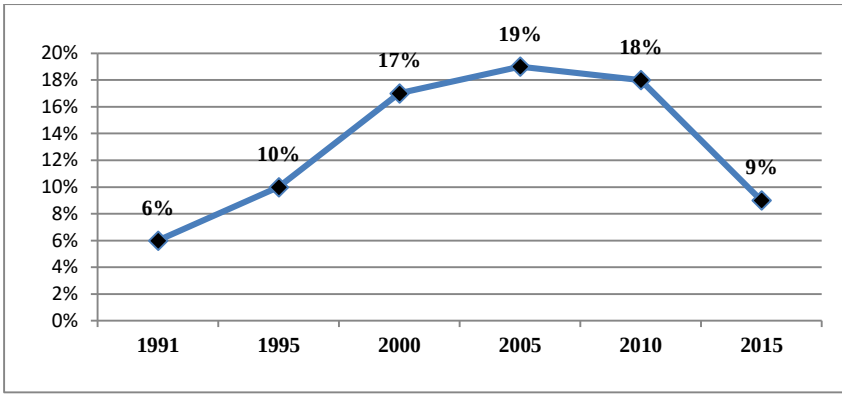

Fig. 2. Tuberculosis mortality in the Republic of Moldova between 1991 and 2015 (per to 100.000 population).

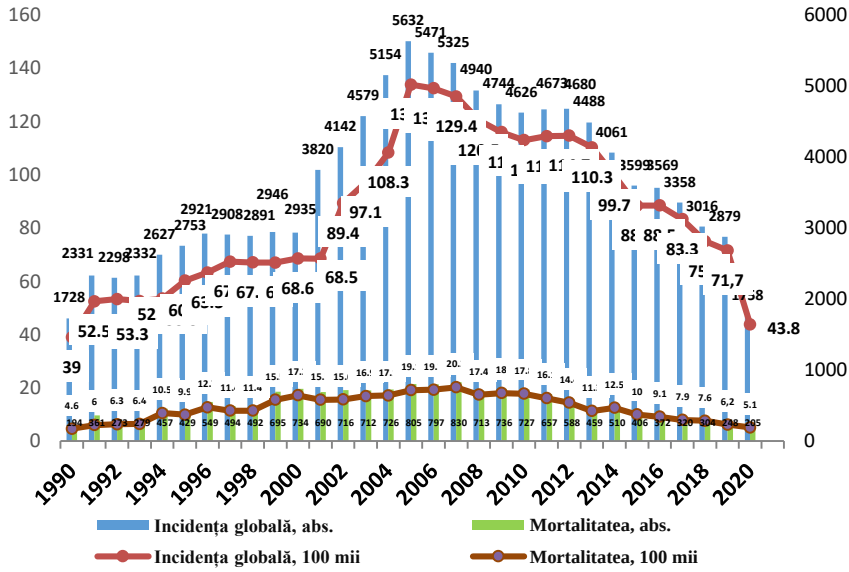

Figure 3. The tuberculosis global incidence and mortality in the Republic of Moldova.

Below were exposed the most important indicators, which characterize the evolution of the epidemiological situation of tuberculosis in the Republic of Moldova. The global incidence (the number of new cases and relapses) for 2018 was 75.1 per 100.000 population (3.022 cases), in 2017-83.3 per 100.000 population (3352 cases), in 2016-88.5 per 100.000 population (3.569 cases). There is a 15% decrease rate during the years 2016-2018 of the global incidence. From the number of registered patients, the new cases of tuberculosis in 2018 were 2.454 with the incidence 61 per 100.000 population, compared to 2017 when were registered 2.681 cases or 66.6 per 100.000 population. In 2016, 2.843 new cases were registered, which means 70.5 per 100.000 population. There is a decrease of 13.5% during 2016-2018 in the incidence of new cases. The incidence of relapses in 2018 decreased by 21.7% (568 cases or 14.1 per 100.000 population) compared to 2016 (726 cases 18 per 100.000 population). The mortality rate for tuberculosis in 2018 constituted 7.6 per 100.000 population (304 cases), compared to 2017-7.9 per 100.000 population (320 cases) and 2016 - 9.1 per 100.000 population (372 cases). The mortality rate for tuberculosis decreased by 16.5% compared to 2016. The success rate of anti-tuberculous treatment for the 2017 cohort of patients with susceptible tuberculosis was 82.2% (903 from 1099 cases), compared to the previous cohort in 2016 - 82.5% (991 from 1202 new cases of susceptible pulmonary tuberculosis, bacteriologically confirmed) and 2015 cohort - 82.2% (1.032 out of 1255 cases). It was determined the increase of the treatment success rate in patients with multidrug-resistant tuberculosis. The success rate of the MDR-TB treatment in new cases for a cohort of 2016 year was 68.6% (302 from 440 cases), for MDR-TB treatment in new cases for a cohort of 2015 - 66.7% (269 from 403 cases) and for MDR-TB treatment in new cases for a cohort of 2014-64.1% (259 from 404 new cases TB MDR). The rate of TB / HIV co-infected patients among new cases and relapses of tuberculosis in 2018 was 8.2% (248 cases), compared to 2017-8.4% (281 cases) and 2016-8.5% (301 cases).

The global incidence, estimated by the WHO for the Republic of Moldova in 2018 was 86.0 per 100.000 population with a gap of 10.0%

between the estimated and registered incidence (75.1 per 100.000 population). The late detection of patients with tuberculosis was confirmed by the rate of 33% of the patients with lung destruction.

Epidemiological indicators worldwide

Tuberculosis (TB) is a communicable disease, a disease of poverty and economic vulnerability. Even if tuberculosis is curable and preventable, it is one of the 10 causes of death worldwide and the leading cause of death from a single infectious agent.

Globally, an estimated 10.0 million (range, 8.9-11.0 million) persons developed tuberculosis in 2019. There were an estimated 1.2 million (range, 1.1-1.3 million) of tuberculosis deaths among HIV-negative persons in 2019 (a reduction from 1.7 million in 2000), and an additional 208.000 deaths (range, 177 000-242 000) among HIV-positive people (a reduction from 678.000 in 2000). Men (aged more than 15 years) accounted for 56% of the persons who developed tuberculosis in 2019; women accounted for 32% and children (aged less than 15 years) were 12%. Geographically, most people who developed tuberculosis in 2019 were living in the WHO regions of South-East Asia (44%), Africa (25%), and the Western Pacific (18%), with a smaller percentage in the Eastern Mediterranean (8.2%), the Americas (2.9%) and Europe (2.5%). Eight countries accounted for two-thirds of the total number of cases: India (26%), Indonesia (8.5%), China (8.4%), the Philippines (6.0%), Pakistan (5.7%), Nigeria (4.4%), Bangladesh (3.6%) and South Africa (3.6%). The other 22 countries from the WHO's list of high MDR-TB burden countries accounted for 21% of the total number of cases.

The drug resistance to anti-tuberculous treatment has currently become a major problem in the control of the disease in the majority of the countries with a high incidence. The main attention actually is attributed to the patients with multi-drug resistance (MDR-TB) and resistance to Rifampicin (MDR/RR-TB). Worldwide 0.5 million people developed rifampicin-resistant tuberculosis (RR-TB), including 78% were microbiologically confirmed with MDR-TB. Only 3 countries included the majority of MDR-TB cases: India, China, and the Russian Fede-

ration. Globally, 3.3% of new cases and 17.7% of those previously treated, were confirmed with MDR-TB. The highest proportion (more than 50% of the total number of confirmed cases) was identified in the post-Soviet countries, where the patient hospitalization system is maintained.

Tuberculosis remains one of the top 10 causes of mortality globally. Annually the number of dead patients decreases constantly. The WHO European region met the milestone in 2020 to reduce the rate of deaths by 31% from 2015 to 2019.

The BCG vaccination rate is over 90% in 163 countries, where vaccination is compulsory.

Synthesis of the information from the second chapter

- Tuberculosis is a socially determined disease, affecting the social-economical vulnerable population, belonging to high-risk groups.
- The management of tuberculosis patients is defined in the WHO recommendations transposed into the global strategies implemented successively worldwide: DOTS, STOP TB, and End TB.
- The current policy of the World Health Organization (WHO) on tuberculosis control is based on the End TB Strategy (Strategy), with the milestones: to reduce tuberculosis deaths by 75% compared with 2015, to reduce the global incidence by 50%, and zero families facing catastrophic costs due to TB.
- The World Health Organization's 2020 report estimated 10.4 million new cases globally (average 8.9 - 11.0 million) in 2019, including 5.9 million (56%) men, 3.5 million (32%) women, and 1 million (12%) children.
- The global incidence, estimated by the WHO for the Republic of Moldova in 2018 was 86.0 per 100.000 population with a gap of 10.0% between the estimated and recorded incidence (75.1 per 100.000 population). Among diagnosed with MDR/RR-TB cases new cases were 33% and previously treated cases - 60%

CHAPTER III. ETIOLOGY OF TUBERCULOSIS

The aim of the practical lesson is to create specific competencies in the field of etiology of tuberculosis and the peculiarities of Mycobacterium tuberculosis.

The objectives are:

- To study the phylogenetic taxonomy of Mycobacterium tuberculosis and the main peculiarities,
- To study other species of Mycobacterium of the Genus Mycobacterium and their peculiarities,
- To identify the physical and chemical agents against which Mycobacterium tuberculosis is resistant and susceptible.

The type of activity is theoretical and involves the study of the information presented in this chapter.

The duration of the study for the introductory course is one academic hour and the associated practical activity will take place over a period of five academic hours.

Theoretical framework

Tuberculosis is an infectious and contagious disease caused by the Mycobacterium tuberculosis complex, which includes Mycobacterium tuberculosis hominis, Mycobacterium africanum, Mycobacterium bovis, Mycobacterium microtti. Tuberculosis can affect any organ or part of the body, but more frequently the lungs. Mycobacterium tuberculosis complex belongs to the genus Mycobacterium, family Mycobacteriaceae, order Actinomycetalis, class Actinobacteria, and domain Bacteria. Infection with Mycobacterium tuberculosis hominis is diagnosed in approximately 90% of tuberculosis patients. Infection with Mycobacterium bovis is established in approximately 5% of the patients. The decrease in epidemiological indicators of the infection with Mycobacterium bovis is determined by the control of infection in cattle and the pasteurization of dairy products. Mycobacterium africanum causes infection in people living in Western and Central Africa. The rate of tuberculosis cases caused by Mycobacterium africanum is about 3%.

The aspect of the Mycobacterium tuberculosis is a thin stick or bacillus of 0.2 - 0.5 μm diameter, with a length of 2 - 5 μm, straight or slightly curved, nonencapsulated and immobile. The bacilli are acid-alcohol-resistant and visible at Ziehl-Neelsen staining. Grows slowly on solid Lowenstein-Jensen medium with the doubling time of 21 hours. It contains granules with a diameter similar to its thickness, which gives them the appearance of rosettes. When stained with fuchsin used by the Ziehl-Neelsen method, they are visualized as pink sticks, located as one, two or in groups on a blue background of the cellular components of the sputum smear. The characteristic location of the bacterial cells is in the form of the Roman Numeral V. Spherical structures or mycelium form can be identified and these are the latent mycobacterial forms.

The genus Mycobacterium includes more than 50 species and subspecies of pathogenic and saprophytic mycobacteria, widespread in nature. Their common features are bacillary form, immobility, acid-alcohol resistance, slow growth and division, and aerobiosis.

Mycobacteria species pathogenic for people are classified according to criteria:

1. Pathogenicity, in: pathogenic, conditionally pathogenic, and saprophytes;
2. Growth rate, in: fast and slow-growing mycobacteria;
3. Pigmentation of the colonies: chromogenic and non-chromogenic;

Actually, more than 70 species of non-tuberculous mycobacteria (Non-Tuberculosis Mycobacteria, abbreviation NTM and Mycobacteria other than tuberculosis, abbreviation MOTT) previously known to have been described as atypical mycobacterium have been described. These NTM species cause mycobacteriosis. Mycobacteriosis was classified into 4 groups depending on the affected organ: lymphadenitis, skin and soft tissue pathologies, lung infection and disseminated pathologies. According to the pathogenicity of NTM, were classified into groups: pathogenic, conditionally pathogenic, and saprophytic Mycobacterium *(Table 1).*

Table 1

The classification of mycobacteria according to the pathogenicity

Pathogenic Mycobacterum	Conditionally pathogenic	Saprophytic Mycobacterium
M. tuberculosis	*M. avium*	*M. gordonae*
M. bovis	*M. intracellulare*	*M. terae*
M. africanum	*M. kansasii*	*M. triviale*
M. microti	*M. malmoense*	*M.phlei*
M. caprae	*M. xenopi*	*M. flavescens*
M. leprae	*M. fortuitum*	*M. gastri*
	M. chelonae	

According to the tinctorial and cultural peculiarities, non-tuberculous mycobacterium was classified by Ernest Runyon in 1959 in groups: photochromogenic, scotochromogenic, noncromogenic, fast-growing mycobacterium species. Photochromogenic mycobacterium have slow growth and the color of the culture is yellow when are exposed to light. Schotochromogenic mycobacterium have slow growth and the cultures turn yellow whether or not they are exposed to light. Mycobacterium szulgai is photochromogenic, if is grown at 24ºC and schotochromogenic if is grown at 37ºC. Nonchromogenic mycobacteria have slow growth. They never produce a pigment regardless of the growing conditions. Fast-growing mycobacterium produces colonies in 5 days and do not produce pigment.

Table 2

The E. Runyon classification of nontuberculous mycobacteria

Photochromogens	Schotochromogens	Nonchromogens	Rapid growing
M. kansasii	*M. scrofulacaeum*	*M.*	*M. fortuitum*
M. marinum	*M. gordonae*	*avium-*	*M. peregrinum*
M. simiae	*M. xenopi*	*intracellulare*	*M. chelonae*
M. asiaticum	*M. flavsecent*	*M. ulcerans*	*M. abscessus*
M. szulgai	*M. thermoresistible*	*M. malmoense*	*M. thermoresistible*
	M.szulgai	*M. xenopi*	*M. smegmatis*
		M. gastri	*M.phlei*
		M.terrae-triviale	
		M.haemophylum	

Resistance to physical and chemical agents

Mycobacterium tuberculosis is resistant to physical and chemical agents due to the peculiarities of the cell wall. The structure of the cell wall is rich in phospholipids, which allows the arrangement of mycobacteria in piles and their coating with an organic tissue formed by the components of the affected tissue. Mycobacterium bacilli are resistant to frost, maintaining their viability up to -180℃ and resistant to dehydration. It is sensitive to heat, light (ultraviolet rays), antiseptic substances (formalin vapors at 50C, chloramine 5-10%, lime chloride 20%, sodium hypochlorite 0.5%, phenol 0.5%, cresol 10%, lysol 1-5%).

Synthesis of the information from the third chapter

1. Tuberculosis is a social, infectious, and contagious disease.
2. Tuberculosis is caused by the Mycobacterium tuberculosis, which includes Mycobacterium tuberculosis hominis, Mycobacterium africanum, Mycobacterium bovis, Mycobacterium microtti.
3. Tuberculosis can affect any organ or part of the body, but more often are affected the lungs because they are the ventilated organs.
4. Mycobacterium tuberculosis complex is part of the genus Mycobacterium, family Mycobacteriaceae, order Actinomycetalis, class Actinobacteria and domain Bacteria.
5. Infection with Mycobacterium tuberculosis hominis is diagnosed in approximately 90% of patients with active tuberculosis.
6. Mycobacterium bovis is established in approximately 5% of patients infected by the digestive way.
7. Mycobacterium tuberculosis has the aspect of a thin rod or bacillus of 0.2-0.5 µm, with a length of 2-5 µm, straight or slightly curved, nonencapsulated, immobile, and non sporulated.
8. Mycobacterium tuberculosis is an acid-alcohol-resistant bacillus when stained using Ziehl-Neelsen staining and is seen as raspberry-pink sticks, located as one, two, or in groups on a blue background of the cellular components of the sputum smear.

9. The characteristic disposal of mycobacterial cells on microscopic examination is in the form of the Roman numeral V. Spherical or mycelial structures can be identified and they are dormant Mycrobacteria (in latent form).
10. Mycobacterium tuberculosis grows slowly on solid Lowenstein-Jensen medium with a doubling time of at least 21 hours.
11. It contains granules with a diameter similar to its thickness which gives them the appearance of rosettes on the microscopic examination of the high power.
12. More than 50 species and subspecies of pathogenic and saprophytic mycobacteria of the genus Mycobacterium are known, and are included in the MOTT group (Mycobacterium other than tuberculosis) known as atypical mycobacteria.
13. Atypical mycobacteria are rose-shaped bacilli, immobile, acid-fast, with slow or rapid multiplication, require aerobiosis, and can be differentiated from Mycobacterium tuberculosis only by cultural methods.

CHAPTER IV. WAYS AND SOURCES OF INFECTION WITH MYCOBACTERIUM TUBERCULOSIS

The aim of the practical lesson is to create specific skills in the field of the epidemiological chain of the transmission of tuberculosis and the groups with a high risk for tuberculosis.

The objectives are:

- To identify the sources of infection and ways of the transmitssion of Mycobacterium tuberculosis,
- To establish the risk factors for the transmission of the infection with Mycobacterium tuberculosis and the risk factors for developing tuberculosis,
- To know the high-risk groups for tuberculosis.

The type of activity is theoretical and involves the study of the information presented in this chapter.

The duration of the study for the introductory course is one academic hour and the associated practical activity will take place over five academic hours.

Theoretical framework

In the actual epidemiological situation, the major way of transmitssion of Mycobacterium tuberculosis infection is the airborne way. The airborne transmission consists of inhalation of nucleosomes (syn. Droplet nuclei) with sizes below 5 μm containing 1 to 5 bacilli. These nucleosomes are highly infectious. They are called bacilli concentrators and keep their infectivity in the ambient air for 8 to 10 days. Droplet nuclei are transported with airflow and contaminate new surfaces and spaces, which become the sources of infection. To infect an adult, it is necessary to inhale a minimum of 2000 viable Mycobacteria.

By speech, sneezing, cough, the patient with pulmonary tuberculosis spread a drop of drops, called Flűgge droplets of different sizes (10-500 μm). Flűgge drops spread around the source of infection at a distance of 0.8-1.5 m. Big Flűgge drops (> 20 μm) float in the air, then

sediment on the ground with a speed of 1 m per hour. Their penetration into the upper respiratory airways of the person who contacted with the source of infection is blocked by the nasal passage, mucociliary clearance, and respiratory protective reflexes. Once released into the environment, the water evaporation from the Flűgge drops transforms them into nucleosomes. Nucleosomes have small sizes (<5 μm) and contain 1-5 bacilli. They penetrate the bronchial tree and, if they are not cleaned by the human physiological protective barriers, reach the terminal bronchiole and the alveoli where develop the primary infection. Certain conditions increase the risk of transmission of tuberculosis infection. Factors relating to the particularities of the exposure to the source of infection have a big importance. The proximity, frequency, and duration of exposure of the contact person with the source of infection are considered epidemiological risk factors. Close contact with the source of the infection and the long duration of contact contributes to a 22% annual probability to get the infection. The frequent coughing, sneezing, inappropriate hygiene of the cough, lung destruction or cavities, inadequate anti-tuberculosis treatment increase the patient's infectiousness and contagiousness.

Poor hygiene of coughing and incorrect handling of the collected specimens are contributing factors to the patient’s infectiousness. If the patient does not cover the mouth and nose when coughing, sneezing, or if he does not expectorate recipients for sputum collection, the close contact is considered to be an infectious risk. The risk of infection transmission is increased in closed, unventilated spaces without access to natural light. Insufficient room ventilation causes an insufficient decrease in the density of the droplet nuclei. Positive pressure in the patient's room causes the dispersion and recirculation of the nuclei. The wrong manipulation of specimens collected from the patient contributes to the spread of the nosocomial infection. Depending on the capacity of the Mycobacteria removal, the duration of the contact, and the receptivity of the organism there are different types of infections:

- Occasional infections – infectious contact, that is not repeated;
- Insidious infections – moderate or persistent infections with bacilli;
- Massive infections – infections with a large number of bacilli, which are present in current tuberculosis outbreaks.

The organism's receptivity to the tuberculous infection is determined by the mucociliary clearance of the bronchial tree from the infectious nucleosomes, as well as by the associated diseases, which diminishes the local and systemic immune reactivity. These risk factors are more frequently established in early childhood.

Another way of transmission, which is rarely encountered is by the digestive tract. It is involved in the transmission of the infection by *Mycobacterium bovis* through unpasteurized milk or its unprocessed derivatives (yogurt, cheese, cream, butter). Elimination of the *Mycobacterium bovis* is produced through the infected milk obtained from tuberculosis mastitis or the feces of the sick animals. The digestive route of transmission is an extremely rare way of transmission the infection to a child fed with breast milk. Due to measures for fighting against bovine tuberculosis, the compulsory skin test of cattle contributed to an important drop-down in the number of cows infected with *Mycobacterium bovis.*

The cutaneous way of the infection with *Mycobacterium tuberculosis* is involved in the development of the tuberculosis chancre and *tuberculosis verrucosa cutis.* Those forms of cutaneous tuberculosis occur in people who manipulate the infected samples, feces, or bodies of tuberculosis patients. There were identified exceptional ways of transmission of the infection with *Mycobacterium tuberculosis* during circumcision, kissing, and mouth-to-mouth breathing.

Rene Laennec (born on February 17, 1781, died on August 13, 1826) was a French physician, who invented the stethoscope and indirect auscultation. He had *tuberculosis verrucosa cutis* on his right hand due to the necropsies and he died of tuberculosis.

The uteroplacental way is rarely encountered in the transmission of tuberculosis from the mother to the newborn. It was reported in the African countries with high epidemic indices, which is associated with

the extension of the HIV infection. Despite the way of transmission and the mechanism of the contamination, the risk of infection is higher, if the contact is closed and the frequency of the contact is higher.

Susceptible groups of the population and the high-risk groups

The susceptible population is the total number of individuals, who came in contact with the source of infection, through different ways and mechanisms of transmission, develop the latent tuberculosis infection and may reactivate into active tuberculosis disease. The population's receptivity is determined by the virulence of *Mycobacteria*, the sources and ways of transmission of the infection, the individual's state of innate immunity and acquired immunity.

Factors that contribute to the vulnerability of the population to the *Mycobacteria* infection are classified as: ***endogenous*** and ***exogenous.*** The ***endogenous factors*** include:

- Age groups;
- Immune resistance of the population;
- Comorbidities.

Age groups

There are several age groups more affected by tuberculosis:

1. The age group of the children aged less than 5 years have an increased receptivity to acquire the tuberculosis infection and to develop rapidly progressive disease. The highest susceptibility has the infant aged less than 1-year-old, often developing severe forms of tuberculosis such as acute disseminated pulmonary tuberculosis (milliary tuberculosis) or generalized form. Children aged between 5 to 14 years have a natural resistance to *Mycobacteria* infection due to the primary infection that occurred in their first years of life.

2. The age group of adolescents aged between 14 and 18 years old is characterized by a high susceptibility of adolescents to develop active tuberculosis, often with extra-pulmonary localization or forms of secondary tuberculosis, more frequently complicated with pleurisy.

3. The age group between 18 and 25 years is characterized by the complexity of the social and epidemiological risk factors, which are more prevalent in patients from high tuberculosis burden countries.

4. The age of the persons older than 65 years, predisposes the reactivation of latent tuberculosis infection localized in the post tuberculous sequelae under the influence of immunosuppressive risk factors and comorbidities.

Immune resistance of the population

The immune resistance of the population is generally effective and can be diminished by a wide range of socio-economical risk factors. Social vulnerability is the first contributing factor to insufficient or inadequate nutrition. Dietary deficiencies in proteins, vitamin D, calcium, and foods with antioxidant activity decrease the body's immune response, predisposing the reactivation of latent tuberculous infection and developing active tuberculosis. BCG vaccination of the newborn provides relative protection of the child against severe forms of tuberculosis: tuberculous meningitis and acute disseminated pulmonary tuberculosis.

The susceptibility of the population to tuberculosis infection can be increased by excluding from the compulsory vaccination program the newborns with contraindications to vaccination or those whose parents have refused vaccination for various reasons. Canceling revaccination with the BCG vaccine of children at the age of 7 years old can increase the epidemiological indicators in adolescents because this age group is vulnerable to the reactivation of the tuberculous infection and extrapulmonary localisations.

Associated diseases

Comorbidities with an increased risk for the development of tuberculosis are HIV infection, chronic nonspecific respiratory diseases, frequently associated with active smoking, gastrointestinal pathologies, chronic or abusive alcohol consumption, intravenous drug use, diabetes, chronic renal failure, mental disorders, neoplastic pathologies and immunosuppressive treatment: corticosteroids, chemotherapy, radiothe-

rapy, biological antiTNFα therapy. Pregnancy and the period after the child's birth (postpartum) are physiological conditions that predispose the development of tuberculosis.

HIV infection

The most severe suppressive condition for the immune system is HIV infection. It can be considered the major risk factor for tuberculosis, both for primary infection and the reactivation of latent tuberculosis infection in active tuberculosis. In people living with HIV, the risk of developing tuberculosis is estimated to be 26-31 times higher than in HIV-negative people. Infection with opportunistic germs *(Pneumocystis jirovecii, atypical mycobacteria, human polyomaviruses* (JC and BK virus*), Cryptococcus neoformans, Cryptosporidium parvum)* as well as those caused by pathogenic germs, for example, *Mycobacterium tuberculosis,* infectious viruses, Toxoplasmosis, determines the death in people living with HIV. In HIV-positive patient's the pneumonia with Pneumocystis can take the clinical-radiological aspect of tuberculosis. Therefore it is necessary for bronchoscopic examination with of bronchoalveolar lavage and Giemsa staining for detection of Pneumocystis. Immunodiagnosis for the detection of circulating antibodies or antigens using ELISA techniques or direct immunofluorescence is also effective for the diagnosis of *Pneumocystis.*

Patients co-infected with TB/HIV have an increased risk of drug resistance (MDR-TB). They have a lower chance to be cured and a higher risk of death. The pandemic nature of HIV infection has transformed tuberculosis from an endemic disease to an epidemic one, considering that HIV infection is the most important factor contributing to the development of tuberculosis. The progression of tuberculosis infection in active tuberculosis occurs in a proportion of 37% of the cases in the first 6 months after the primary tuberculosis infection and in 2-5% in the next 2 years. In people living with HIV, tuberculosis can occur at any stage of the disease, regardless of the level of CD4+ lymphocytes, but more frequently in the advanced stages of HIV infection when immunity achieves the minimum level. The Acquired Immune Deficiency

Syndrome (AIDS) is the final stage of infection, characterized by severe immunodepression (CD4+ lymphocyte count is below 200/mm^3), and the diagnosis of tuberculosis is the clinical criterion for stages B and C.

The effectiveness of the treatment for AIDS-associated tuberculosis is low, with a high rate of mortality and therapeutic failure. The causes of the treatment failure in TB/HIV coinfected patients are severe reduction of all physiological functions, chronic diarrhea with *Cryptosporidium*, intestinal malabsorption. Most often the patients develop primary forms of tuberculosis, extrapulmonary tuberculosis, or generalized tuberculosis. Among the forms with extrapulmonary localization predominates the tuberculosis of the lymph nodes (tuberculous lymphadenitis), tuberculous pleurisy, tuberculosis of the central nervous system (meningitis), tuberculous pericarditis, etc.

In the Republic of Moldova, the rate of TB/HIV co-infection in new cases and relapses was 8.2% (248 cases) in 2018. All patients with tuberculosis in the Republic of Moldova are counseled and tested for HIV markers (Elisa HIV test for antibodies and antigens, p 24 antigen). People living with HIV are exposed to annual radiological screening for tuberculosis by radiological examination in adults and tuberculin skin test in children.

Diabetes mellitus

Patients with diabetes have a risk to develop tuberculosis 5 to 10 times higher than patients without glycemic disorders. The pathway is the reactivation of the latent tuberculous infection located in the mediastinal lymph nodes or the post tuberculous sequelae in the lung parenchyma. The main causes of the reactivation of the latent tuberculous infection are: reduced acid secretion in the gastrointestinal tract, disorders of lipid-protein and mineral metabolism, decreased synthesis of anti-tuberculous antibodies, and consequently decreased immune reactivity of the body. In patients with tuberculosis and diabetes, extensive parenchymal destruction is often established due to the exudative-necrotic features of tuberculous inflammation, pulmonary fibrosis, and hemato-bronchogenic dissemination.

The clinical manifestations and the expressiveness of the symptoms of tuberculosis depend on the severity of diabetes and the level of the compensation of the endocrine disorders. In compensated diabetes mellitus, the limited forms of pulmonary tuberculosis are more frequently diagnosed. In decompensated diabetes, extended forms and fibrocavitary pulmonary tuberculosis are common. Limited forms of tuberculosis in diabetic patients have a low expressed symptomatology. Asthenia, weight loss, night sweats, low-grade fever are more frequently attributed rather to diabetes than tuberculosis. The first signs which are attributed to the decompensation of diabetes are disorders of carbohydrate metabolism and increased need for insulin supplementation. A peculiarity of tuberculosis in patients with diabetes is the localization of tuberculosis in the inferior lobes. The clinical manifestations of tuberculosis in patients with diabetes are characterized by the consequence of the development of tuberculosis. Tuberculosis that occurs in the background of diabetes has an acute evolution, severe lymphogenic and haematogenic dissemination with progressive evolution, and a low response to antituberculosis treatment. Healing occurs with significant fibrosis and lung sequelae which reduce the respiratory dynamics.

Exogenous factors

Exogenous factors with a high risk for tuberculosis are the meteorological conditions specific to the geographical area. Humidity, low temperature, air movements, atmospheric pressure can contribute to an increased risk of the reactivation of the latent tuberculosis infection during the cold season. Environmental pollution causes decreased mucociliary clearance, chronic inflammation of the bronchial mucosa, ireversible changes or fibrosis in lung architecture, which contributes to tuberculosis. Natural disasters through the social and economic impact determine the recrudescence of the epidemiological indicators.

Social economical factors

The World Health Organization defined tuberculosis as a classic example of a social disease with multiple causes. The social economical vulnerability of the population groups at high risk for tuberculosis is asse-

ssed by unemployment, low financial income (students, people with disabilities or retired), migration, detention, etc.

Migration is a challenge for any state's public health system. Young people are more often involved in migration. Migration is associated with poverty, overcrowding, and illegal status. Internal migration is a form of migration within the country with an increased risk for tuberculosis, as well.

Psychosocial stress and wars are among the factors with an increased risk for tuberculosis. War tuberculosis has been described in the literature. It is caused by malnutrition, overcrowding, low sanitation, intense migration, the war industry, and psychosocial stress.

Occupational factors are involved in the development of occupational diseases. Pneumoconiosis, such as silicosis, anthracosis, and asbestosis, are comorbidities with high-risk factors for tuberculosis. The prevalence of tuberculosis in patients with silicosis is 30 times higher than in the general population.

Tuberculosis in medical staff often is associated with nosocomial infection. In medical institutions specialized pneumophthisiology, the indicators of tuberculosis among the medical staff are 5 times higher than in the general population.

Adequate nutrition provides effective immune protection against the reactivation of latent tuberculosis infection. Hyponderability (<10% of ideal weight), fasting, restrictive diet (lack of animal protein or dairy), vitamin deficiency through a diet low in vegetables and fruits s considered high-risk factors for tuberculosis. On the other side, the overweight diminishes, as well the immune resistance of the body, and can reactivate the latent tuberculosis infection.

The low hygienic-sanitary standard appreciated by the precarious living conditions, such as overcrowding and unhealthiness, increases the risk of infection and illness. In this context, Victor Babeş mentioned the following statement: „There is a vicious circle between misery, the cultural and health status of the population, and tuberculosis".

Harmful addictions with an increased risk of tuberculosis are alcohol consumption, smoking, and intravenous drug use. Alcoholism „lays the bed of tuberculosis" through conditions of social misery, metabolic disorders, and immune suppression. Smoking predisposes to chronic non-specific respiratory diseases, which is a risk factor for tuberculosis. Intravenous drug use increases the risk of contracting HIV infection, which is a risk factor for tuberculosis. Co-dependence on psychotropic substances contributes to the decrease of self-criticism and adequate receptivity to the environment. Consequently, the codependent patient has a diminished addressability to the medical services, reduced acceptance of the diagnosis, and reduced compliance to the antituberculosis treatment.

High-risk groups for acquiring tuberculosis

According to the National Clinical Protocol, the groups with an increased risk for tuberculosis are:

1. Contacts with tuberculosis patients, identified in the frame of the epidemiological investigation of the clusters;
2. Persons with post-tuberculosis changes, sequelae;
3. People living with HIV;
4. Persons with compromised immunity or receiving immunosuppressive therapy (cortisone therapy, chemotherapy, radiation therapy for cancer, immunomodulators anti-TNF-α);
5. Patients with diabetes mellitus;
6. Patients with mental illness;
7. Migrants;
8. Homeless people;
9. Staff of emergency healthcare teams;
10. Staff of medical institutions specialized in phthisiopneumology.

Other groups with an increased risk of disease specific to the epidemiological situation in the territory may be adjusted to the mentioned groups. This allows the extension of the area for active screening. Ho-

wever, clinical awareness should be maintained for all risk groups not included in the above list.

The category of persons in need of increased vigilance includes:

- Social vulnerable people: unemployed and people with low-income;
- People with medical-biological risk factors: patients with chronic non-specific respiratory diseases, active smokers, chronic or abusive alcohol users, intravenous drug users, patients with chronic renal failure, gastrointestinal pathologies, pregnant women, BCG unvaccinated children;
- Staff of the institutions: asylums, prisons, hospices, placement centers;
- Students of the medical colleges, of the University of Medicine and Pharmacy, residents;
- Staff of all types of medical institutions;
- Nurses.

Synthesis of the information from the fourth chapter

1. Tuberculosis is an infectious-contagious disease with airborne transmission.
2. Infection occurs after the inhalation of nucleosomes, which may contain 1-5 *Mycobacterium tuberculosis* sufficient to infect the human body.
3. The main source of infection is the patient with pulmonary tuberculosis. The source of infection through the respiratory reflexes eliminates Flűgge droplets of different sizes (10 - 500 μm) that spread *Mycobacterium tuberculosis* at a distance of 0.8-1.5 m.
4. The risk of the infection depends on the proximity, frequency, duration of the contact with the source of infection, and the particularities of the environment (ventilation, humidity, exposure to sunlight/ultraviolet rays).

5. Tuberculosis caused by the *Mycobacterium bovis* can affect any organ but more frequently the gastrointestinal tract and was eradicated through the tuberculin skin testing of the cattle and slaughter of the positive reactive animals.
6. The cutaneous and placental way of transmission is episodic, absent in the current epidemiological context of the Republic of Moldova.
7. The receptive population is higher in the phthisiogenic age groups: 0-4 years, 14-18 years, 18-25 years, and over 65 years.
8. The immune resistance of the population depends on the complexity of the contributing factors: socio-economic conditions, food quality, the rate of the subpopulation with harmful habits (smoking, alcoholism, drug addiction), the prevalence of BCG non-vaccinated newborns, the prevalence of HIV infection, etc.
9. There is currently an increased rate of comorbid patients in hard-to-reach groups (homeless, migrants, detainees, or persons with a personal history of detention), which keeps the epidemiological situation endangered.
10. The groups at high risk are: contacts with tuberculosis patients, identified during the epidemiological investigation of the clusters; people who were diagnosed with tuberculosis before or have post-tuberculosis sequelae; HIV-infected people; persons with impaired immunity or receiving immunosuppressive treatment; patients with diabetes; patients with mental illness, migrants; homeless people; staff of the urgent healthcare teams; staff of the institutions specialized in phthisiopneumology.

CHAPTER V. IMMUNE RESPONSE AND THE PATHOGENESIS OF TUBERCULOSIS

The aim of the practical lesson is to create specific skills in the field of immune response and the pathogenesis of tuberculosis infection.

The objectives are:

- To study the immune response involved in the protection against the infection with *Mycobacterium tuberculosis,*
- To study the cycle of tuberculosis infection in humans,
- To identify the criteria for differentiation of the latent tuberculosis infection from active tuberculosis.

The type of activity is theoretical and involves the study of the information presented in this chapter.

The duration of the study for the introductory course is one academic hour and the associated practical activity will take place over five academic hours.

Theoretical framework

The human immune response to mycobacterial infection is effecttive against *Mycobacterium tuberculosis.* Successive epidemic waves in Europe have contributed to the selection of genetically resistant individuals, eliminating susceptible people. The different evolution of epidemiological indicators in different regions of the globe is explained by the genetic susceptibility of the black race and the American aboriginal race to tuberculosis infection.

The body's reactivity to mycobacterial infection is explained by the Koch phenomenon described by Robert Koch in 1891. In guinea pigs, subcutaneous inoculation of a virulent dose of bacilli causes the appearance of a nodule, which after 2 weeks' progresses into ulceration with the elimination of a caseous mass. The guinea pig shows all the clinical signs of active tuberculosis: loss of appetite, weight loss, apathy, fever, tachypnea, and cachexia. If a new inoculation will be performed at least 8 weeks later at another region of the body, the body reactions will be

different compared with the first inoculation. In 24-48 hours, a necrotic nodule will constitute and will eliminate the caseum. The local ulcer will heal spontaneously. Differences in the evolution of the lesions demonstrate the appearance of delayed hypersensitivity reaction, which will confer an immune resistance to a new infection. This hypersensitivity causes the response to the pathogen to be rapid and brutal with acute tissue necrosis, and immune resistance contributes to the blocking of the infection at the site of inoculation. The morphological substrate of the Koch phenomenon represents the cell-mediated response. The effector immune cells involved in the Koch phenomenon are: circulating monocytes that migrated into the inoculated tissue and transformed into macrophages, epithelioid cells, giant Langhans cells, as well as T lymphocytes (CD4+ and CD8+ phenotype). The localization in the cockade of these cells is characteristic of tuberculous granuloma. The center of the granuloma consists of caseous necrosis, surrounded by a crown of macrophages, epithelioid cells, giant Langhans cells, and lymphocytes. Macrophages located in the granuloma differentiate into epithelioid cells, giant Langhans cells, and foamy macrophages. At the cross-section, the caseous necrosis is surrounded by a crown of giant Langhans cells, surrounded at the periphery by macrophages, epithetlioid cells, lymphocytes, and a crown of the B lymphocytes and neutrophil leukocytes. The center of caseous necrosis is a necrotic lesion of the tissue with a low concentration of oxygen. Hypoxia from caseous necrosis forms an unfavorable environment for the growth and multiplication of mycobacteria. Foamy macrophages are located at the demarcation line of classification necrosis with the cellular component. The induction of foamy macrophage formation is done by mycolic acids from the mycobacterial wall. After phagocytosis of the mycobacteria, foamy macrophages lose their capacity for phagocytosis and enzymatic degradation but are secretors of tumor necrosis factors (TNF-ά). Through secreted cytokines, foamy macrophages stimulate tissue necrosis. Under the action of the hypoxic intracellular environment, the mycobacteria internalized in the foamy macrophages are induced in the dormant state,

also called latent (dormant) form (L-form) of Mycobacterium tuberculosis. Under certain conditions, foamy macrophages lose their ability to keep the infection dormant and allow the spread of active bacilli in the body. The lymphocyte complex of the tuberculous granuloma consists of CD4 T lymphocytes, CD8 T lymphocytes, and B lymphocytes. At the periphery of the granuloma increases the number of fibroblasts and the synthesis of collagen with the formation of a capsule. Within 5-10 years the granuloma is completely replaced by a connective tissue impregnated with calcium salts and calcination is constituted. In calcinates, mycobacteria turn into the latent form L, which keeps the tuberculous infection asleep. Under the action of a wide range of immunosuppressive risk factors, Micoobacteria can switch from a latent form to an active form. Clinically, appear the signs of tuberculous intoxication.

Infection and disease in humans are cyclical. The penetration of mycobacteria by air into the lung alveoli in an uninfected person causes the development of a lesion during 3 to 8 weeks, which is called the primary focus. The lymphatic vessels are affected by the development of lymphangitis and intrathoracic lymphadenopathy. At the same time, the organism acquires delayed hypersensitivity, also known as hypersensitivity type IVth. The method that determines the appearance of the delayed hypersensitivity to mycobacterial antigens is the tuberculin skin test. The positive or hyperergic result of the tuberculin skin test identifies the tuberculosis infection, without differentiation from the active disease.

Latent tuberculosis infection (LTBI) is a condition of the body in which mycobacteria are kept in latent form (dormant) and a person is conventionally healthy. Indicators of the presence of LTBI are the positive/hyperergic result of the tuberculin skin test, the tuberculin skin test conversion, the positive result of the in vitro interferon γ release test (IGRA test).

Infected people are not infectious and contagious, but under the influence of complex risk factors, they can reactivate the infection and develop the active disease. Several criteria for differentiating LTBI and active tuberculosis were formulated: the clinical manifestations of the pa-

tient, the results of the laboratory tests: microbiological, radiological, immunological, as well as the requirement of the antituberculosis treatment.

The infected person is asymptomatic, the results of laboratory tests are negative, the radiological examination is normal, immunological tests (tuberculin skin test, IGRAs test) show positive results. The patient will not be treated for tuberculosis.

In children who are contacted with a patient with tuberculosis and have a positive or hyperergic tuberculin skin test, and have not been diagnosed with active tuberculosis, will be treated with chemopreventive treatment with isoniazid 10 mg/kg bodyweight for 6 months.

The patient with pulmonary tuberculosis has some manifestations of tuberculous intoxication syndrome: asthenia, fatigue, anorexia, weight loss, hypoponderability, fever or low-grade fever, profuse sweating, and bronchopulmonary syndrome: cough for more than 3 weeks, expectoration and hemoptysis, rarely dyspnea and chest pain. Laboratory microbiological and molecular genetic tests can identify the etiological agent, the radiological examination establishes the clinical-radiological form, and the immunological tests (tuberculin skin test, IGRAs test) are positive. In severe forms associated with immune suppression, the tuberculin skin test may be false negative. The patient requires treatment that should be administrated corresponding to the case type and the spectrum of susceptibility of mycobacteria. Lack of adequate treatment determines the death of the patient within two years.

An estimated one-third of the human population is in a state of latent infection with Mycobacterium tuberculosis. About 10% of infected people will get sick, including 5% in the first two years after infection and 5% later. Tuberculosis develops in certain susceptible individuals, whose predisposition is determined by factors with an increased risk of disease. HIV infection is the most important risk factor for tuberculosis. It has been found that the person lives with HIV but dies of tuberculosis.

Table 3

Differentiation between the latent tuberculosis infection and active tuberculosis

Indicator	Latent tuberculosis infection	Active tuberculosis
Epidemiological anamnesis	TB contact established / absent	The TB contact established
Clinical symptomatology	Asymptomatic	Clinical signs of the intoxication syndrome and bronchopulmonary syndrome
Tuberculin skin test with 2 UT	Positive/hiperergic	Negative (fals) /positive/hyperergic
Radiological examination	No pathological changes or post tuberculosis consequences (sequelae)	Infiltrative opacities, nodules, cavities in „alarm zones", enlarged hilum, pleural effusion
Microbiological examination	AFB negative, culture-negative, GeneXpert MTB/RIFf negative	AFB positive, culture-positive, GeneXpert MTB/Rif positive
Treatment	Chemoprevention	Anti-tuberculosis treatment according to case type and drug susceptibility

The annual risk for tuberculosis in the co-infected person is 7-10%. Patients with diabetes mellitus, chronic end-stage renal disease, neoplastic pathologies, and patients treated with immunosuppressive drugs (corticosteroids, chemotherapy, immunomodulatory therapy, radiation therapy) are likely to develop tuberculosis. Non-specific respiratory disorders reduce mucociliary clearance and non-specific respiratory resistance. As a result, there is an increased risk for both infection and disease. Pathologies of the gastrointestinal tract, which are associated with maldigestion and malabsorption, also lead to an increased risk of tuberculosis. The causes of gastrointestinal maldigestion are gastrectomy, gastroenteroanastomosis, and Zollinger Ellison syndrome. The biliary causes of maldigestion are chronic liver disease and chronic biliary obstruction. The pancreatic causes of maldigestion are chronic pancreatitis, cystic fibrosis (cystic fibrosis of the pancreas). Intestinal enzyme

deficiency, celiac disease, Crohn's disease, short bowel syndrome (post-surgical, enterocolitis fistulas, intestinal bypass) are the intestinal causes of maldigestion.

Synthesis of the information from the fifth chapter

- Tuberculosis is a disease with lymphatic tropism because it affects the cells involved in the cellular immune response (lymphocytes, plasmatic cells).
- The morphological criteria of tuberculous inflammation is the tuberculous granuloma defined by Robert Koch in 1882.
- The immunological reactivity in tuberculous infection is the delayed hypersensitivity, known as the IVth type hypersensitivity.
- The cellular elements of tuberculous granuloma are the circulating monocytes that migrated into the inoculated tissue and transformed into macrophages, epithelioid cells, and Langhans giant cells, as well as T lymphocytes (CD4+ and CD8+ phenotype).
- Tuberculous granuloma consists of the caseification necrosis, which is surrounded by a crown of giant Langhans cells, at the periphery by macrophages, epithelioid cells, lymphocytes, and marginally B lymphocytes and neutrophils.
- The center of the caseous necrosis is a necrotic lesion of the tissue that differentiates the tuberculous granuloma from the tuberculoid granuloma. The tuberculoid granuloma does not show caseous necrosis.
- One-third of the global population is infected and remains in the latent tuberculosis infection (LTBI), without being contagious. LTBI is a condition of the body in which Mycobacteria are kept in dormant form. The criteria for the diagnosis of LTBI are positive/hyperergic result of the tuberculin skin test, the tuberculin skin test conversion, or the positive result at the IGRAs test.
- LTBI is often detected in the high-risk groups, the management of which is clearly defined in the national clinical protocol.

- Active tuberculosis develops after the reactivation of LTBI or exogenous infection.
- The symptomatic patient has the clinical signs of tuberculous intoxication syndrome: asthenia, fatigue, anorexia, weight loss, hypoponderability, fever or low-grade fever, profuse sweating, and bronchopulmonary syndrome: cough for more than 2 weeks, expectoration, hemoptysis, chest pain, and dyspnea.

TESTS FOR SELF-CONTROL OF KNOWLEDGE

In the practical classes, the student is evaluated based on control work, the activity at the patient's bedside, the practical knowledge of the subject, and the clinical case study. At the exam, the student is evaluated based on a test and clinical case study. The tests and clinical cases set out below are useful for the individual preparation of students by verifying knowledge.

The tests for the self-assessment of knowledge are randomly distributed in single-complement (SC) and multiple-complement (MC) response tests:

1. Specify the correct definition of tuberculosis prevalence (SC):
1) The number of new cases of tuberculosis and relapses recorded during a year reported per 100.000 population.
2) The number of all tuberculosis cases registered during a year reported per 100.000 population.
3) The number of tuberculosis deaths registered during a year reported per 100.000 population.
4) The number of new cases of tuberculosis registered during a year reported per 100.000 population.
5) Number of all radiologically investigated cases for the diagnosis of tuberculosis.

2. Identify the correct definition of the global incidence of tuberculosis (SC):
1) The number of relapses registered during a calendar year reported per 100.000 population.
2) Number of the new cases and relapses registered during a calendar year reported per 100.000 population.
3) Number of the new cases confirmed bacteriologically during a calendar year reported per 100.000 population.

4) Number of the cases that initiated antituberculosis treatment during a year reported per 100.000 population.
5) The number of new cases of tuberculosis registered during a year reported per 100.000 population.

3. Specify the correct definition of tuberculosis incidence (SC):
1) Number of new cases of tuberculosis during a calendar year reported per 100.000 population.
2) The number of all tuberculosis cases registered during a calendar year reported per 100.000 population.
3) The number of tuberculosis deaths registered during a calendar year reported per 100.000 population.
4) Number of new cases of tuberculosis registered during a calendar year reported per 100.000 population.
5) Number of all radiologically investigated cases for the diagnosis of tuberculosis.

4. Identify the correct definition of tuberculosis mortality (SC):
1) The number of patients who died from tuberculosis in hospital registered during a calendar year reported per 100.000 population.
2) The number of patients who died from other causes than the progression of tuberculosis registered during a calendar year compared to 100.000 population.
3) The number of patients who died from the progression of tuberculosis registered during a calendar year reported per 100.000 population
4) The number of patients diagnosed with post-mortem with tuberculosis during a calendar year reported per 100.000 population.
5) The number of the patients that initiated antituberculosis treatment and died from tuberculosis during a year reported per 100.000 population.

5. To Robert Koch is attributed (CM):
1) Discovery of the etiological agent of tuberculosis.
2) Extraction of tuberculin from mycobacterial culture.

3) Presenting on 24th March 1882 the etiological agent of tuberculosis and establishing it as the world's day for the fight against tuberculosis.
4) The discovery of the first anti-mycobacterial drug.
5) Initiation of tuberculosis vaccination.

6. The End TB Strategy targets are (CM):
1) Eradication of tuberculosis worldwide.
2) Reducing the number of tuberculosis deaths by 95% by 2035 compared to 2015.
3) Reducing the incidence of tuberculosis by 90% by 2035 compared to 2015.
4) Increasing the living standard of the population.
5) Reducing to zero the number of families affected by the catastrophic costs of tuberculosis.

7. The causes that aggravated the epidemiological situation of tuberculosis in the Republic of Moldova (CM) are:
1) Decreased the living standard of the population.
2) Massive economical migration of the population.
3) A large number of people incarcerated in the prisons or who were in detention.
4) A large number of sources of multidrug-resistant tuberculosis.
5) Implementation of the National Tuberculosis Control Program.

8. The etiological agents of tuberculosis in humans are (CM):
1) Mycobacterium tuberculosis hominis
2) Mycobacterium tuberculosis africanum
3) Mycobacterium bovis
4) Mycobacterium avium intracelullarae
5) Mycobacterium microtti

9. The ways of the infection with *Mycobacterium tuberculosis* of the human body are those listed with the exception (CS):
1) Respiratory (airborne).
2) Cutaneous or mucocutaneous.

3) Transplacental.
4) Sexual.
5) Digestive.

10. The nonspecific resistance of the respiratory tract against the infection with *Mycobacterium tuberculosis* consists of (MC):
1) Mucociliary clearance.
2) The nasal passage.
3) Coughing and sneezing.
4) Secretion of IgA.
5) Secretion of the antibodies against *Mycobacterium tuberculosis*.

11. Sources of the *Mycobacterium tuberculosis* infection can be (MC):
1) Patient with pulmonary tuberculosis.
2) Patient with extrapulmonary tuberculosis.
3) Cattle with tuberculous mastitis.
4) Mice and rats.
5) Insects.

12. Factors that increase the risk of the transmission of the tuberculous infection are (MC):
1) Susceptibility of the exposed individual to the infection.
2) Contagiousness of the source of infection.
3) Environmental factors.
4) Tuberculosis in anamnesis.
5) The low hygienic level of the population.

13. Identify the correct statements about the digestive way of the transmission of the tuberculosis infection (MC):
1) It is the main way of transmission of Mycobacterium bovis.
2) Sick cattle are usually affected by tuberculous mastitis.
3) It is an exceptional way of transmission of the infection from the mother to child
4) It is detected in a large proportion of cases in the Republic of Moldova.
5) It has been eradicated due to tuberculin testing of the cattle.

14. The risk of Mycobacterium tuberculosis infection depends on (MC):
1) Topographic localization of tuberculosis at the patient source of infection.
2) The degree of emission of the acid-fast bacilli by the patient source of infection.
3) Associated diseases of the exposed person.
4) The type of contact with the patient source of infection.
5) Frequency and duration of the exposure with the patient source of infection.

15. The age groups with the highest susceptibility for tuberculosis are (MC):
1) Children aged less than 5 years old.
2) Adolescents aged between 14 and 18 years old.
3) Persons older 65 years.
4) Young adults between 25 and 34 years old.
5) Newborns.

16. The vulnerability of the population to tuberculosis infection is determined by (MC):
1) Social economical state of the population.
2) The distribution of the age groups.
3) Immune resistance of the population.
4) Comorbidities.
5) Geographical situation of the population.

17. Associated diseases that increase the risk of tuberculosis are correctly listed, with the exception (SC):
1) Diabetes.
2) Hepatitis B, C, and/or D.
3) Mental diseases.
4) Immunosuppressive treatment.
5) Neoplastic diseases.

18. The social factors that increase the risk of tuberculosis are (CM):
1) low economic status: unemployment, low income, migration.
2) Absence of medical insurance and other barriers in the provision of medical assistance.
3) Low social status associated with a low hygienic-sanitary standard.
4) The high social status.-
5) Associated diseases and age groups with increased risk.

19. The occupational factors associated with an increased risk for tuberculosis are (CM):
1) Silicosis.
2) Anthracosis.
3) Professional activity in specialized medical-sanitary institutions.
4) Staff of the primary schools and kindergartens.
5) Persons with business activity.

20. Groups with an increased risk to be infected with Mycobacterium tuberculosis are (CM):
1) Contacts with tuberculosis patients.
2) Staff of medical-sanitary institutions specialising in phthisiopneumology.
3) Persons working in the public service.
4) Staff of medical-sanitary institutions.
5) Staff of the schools.

21. Mycobacterium tuberculosis is sensitive to (CM):
1) Boiling, pasteurization.
2) Drying.
3) Chlorinated chemical disinfectants.
4) Ultraviolet rays.
5) Humidity.

22. The peculiarities of the Mycobacterium tuberculosis are (CM):
1) Acid-fastness.
2) At Ziehl-Neelsen staining is colored in pink.

3) Aerobic.
4) Anaerobic.
5) Sporulated.

23. Nontuberculous mycobacteria cause (CM):
1) Mycobacteriosis.
2) Tuberculosis.
3) Lymphadenitis, infection of the skin and soft tissues, lung infections.
4) Generalized infections.
5) Leprosy.

24. Differentiation criteria of latent tuberculosis infection (LTBI) from active tuberculosis are (CM):
1) Clinical.
2) Microbiological.
3) Immunological.
4) Radiological.
5) Psychological.

25. Identify the components of tuberculous granuloma (CM)
1) Lymphocytes.
2) Plasma cells.
3) Eosinophils.
4) Caseification necrosis.
5) Langhans giant cells.

26. Identify the correct statements (CM)
1) More than 90% of people infected with Mycobacterium tuberculosis will never be sick.
2) About 5% of people infected with Mycobacterium tuberculosis will get sick in the first two years after the primary infection
3) People with latent tuberculosis infections are infectious and contagious.

4) About 5% of people infected with Mycobacterium tuberculosis will get sick after 2 years from the primary infection.
5) The annual risk for tuberculosis in TB/HIV co-infected patients is 7-10%.

27. All statements that characterize latent tuberculosis infection (LTBI) are correct with the exception (CS):
1) Mycobacterium tuberculosis achieves the alveoli, where are maintained in a dormant state.
2) LTBI follows delayed hypersensitivity.
3) LTBI can be detected using the tuberculin skin test or IGRA test.
4) LTBI is caused by active Mycobacterium tuberculosis.
5) LTBI will never reactivate.

28. Identify the particularities of the persons with the latent tuberculous infection (CM):
1) They are asymptomatic.
2) Complain diminished symptomatology.
3) They have positive results at the microbiological examinations.
4) Requires the anti-tuberculous treatment.
5) They are immunosuppressed.

29. The risk of transmission of Mycobacterium tuberculosis increases due to:
1) Poor cough hygiene.
2) Insufficient natural ventilation.
3) Insufficient natural lighting.
4) Closed air ventilation.
5) Wrong handling of specimens collected from the patient.

30. Social economical risk factors, which increase the risk for tuberculosis are (CM):
1) Contact with the tuberculosis patient.
2) Diabetes mellitus.
3) Low level of economic status.
4) Migration.
5) Adolescence.

PRACTICAL WORK

This compartment was created to apply the definitions and formulas provided in the theoretical framework of the methodological recommendations. The calculation method is standardized and described in Chapter 1. Random numbers were used.

1. In Chisinau in 2014, 956 patients with tuberculosis were registered (new cases, relapses, recovered after loss to follow-up, treatment failure. Calculate the prevalence of tuberculosis in the population of Chisinau, considering that the total population was 956.000 people.
2. In Bălţi in 2015 there were registered 734 cases and relapses. Calculate the global incidence considering that the total population was 146.000 people.
3. In all districts of the Republic of Moldova in 2013 were registered 2.233 new cases of tuberculosis. Calculate the incidence considering that the total population in the Republic of Moldova was 2.596.511 people.
4. Calculate the incidence of tuberculosis in children (0-17 years 11 months 29 days) from Chisinau in 2015, considering that 49 children were diagnosed, and the total number of children was 153.000.
5. In 2013 in the Republic of Moldova 406 patients died during the anti-tuberculosis treatment. Calculate the mortality if the total population was 2.596.511 people.
6. Calculate the incidence of multidrug-resistant tuberculosis (MDR-TB) in Bălţi in 2015 if 96 MDR-TB cases were registered, and the total population was 152.941 people.
7. Calculate the incidence of tuberculosis relapse in Chisinau in 2015, if 187 patients were diagnosed with relapse, and the total population living in Chisinau was 984.000 people.

8. Calculate the rate of patients with HIV co-infected patients among new cases and relapses in 2015 in Chisinau, if 624 new cases and relapses were registered, and among them, 76 were HIV co-infected.

9. Calculate the rate of patients - new microbiologically confirmed cases (microscopy, culture, or GeneXpert MTB / Rif) in 2015 in Chisinau, if it is known that 478 new cases were registered, of which 263 were microbiologically confirmed.

10. Calculate the treatment success rate in patients diagnosed with drug-susceptible tuberculosis in 2014 in Chisinau, considering that 775 patients were enrolled in the treatment and 555 patients finished it as „cured" or „treatment completed".

11. Calculate the treatment success rate in patients diagnosed with multidrug-resistant tuberculosis in 2012 in Balti, considering that 196 patients were enrolled in the treatment for MDR-TB and 114 patients finished it as „cured" or „treatment completed".

12. Calculate the rate of patients who died from the progression of tuberculosis diagnosed with TB / HIV co-infection in Chisinau in 2015, if it is known that the total number of people who died of tuberculosis was 80, and 30 of them were diagnosed with TB / HIV co-infection.

13. Calculate the rate of multidrug-resistant tuberculosis (MDR-TB) patients who died during the anti-TB treatment, if it is known that the total number of dead patients was 80, and 24 of them were diagnosed with MDR-TB.

14. Determine the rate of men diagnosed with tuberculosis in 2015 in the Republic of Moldova, considering that the total number of diagnosed patients was 3.082 patients and among them 2.343 were men. Calculate the men/women rate in the cohort of tuberculosis cases.

15. Calculate the rate of the patients - new cases and relapses investigated for HIV, if it is known that 3.599 cases with tuberculosis were reported of which 3.527 were investigated for HIV.

16. Calculate the rate of acquired multidrug-resistant tuberculosis (MDR-TB) in the Republic of Moldova in 2015 among the cases following the antituberculosis treatment, if it is known that the total number of registered cases with MDR-TB was 643, and among them, 418 had received the antituberculosis treatment in the past.

CLINICAL CASES

Clinical case study 1

An 18-year-old college student was diagnosed with pulmonary tuberculosis. From the anamnesis, it was established that the patient was in permanent contact with her father suffering from multidrug-resistant tuberculosis, treated in outpatient condition, and the treatment was completed 2 years ago. Ten days before the diagnosis of tuberculosis, the patient had an upper respiratory infection after exposure to the cold. She reported a fever of 39.7°C, profuse night sweating, coughing, and mucous sputum, loss of appetite.

General practitioners recommended the broad-spectrum antibiotic treatment, anti-inflammatory drugs, and vitamin therapy. The patient's condition improved insignificantly. Chest radiography was performed, which identified the pattern of acute disseminated tuberculosis (miliary). The patient was microbiologically investigated, all examinations gave negative results. The tuberculin skin test identified an induration of 8 mm with a 5 mm BCG vaccine scar.

- Identify the risk factors for tuberculosis.
- Identify the peculiarities of the case management and the errors made.
- Comment the results of laboratory tests and their significance.

Clinical case study 2

A 45-year-old woman, a nurse in a hospital, lives in a 2-rooms apartment with her husband's family and a 14-year-old son. For rheumatoid arthritis, she received continuous treatment with non-steroidal anti-inflammatory drugs and immunomodulatory drugs which kept arthritis in a state of remission. For more than a year, the patient's general condition deteriorated with the appearance of deep asthenia, weight loss (current weight 52 kg and height 168 cm), cough, mucopurulent sputum, increased intensity of dyspnea. She went to the family doctor for an investigation. The general condition was deteriorated moderately, malnutrition, pallor, and at the auscultation were established the rales in the upper third of the right hemithorax. Conventional radiological examination of the rib cage determined the pattern of a thick-walled cavity in the lung parenchyma, microopacities in the adjacent parenchyma, and thickening of the peribronchovascular pattern. Microbiological examination identified acid-fast-bacilli. The genetic molecular method is positive and sensitive to rifampicin. The patient was hospitalized in the clinical department for the initiation of the anti-tuberculosis treatment.

- Identify the risk factors in the evaluated patient and the mechanism by which they influenced the development of tuberculosis.
- Identify the peculiarities of case management and the mistakes made.
- Comment the results of laboratory tests and their significance.

Clinical case study 3

A 37-year-old man, an unskilled worker, lives with his wife and 2 children in a 2-room apartment. He is a smoker of 20 PY (packages per year), with daily consumption of 20 cigarettes. He is an occasional drinker. He carried out his professional activity in open spaces. For more than 6 months he has been coughing, expectorating mucopurulent sputum, for a week he has noticed streaks of blood, which made him to consult the family doctor. At the objective examination, a moderately worsened general condition was identified, normoponderal patient (cu-

rrent weight 78 kg, height 171 cm) and at the auscultation - diffused audible rales at the level of the right hemithorax. At the radiological examination was established the pattern of infiltrative opacities with unclear borders, located in the segments 1, 2, and 6 of the right lung, and microopacities in the neighboring segments. Acid-fast-bacilli were identified on microscopic examination. The GeneXpert MTB Rif genetic molecular method was positive and resistant to rifampicin. The patient was hospitalized in the service specialized in the management of multidrug-resistant tuberculosis for the initiation of antituberculosis treatment.

- Identify the risk factors for tuberculosis.
- Identify the peculiarities of case management.
- Comment on the results of laboratory tests and their significance.

Clinical case study 4

A 45-year-old man, without a stable place to live and unemployed, went to the emergency department, accusing him of frequent hemoptysis, severe asthenia, high fever, mucopurulent sputum, which persist for more than a year, and short-term dyspnea. The patient perceives the change in the timbre of the voice with slight hoarseness of several months. He also noticed the pain of swallowing. He is a smoker with a history of 25 PY (packages per year), chronic alcohol consumer, and has a short history of detention in a penitentiary from the Republic of Moldova. The objective examination identified a hypoponderal patient (actual weight 54 kg, height 168 cm). At auscultation were revealed subcrepitant and crackling rales in both lung areas. On radiological examination, multiple micro-and macroopacities were spread on both areas of the lungs, located mainly in the upper two-thirds of the lungs. The molecular genetic method GeneXpert MTB/Rif established a positive and sensitive result for rifampicin. He was transferred to the hospital for the anti-tuberculosis treatment.

- Identify the risk factors for tuberculosis and the mechanism by which they contribute to the development of the disease.
- Identify the peculiarities of case management.
- Comment on the results of laboratory tests and their significance.

Clinical case study 5

A 14-year-old child lives with his parents in a private house and is a student at the local gymnasium. In the summer, he regularly visited his grandparents in the village, where he came in contact with his uncle, who had tuberculosis and was treated with multiple interruptions. After the last return from the village, the child complained of severe chest pain in the left hemithorax, which changes its intensity with a change in body position and is associated with a cough. The parents noticed an increase in evening body temperature and profuse sweating. The child was given non-steroidal anti-inflammatory drugs without any improvement in symptoms. The parents called the Emergency Service of the National Health Care Center. At the objective examination, a moderately worsened condition was established, dullness on the percussion of the left hemithorax and the absence of vesicular murmur in the lower third of the left hemithorax. Conventional chest radiography was performed, which established a high-intensity homogeneous opacity with the concave superior border, changing its appearance with body movement. The diagnosis of pleurisy was established and thoracentesis was performed. An amount of 400 ml of serocitrin liquid was extracted. Biochemical examination of the collected fluid identified a serocitrin exudate with a positive Rivalta reaction, an LDH concentration greater than 200 U/L, adenosine deaminase activity greater than 42 U/ml, and the lymphocyte count over 90% of all cells identified on microscopic examination. The molecular genetic method GeneXpert MTB/Rif established a negative result. The tuberculous etiology of pleurisy was suspected and the patient was transferred to the pediatric tuberculosis ward.

- Identify the risk factors for tuberculosis.
- Identify the peculiarities of case management.
- Comment on the results of laboratory tests and their significance.

BIBLIOGRAPHY

1. BOTNARU, Victor; coaut. *Pneumologia.* Chișinău: Tipografia „Balacron", 2018. 732 p. ISBN 978-9975-3255-5-4.
2. CRUDU, Valeriu., ROMANCENCO, Elena. *Diagnosticul microbiologic al tuberculozei.* Chișinău: „Tipografia Elan Poligraf" 2012. 244 p. ISBN 978-9975-66-297-0.
3. EȚCO, Constantin, CALMIC, Varfolomei, BAHNAREL, Ion. Chișinău, *Promovarea sănătății și educația pentru sănătate.* Chișinău: „Epigraf", 2013. 600 p. ISBN 978-9975-125-30-7.
4. LANGE, Christoph, MIGLIORI, Giovanni. *Tuberculosis.* Sheffield, UK: European Respiratory Society, 2018. 260 p. ISBN: 978-1-84984-100-9
5. SPINEI, Larisa, LOZAN, Oleg, BADAN, Vladislav. *Biostatistica medicală.* Chișinău: Î.S.F.E.P. „Tipografia Centrală", 2009. 186 p. ISBN 978-9975-78-743-7
6. VILC, Valentina., ALEXANDRU, Sofia, CRUDU, Valeriu, IGNAT, Rodica. *Controlul tuberculozei la nivelul asistenței medicale primare.* Chișinău: Tipografia „Foxtrot", 2015. 146 p. ISBN 978-9975-120-82-1.
7. *Ministerul Sănătății, Muncii și Protecției Sociale al Republicii Moldova.* Protocol Clinic Național. Tuberculoza la Adult. PCN-123. Chișinău, 2020. 152 p. Available on: https://msmps.gov.md/wp-content/uploads/2021/02/PCN-123-Tuberculoza_la_adult.pdf
8. Ministerul Sănătății, Muncii și Protecției Sociale al Republicii Moldova. Protocol Clinic Național. Tuberculoza la copil. Chișinău, 2020. 180 p. Available on: https://msmps.gov.md/wp-content/uploads/2021/02/PCN-55-Tuberculoza_la_copil.pdf
9. Programul Naţional de Control al Tuberculozei pentru anii 2011-2015, aprobat prin Hotărârea Guvernului RM nr.1171 din 21.12.2010. In: *Monitorul Oficial al Republicii Moldova,* 2011, Nr. 259-263.
10. Programul Național de Control al Tuberculozei pentru anii 2016-2020, aprobat prin Hotărârea Guvernului RM nr.1160 din 20.10.2016. In: *Monitorul Oficial al Republicii Moldova,* 2016, Nr.369-378.
11. CENTERS FOR DISEASE CONTROL. Core Curriculum on Tuberculosis: What the Clinician Should Know. Sixth Edition, 2013. CDC. Available on: https://www.cdc.gov/tb/education/corecurr/pdf/corecurr_all.pdf
12. *International Classification of Diseases.* 11th Revision. The global standard for diagnostic health information. Available on: https://icd.who.int/en.
13. Global Laboratory Initiative. Model TB diagnostic algorithms. Available on: http://www.stoptb.org/wg/gli/assets/documents/GLI_algorithms.pdf

14. *WHO:* The immunological basis for immunization series: module 5: tuberculosis, 2011. Available on: https://www.medbox.org/the-immunological-basis-for-immunization-series-module-5-tuberculosis/preview
15. *WHO*. Recommendations for investigating contacts of persons with infectious tuberculosis in low- and middle-income countries, 2012. Available on: https://apps.who.int/iris/bitstream/handle/10665/77741/9789241504492_eng.pdf?sequence=1&isAllowed=y
16. *WHO*. Management of tuberculosis and HIV coinfection. Clinical Protocol, 2013. Available on: http://www.euro.who.int/__data/assets/pdf_file/0004/78124/E90840_Chapter_4.pdf
17. *WHO:* Guideline: Nutritional care and support for patients with tuberculosis, 2013. Available on: https://apps.who.int/iris/bitstream/handle/10665/94836/9789241506410_eng.pdf?sequence=1
18. *WHO:* Guideline: Nutritional care and support for patients with tuberculosis, 2013. Available on: https://apps.who.int/iris/bitstream/handle/10665/94836/9789241506410_eng.pdf?sequence=1
19. *WHO:* Companion handbook to the WHO guidelines for the programmatic management of drug-resistant tuberculosis, 2014. Available on: https://www.who.int/tb/publications/pmdt_companionhandbook/en/
20. *WHO:* Systematic screening for active tuberculosis: an operational guide, 2015. Available on: https://www.who.int/tb/publications/systematic_screening/en/
21. *WHO: Chest radiography in tuberculosis detection. Summary of current WHO* recommendations and guidance on programmatic approaches, 2016. Available on https://www.who.int/tb/publications/chest-radiography/en/
22. WHO: Guidelines for treatment of drug-susceptible tuberculosis and patient care, 2017. Disponibil: https://www.who.int/tb/publications/2017/dstb_guidance_2017/en/
23. *WHO:* Latent tuberculosis infection. Updated and consolidated guidelines for programmatic management, 2018. Available on: https://www.who.int/tb/publications/2018/latent-tuberculosis-infection/en/
24. *WHO*. Global tuberculosis report, 2019. Available on: https://www.who.int/tb/publications/en/