

UNIVERSITY OF MEDICINE AND PHARMACY OF CRAIOVA

**CLINIC-EPIDEMIOLOGIC ASPECT BOF THE
TUBERCULOSIS ENDEMIC EVOLUTION IN SECTOR 5
OF BUCHAREST MUNICIPALITY DURING 2007-2011**

PH.D.THESIS-ABSTRACT

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CONTENTS

GENERAL PART.....	3
I. Importance of the issue.....	3
III. Multidrug-resistance – challenge of the present TB endemic in Romania.....	5
<i>III. 1. Bacteriological diagnostic of the resistant tuberculosis.....</i>	5
SPECIAL PART.....	11
I. Motivation for choosing the subject.....	11
II. Purpose and objectives of the work.....	12
<i>II.1. Purpose of the study.....</i>	12
<i>II.2. Objectives of the study.....</i>	12
III. Material and method. Work protocols.....	13
IV. Results.....	15
<i>IV.1. General analysis of the researched sample.....</i>	15
<i>IV.2. Analysis of the TB cases in children.....</i>	19
<i>IV.3. Analysis of the HIV-TB co-infection cases.....</i>	19
<i>IV.4. Analysis of the MDR - TB.....</i>	21
<i>IV.5. Analysis of the mortality indicator.....</i>	22
V. Conclusions.....	23

GENERAL PART

I. Importance of the issue

Although tuberculosis is a disease present among us for millennia, the causing microbial agent was discovered more than 130 years ago by Robert Koch, while the chemical antibiotics began to be used at the mid of the past century. Tuberculosis affects mainly the adult population in the most productive years of life, with serious social direct and indirect consequences, and the fight against it requires important financial resources. Being a specific infectious-contagious disease, widely spread in the world, it has a chronic evolution generating an important fatality rate and is recognized as a *worldwide major public health problem*.

This infection still remains the most frequent cause of illness and death among all infectious diseases in the world, due to its high prevalence in the high density populations of the developing countries, and its eradication shall be extremely difficult because of poverty overcrowding and HIV infection. Nonetheless, TB remained the first avoidable cause of death in the developing countries.

The increase of TB incidence at global level is due to a series of factors: AIDS epidemics, appearance of resistant strains, and expansion of the number of immigrants coming from countries with endemic TB.

Resistant strains to some anti-TB drugs were identified in all countries monitored by WHO. A extremely severe of TB is multidrug-resistant (MDR-TB), with its recent version of extended resistance to anti-TB drugs (XDR-TB), which proved to be an unbeatable barrier by the institutional efforts to control and reduce this malady.

According to the World Health Organization data, 1.3 million people die each year in the world because of TB, which is the 7th general cause of death in the world and the 2nd infectious cause of death, following AIDS. In 2012, 1.4 million deaths of TB were registered, which means 3,800 deaths per day.

It's worrisome to observe that around 3 million people with TB or contacts of TB patients are not provided with necessary medical care and over 95% of the cases appeared in countries with low and average income.

Tuberculosis is among the first 3 causes of death at women aged 15-44. In 2012, around 530,000 children were diagnosed with TB, and 74,000 children died because of this epidemic. Statistically speaking, each year there are registered 8.6 million new cases, global incidence at global level being 122‰, most of the cases being notified in Asia (59%) and Africa (26%).

From the point of view of TB global incidence, in the recent years Romania is among the first place in WHO European Region (6th place in 2009, after Kazakhstan, Republic of Moldova, Georgia and Kyrgyzstan), after it occupied the first place among the European Union member states since the accession, in 2007.

In the recent years, important progresses were registered in the disease control, a consequence of implementing DOT Strategy recommended by the World Health Organization (WHO) aiming at TB control, which has 100% coverage since 2005.

The positive aspects noted related to the TB endemic evolution are deduced from the values of epidemiologic impact indicators. Thus, the same time with the reduction of the global incidence, a remarkable increase of the success rate was noted for the new cases bacteriologically confirmed, from 51% in 1995 to 85.8% in 2009, values maintained in 2011 (85.9%), according to the most recent available data (TB Surveillance 2014, ECDC). With this success rate of the new cases bacteriologically confirmed, Romania meets the global WHO target of 85%, considered the only way to control the TB endemic.

II. TB – HIV infection association

TB is the most common and frequent infection at patients with HIV worldwide, the two infections being a mutual disadvantageous association. Starting with 1993, pulmonary TB was one of the definition criteria for the AIDS case in adults, while extra-pulmonary TB and disseminated atypical mycobacteriosis were considered definition criteria for the IV clinical stage of AIDS disease since 1987.

HIV infection, through the progressive alteration of the immune response capacity of the organism, determines a high TB illness risk at the persons with double infection. It changes not only the illnesses proportion of the infected persons, but can also determine the augmentation of the annual infection risk by the extension of the number of sources and the infection rate

determined by it. The persons previously infected with *M. tuberculosis* developing an HIV infection have a very high rate of reactivation of the bacillary process.

The TB clinic aspect at the HIV infected patients varies from the typical characteristics of the basic condition to those of the atypical disseminated illness. Because patients rarely have cavitory anatomic-radiologic lesions, the number of acid-fast bacilli in the sputum can be reduced and the efficiency of the smear-tests is low. The tuberculin skin test remains an important clinical and epidemiologic tool for the detection of the persons infected with *M. tuberculosis*. Pulmonary TB at HIV positive patients can appear on X-ray film with focal infiltrates, diffuse infiltrates, cavities, pleural effusion, hilar or mediastinal adenopathies. There may be patients with normal chest X-ray, but with positive culture.

III. Multidrug-resistance – challenge of the present TB endemic in Romania

The two major causes of the development and spread of the drug-resistance in clinic are lack of adherence to the prescribed treatment and use of inappropriate treatment regimens. At these there should be added the diagnostic delay caused by the rapid detection techniques of the chemo-resistance, inappropriate treatment due to lack of some essential drugs, immune-depression and lack of TB control measures. If the drugs are not administered according to the prescription, the bacilli shall be exposed to only one drug for long period of times, which allows the multiplication of chemo-resistant germs.

More than that, some treatment regimens may contain several drug, while the germs are susceptible to only one, which can occur in the case of a unsuspected primary chemo-resistance or if only one drug is added to a failed regimen. These regimens are equivalent with a monotherapy and can lead to the selection of poly-chemo-resistant germs. The acquired poly-chemo-resistance is usually a result of mixing non-adherence with inappropriate treatment.

The appearance of chemo-resistance is much less probable at most forms of extra-pulmonary TB, where bacilli population is much smaller.

III. 1. Bacteriological diagnostic of the resistant tuberculosis

After finishing the intensive phase of the treatment (2 or 3 months), the first element which can suggest the possibility of a chemo-resistance is the positive microscopic exam. It is very important for these cases to run a direct rapid testing (to the positive sputum), with a result obtained in 1-2 days in case of genetic tests and in 10-17 days for phenotypic tests. If MDR TB or any other mono-resistance is confirmed, extensive drug-sensibility testing must be done from the culture of the product, in order to highlight the resistances to the other anti-TB substances.

III. 2.1. Drug susceptibility testing (testing the susceptibility to anti-TB substances)

It is necessary to run the drug sensibility test only for strains belonging to *M. tuberculosis* complex, and the method used for testing can be different based on the patient category.

*Methods of testing susceptibility to anti-TB drugs*³²³

A. Direct drug susceptibility test

It uses as inoculant the pathologic product with positive result at the microscopic exam, with a high AFB content, employed after the decontamination of the product.

B. Indirect drug susceptibility test

The indirect methods as validated and standardized, considered at present as gold standard. The inoculant is prepared from the mycobacterian culture, with the possibility to use three standardized methods in Löwenstein-Jensen medium and one method using liquid media, in which the obtained results do not differ significantly for the 1st line substances.

The testing methods are:

a. *The proportion method* in Löwenstein-Jensen medium (Canetti et col., revised). At present, this method is preferred especially in the case of strains with very slow growth.

b. *The absolute-concentration method* (Meissner et col.) has less confident results compared to those obtained with the proportion method, but the technical conditions are more accessible and the cost is lower. Reading of the results is done at 21 days after inoculation.

c. *The resistance-ratio method* (Mitchison) uses simultaneous testing of the patient's strain and the reference sensitive strain, in 5 tubes with different concentration of each TB drug.

d. *DST on liquid media in automatic systems. MGIT 960* is the golden standard recommended at present for short series DST at the essential drugs and extended series at 2nd line. The results are obtained in 4-5 days. The disadvantage of this test is the high cost and that it requires experienced and trained staff.³⁰⁰

e. *Rapid phenotypic techniques.* Have been approved and recommended by WHO as a provisional alternative until the routine application of the liquid media testing and/or genetic tests. They have the advantage of being rapid (the result is obtained in 7 – 10 days) and relatively low cost compared to the other methods.³²³

- Nitrate-reduction assay in Löwenstein-Jensen solid media can be used as direct or indirect method.
- Microscopic-observation drug-susceptibility assay (MODS) is based in the microscopic observation of cords formation in the liquid media used for testing.
- Colorimetric assays.

III. 2. 2. Impact of the molecular biology. Genetic methods

The amplification methods of the nucleic acids which theoretically insure means to highlight one microorganism from a single clinic sample represent an alternative for the cultivation and identification of microorganisms. Besides the fact that insures much more rapid results, these new method are much more specific than the classical ones based on the growth characteristics and biochemical tests.

The same time, the research in the field of molecular biology provided cloned genes which can be used for the antigens production for the serologic tests and for the production of monoclonal antibodies.

The first amplification method was the *polymerase chain reaction – PCR*, the method preferentially used by the researchers. To highlight the mycobacteria, other amplification methods were adjusted, including the transcription based amplification, amplification by catenary replacement and ligase-amplification reaction.²⁸¹

Polymerase chain reaction – single strand conformation polymorphism (PCR-SSCP) analysis represents a direct genotypic method of determining the chemo-resistances, transferring strain susceptibility assessment at genotype level, instead of phenotype. The same time, the detection of resistance is done rapidly, by avoiding the time-consuming culture procedures.

Spoligotyping. This method uses the presence of a chromosomal locus, containing a large number of sequences – direct repeat sequences, of 36 basis pairs, invariably and exclusively present in the *M. tuberculosis* complex strains. The method can be successfully applied on biologic products, without the culture development. It is rapid, sensitive and specific, less laborious and cheaper than RFLP, having the same utility in epidemiologic research and indirect identification of the chemo-resistances.²⁰³

DNA genetic prints. Fingerprinting methods^{199, 242, 282}

The methods based on DNA genetic prints (fingerprinting) provided highly specific markers for the isolates typing. The fingerprinting proved to be valuable in detecting the multidrug-resistant strains spread. In the MDR-TB clusters, the drug-resistance profiles indicated an epidemiologic link between the cluster's strains, while the fingerprinting provided the ultimate proof. The printing method is even more valuable in supporting the investigations regarding the drug-susceptible isolates, being the only lab proof regarding strains patterns.

The commercial genetic methods diminish the TB or MDR-TB confirmation duration, to 24-48 hours. They were endorsed by WHO in 2008 for MDR detection at the smear-positive

cases or positive only in culture. They do not replace the standardized actual methods for detection of *M. tuberculosis* complex and of resistance profile.

Molecular biology (genetic) tests

A. Line probe assay (LPA) uses the polymerase chain reaction to amplify certain regions of the mycobacterian genome, continuing with reverse-hybridization in order to identify the probe specific sequences. In 2008 WHO endorsed and recommended their direct use for all smear-positive sputa in order to rapidly detect RMP resistance and consequently the MDR-TB.

a. GenoType MTBDRplus test is an improved version and can detect resistance to RMP and INH). The medium turnaround for this test is 2 days when applied directly to the sputum.

b. INNO-LiPA Rif TB test can simultaneously detect the *M. Tuberculosis* complex and mutations in *rpoB* gene, thus resistance to RMP.

B. GeneXpert MTB/RIF is a closed system, completely automatic in order to simultaneously detect and identify the *M. tuberculosis* complex and the RMP resistance with the real time polymerase chain reaction (RT PCR). For detection there can be used different biologic specimen, such as bronchial aspirate, tissue, sputum, urine, generating results in less than 2 hours.

III. 2. Chemo-resistance treatment

From a classical perspective, the anti-TB drugs were divided in two categories: 1st line drugs (used in the standard treatment) and 2nd line drugs (associated in individualized regimens), relevantly grouped in therapeutic regimens (table 1).

Table 1. Present mode of grouping the anti-TB medicines

Group	Anti-TB medicines
Group 1 First line medicines	Isoniazid (INH, H) Rifampicin (RIF, R) Pyrazinamide (PZA, Z) Ethambutol (EMB, E) Streptomycin (SM, S)
Group 2 Injectable drugs	Streptomycin (SM,S) Kanamycin (KM, K) Amikacin (AK) Capreomycin (CM)
Group 3 Fluoroquinolones	Ofloxacin (OFX) Levofloxacin (LFX) Moxifloxacin (MFX) Gatifloxacin
Group 4 Oral bacteriostatic 2 nd line	Ethionamide / Prothionamide (E/PTM) Cycloserine / Terizidone (CS/TRD) p-Aminosalicylic acid (PAS)
Group 5 Drugs not recommended by WHO for routine use	Clofazimine (CFM) Linezolid (LZD) Amoxicillin / Clavulanate (AMX/CLV) Thiacetazone (THZ) Clarithromycin (CLR) Imipenem / Cilastatin (IPM / CLN) Isoniazid – high dosage (INH)

Source: Guideline for the DR TB Cases Management, 2013

Formulation of the different individualized treatment regimens is presented in the Guideline for the DR-TB Cases Management by specifying some steps to be followed (table 2).

Table 2. Formulation of the individualized treatment regimen

STEP 1	GROUP 1 1st LINE	Use one 1 st line drug: Pyrazinamide Ethambutol
STEP 2	GROUP 2 INJECTABLE	MDR patients have high resistance to Streptomycin Use based on DST and history: Kanamycin Amikacin Capreomycin
STEP 3	GROUP 3 QUINOLONES	Do not use Ciprofloxacin Increasing efficiency is Ofloxacin < Levofloxacin < Moxifloxacin Use based on DST and history: Ofloxacin Levofloxacin Moxifloxacin
STEP 4	GROUP 4 BACTERIOSTATIC 2nd LINE	Use group 4 bacteriostatics to complete the therapeutic minimum: Ethionamide / Prothionamide Cycloserine / Terizidone PAS
STEP 5	GROUP 5 NOT ROUTINELY RECOMMENDED	Use group 5 drugs if the regimen does not include minimum 4 TB drugs from the 1-4 groups: Clofazimine Linezolid Amoxicillin/Clavulanate Thiacetazone Imipenem / Cilastatin Clarithromycin Isoniazid – high dosage

Source: *Guideline for the DR TB Cases Management, 2013*

Rules required to establish the treatment regimen for the MDR-TB patients:

1. must use one fluoroquinolone;
2. must rather use one newest generation fluoroquinolone;
3. must use ethionamide or prothionamide;
4. in the intensive phase, four supposedly efficient 2nd line drugs shall be used (including one parenteral agent), as well as pyrazinamide;

5. the regimen must include at least one pyrazinamide, one fluoroquinolone, one parenteral agent, ethionamide (or prothionamide) and cycloserine (or PAS – p-Aminosalicylic acid, if cycloserine cannot be used).

In case XDR-TB of patients the same guidelines for establishing treatment shall be used, based on DST (compulsory for quinolone and aminoglycoside), in order to choose the drugs for which there is susceptibility.

In the XDR-TB there are recommended:

- the use of any drug from Group 1 still efficient;
- the use of the injectable drug to which the stain is sensible and considering the extension of the administration duration;
- the use of one latest generation fluorquinolone (levofloxacin, moxifloxacin);
- the use of all drugs from Group 4 still efficient or which haven't been used previously;
- the use of one or more drugs from Group 5;
- the analysis of the surgical intervention in the localized form of MDR/XDR TB.

Regarding the treatment duration of the MDR-TB patients, the same Guideline recommends:

1. duration of the intensive phase (defined by the administration of the injectable) of at least 8 months;
2. total duration of the treatment of at least 20 months in case of the patients without previous individualized treatment.

SPECIAL PART

I. Motivation for choosing the subject

Tuberculosis was and still is a disease spread in the entire world. Archaeological studies and historical evidence suggest that, sporadic at the beginning, then epidemic and finally endemic, TB affects human species for at least 6000 years. In the past, because of its extension in the population and its basically incurable nature, TB was considered for many centuries a real social danger, for which there weren't efficient remedies. TB represents a perpetual threat in the whole world, frequently materialized in dramatic epidemic outbursts, such as the TB explosion in

some countries of Africa and Asia, in association with the present epidemic of HIV infection, which pandemic nature determined the metamorphosis the TB endemic nature in an epidemic one.

In the 21st century TB still remains one of the most frightening illnesses with multiple medical and social implications. Spread around the world, it has a different incidence, from 10-12 cases to 400-600 cases at 100,000 population. At this adds up the alarming increase of primary and acquired multidrug resistance. With the occurrence and development of the HIV/AIDS pandemic, the TB infection and illness gained new dimensions regarding the incidence, as well as the clinical manifestation and evolution.

In the recent years, TB incidence decreased continuously, but the human, material and financial resources allocated to the important public health problem are limited, which requires their routing especially towards the population groups with the highest TB incidence, high-risk or vulnerable groups.

At present, the evolution of TB endemic in sector 5 of the capital city registers a decreasing documented trend, although slow, which needs an evaluation of the current situation from clinical and epidemiologic point of view, in a territory with ethnical, social and economic particularities.

II. Purpose and objectives of the work

II.1. Purpose of the study

Considering the high values of the epidemiologic indicators of the TB endemic in sector 5 of the Bucharest municipality, I proposed to analyze the clinical forms of the TB cases at the moment of the detection, their evolution under treatment, as well as the causes which generated these forms.

Analyzing the evolution of the epidemiometric impact indicators, I tried to identify if the incriminated factors in maintaining these high values are found also in sector 5 of the capital city in unfavorable circumstances generated by the HIV endemic and the increase of the number of chemo-resistant cases.

II.2. Objectives of the study

I set to study:

- the factors maintaining the high values of the main epidemiometric indicators of the TB endemic, meaning the transmission way, supporting factors, impact of the interventions took in

time by the state stakeholders involved in health policies upon the improvement of the health conditions;

- involvement of the non-governmental organization (if the case) and the moral and financial support provided to the vulnerable persons, with special view to the areas representing a subject matter for discussions at different levels of social and political representation;
- situation of the academic network, the evolution of the health network and, first of all, the primary care network in monitoring the health status of the population in the territory;
- if there was a higher frequency of the severe TB forms at detection, disregarding age and if there were microepidemics;
- the existence of a link between the above-mentioned conditions and the occurrence of the epidemiologic events;
- if there was appropriate communication regarding TB and the way in which the pneumophthisiology ambulatory received requests and answered to the problems specific to its activity;
- highlighting the vulnerable population groups in sector 5;
- the evaluation of the population vulnerable groups' impact on TB epidemiologic indicators in this sector;
- framing the gathered information in the current trends of the epidemiologic indicators of TB in Bucharest and Romania
- analysis of the associated pathology impact on TB forms;
- evaluation of the anti-TB treatment outcome according to the neighborhood distribution.

III. Material and method. Work protocols

Approaching TB based on the clinical, epidemiologic and therapeutic aspects represents the subject of the permanent, coherent and competent preoccupation of all responsible stakeholders for fighting this malady, at national, regional and world level, but the deficits registered time and again in optimization of the fight against TB prove the need to use some new concepts and specific methods.

In the study I proposed, having as theme “Clinic-epidemiologic aspects of the tuberculosis endemic evolution in sector 5 of Bucharest municipality during 2007-2011”, I pursued that it complies with the principle to be ethic, pertinent, achievable and new. Thus, patients' inclusion in the sample was done based on the professional ethics, such as each of them benefit of the

necessary investigations and drug treatment, meeting the principal of respecting the person and their confidentiality. I wanted that the collected data to allow a better knowledge of the disease, the TB evolution phenomenon in general, to contain substantial new elements, rejecting the possibility to be in-line with other eventual existing studies. The evaluation of ability of finalizing the study took in consideration all the elements proposed for analysis.

The studies composing the work are based on the comparative analysis of the TB endemic level, its association with the increasing values of the HIV/AIDS infection, the chemo-resistance level of Mycobacterium tuberculosis at anti-TB antibiotics for the chemo-resistance cases in sector 5 of the capital city, as well as the evaluation of the treatment outcome applied for registered cases.

The following were used:

- TB register in electronic and printed format;
- reference medical document for each patient (consultation form, medical test reports, lab bio-chemical tests, different mailings with other medical echelons, statistical listings of Romania);
- demographic information and other statistical data available at the TB ambulatory, local city halls and Bucharest municipality;
- electronic database of the National TB Control Program in Romania;
- Statistical Documentation Center of the Ministry of Health;

The study type used for the elaboration of the work is analytical observational epidemiologic of descriptive-retrospective type, possible to use for the chronic diseases, which simultaneously measures the exposure factor or variable and the existence of a certain condition, at the level of a population sample.

The research pursued chronologically the studied years, being followed by the development of a program which included the research data for patients and pursued criteria. This offered the possibility of the statistical analysis which, eventually, through the acquired significances allowed us to redirect NTPSCP specific interventions.

Annually we proceeded to evaluate the development stage of the research. This didn't involve funds allocation, the office or transportation supplies within the patients' circuit being obtained either through the capitalization of the existing compulsory informatics system of NTPSCP, or with personal means.

IV. Results

IV.1. General analysis of the researched sample

The research was done in sector 5 of Bucharest, on 1427 patients (adults and children) registered during 2007 – 2011. Out of the total TB cases, 908 were *males* (63.6 %) and 519 *females* (36.4 %), with a 1.75/1 gender report.

Analysis on *residence area* showed the net higher share of the cases in urban area (93.3 %) compared to the cases in rural area (6.7 %), the proportion of males in urban area being obviously dominant, given the structure of the sample.

Analysis of the sample relative to the *social-economic level* provides one of the biggest discrepancies between poor and wealthy area. This is the area of major contrasts in which the values of the TB incidence varied from 360.29‰ in disadvantaged area to 20.4‰ in area with higher economic level.

Case distribution according to *age groups and gender* indicates that most of the TB patients, for both genders, were registered at the 41-50 years age group, comprising 313 cases (22% out of the total).

Based on the patients' *occupation*, the highest share is of patients with different occupations (61%), this value being twice higher compared to the number of patients without occupation (32%).

Most of the patients were insured (976 cases – 68.4%), compared to those not insured (443 cases – 31.6%).

According to the *TB localization* criteria, 1196 cases were pulmonary (84%), compared to 231 extrapulmonary cases (16%), with a report of 5.25/1 in favor of the pulmonary localization.

The analysis of the *therapeutic history* of patients showed that the most frequent categories were the new cases – 1053, relapses – 247 and defaults – 64 patients, followed (with much lower representativeness) by failures – 23, chronic cases – 22 and transfers – 18 patients.

The criterion of *main localization* of TB creates the following categories in the studied sample:

- nodular infiltrative TB 533 cases (37,35%),
- unicavitary TB 410 cases (28.73%),
- TB lobitis 140 cases (9.81%),
- TB pleurisy 109 cases (7,63%),

- extended polycavitary TB 56 cases (3.92%),
- extensed caseous TB 49 cases (3.43%),
- the least frequent diagnostics were “other poliserositis” and “TB of the pulmonary parenchyma without other details“, each with 1 case (0.07%).

The *extended, severe forms* of pulmonary TB (cavitary, polycavitary, caseous, extensive caseous extensive, miliary) represented a percentage of 47.43% of the total.

Other extra-pulmonary localizations at the level of other systems and organs: bone TB, genital, digestive, kidney, cutaneous, TB of the central nervous system, extra- and intra-thoracic adenopathies, pericarditis and pleuro-pericarditis represented 0.07% of the cases.

The patients with *secondary localization* of the TB were 109, representing 7.64% of the total cases. The pleurisies were 52%, corresponding to 57 cases.

A number of 233 TB cases (16.3% out of the total general) had *associated illnesses*, out of which 50.6% were liver infections and sugar diabetes (118 cases, aggregated). Of the total patients with TB, 16.33% had another important condition associated and 7.64% presented a second bacillary localization.

Evaluation of the results to treatment for the entire sample under research shows that the proportion of cured patients and those with complete treatment represents 84.23%.

29 patients were evaluated as failures (2%), 106 patients (7.43%) represent „default“; 43 patients (3.0%) were invalidated at the intermediate evaluation and were excluded from the sample at the final evaluation; 34 patients (2.38%) changed their address after registration until the evaluation moment and out of them, 11 patients were evaluated as “lost” after 2 months after transfer; 34 patients died (2.38%) – figure 1.

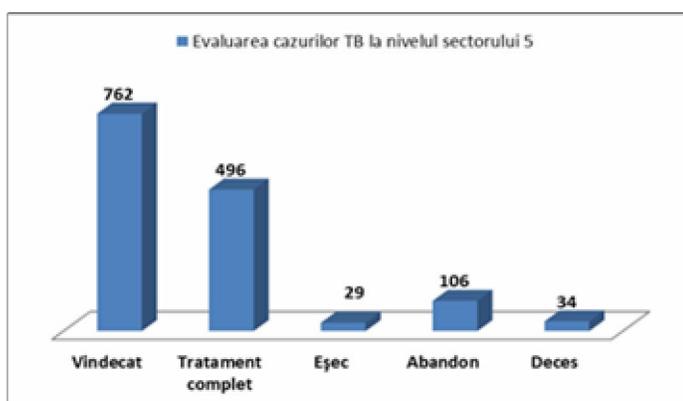


Figure 1. Evaluation of the treatment outcome for the entire sample

For the period 2007 - 2011, with a first analysis we pursued the identification of the *bacilliferous cases and calculation of the sputum conversion rate* at 2 months after initiation of treatment.

Out of the 1427 patients registered during the above-mentioned period, 876 cases (61.38%) had positive BK microscopy exam and culture at the initiation of treatment, 320 cases (22.42%) had negative BK microscopy exam and culture, and from the remaining 231 cases (representing the extra-respiratory forms of TB), a number of 42 were hysto-pathological confirmed.

Out of the 876 patients with positive BK microscopy exam and culture at the beginning of the treatment, 171 cases (19.52%) were *uni- and policavitary forms* (140 unicavitary cases, 31 policavitary cases).

The policavitary cases were included in the 2nd *lesional score* (moderate advanced forms) and in the 3rd score (very advanced forms – 38.8%), with a *cavitary score* from 1 to 3.

The analysis of the *cavitary cases* registered with *positive microscopy* at the beginning of treatment shows the following aspects:

- 134 patients were males (78.36%) and 37 females (21.64%);
- 163 cases were from urban area (95.32%), and 8 from rural area (4.68%);
- 79 patients were insured (46.20%), and 92 uninsured (53.80%);
- out of 171 cavitary patients, 121 were registered as “new case“ (70.76%) and 50 patients as “retreatment“ (29.24%);
- 63 patients presented co-morbidities (36.84% of the total number of cavitary cases with initial positive microscopy), prevailing the liver condition;
- 147 patients (85.96%) followed the standard treatment regiment and 24 of them (14.04%) the individualized regimen.

Out of the 876 patients with initial positive BK microscopic exam and culture, after 2 months of treatment 92 cases (10.5%), including 41 cavitary cases, continues to have positive microscopic exam.

From the entire sample of patients notified with TB, 31 policavitary confirmed cases belonged to the group of patients with extended forms, formed of 56 cases. They were registered as new cases, after detection, but certainly they were forms with late diagnostic, high degree of immune-suppression, enabling rapid extension of lesions. The registration was done as “new case” according to the case-definitions, and the applied regimens were according to the treatment guidelines valid at the moment of the analysis.

After hospitalization in the medical units where isolation took place, standard R1 (HRZE) anti-TB treatment was initiated. The average of the hospitalization duration was of 33 days and, even if the patients remained smear positive, the discharge was done according to the legislation in force of the National Health Insurance House. In ambulatory, the patients continued the treatment standard regimen corresponding to the regimen initiated in hospital.

Because during the follow-up of the 4 cases with bilateral extended polycavitary forms at the moment of the detection they remained smear positive, at 4 months after beginning the anti-TB treatment I took the decision that the other cases with the same characteristics of the mentioned group use streptomycin as the 4th anti-ZB drug, after BK confirmation. At 10 of the followed cases, standard treatment was administered with ethambutol as the 4th drug, and for the other 21 cases, it was replaced by streptomycin.

At the bacteriology monitoring after 2 months, 9 of the 10 patients with ethambutol in the treatment regimen were still smear positive, and after 4 months of treatment they were still positive in culture. These patients were evaluated as “failure” of treatment and a new treatment regimen began, in which the 5th drug introduced was streptomycin. At the final evaluation of the treatment outcome, all the 9 failures were considered “cured”. Out of the 21 patients which used streptomycine as the 4th drug, at 2 months after treatment initiation only 1 case was still smear positive at microscopy and culture. The DST done on the sputum collected at the beginning of the treatment regimen confirmed the presence of primary chemo-resistance at isoniazide and rifampicine, the case being registered as MDR and switched to individualized treatment.

The duration of the treatment was extended for all the 31 cases analyzed over 6 months. The shortest treatment regimen lasted 8 months for 2 of the cases, the other having the duration of 12 months. The intensive phase of the treatment was also extended to 4 months, with daily drug administration.

All 20 cases which became BK negative after 2 months of treatment, with streptomycin as the 4th drug at the initiation of the treatment, were evaluated as “cured” after the treatment was finalized.

The sputum conversion rate, faithful indicator of the debacillization and reduction of the TB spreading risk, was of 89.49% at 2 months after treatment initiation in new cases with pulmonary localization. Patients still smear positive at 2 months after treatment initiation were in percentage of 10.51%.

IV.2. Analysis of the TB cases in children

During the analyzed period, in sector 5 of Bucharest municipality 57 TB cases at children aged 0-14 years were notified, representing 1.26% of the total of 4494 cases registered at children at national level, in the same timeframe.

Out of the total number of cases in children, 10.52% presented *severe forms* of TB (TB meningitis – 5 cases, milliary TB – 1 case).

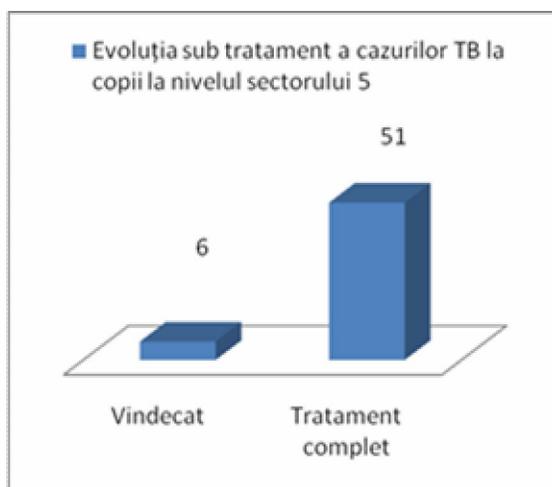


Figure 2. Evaluation under treatment of children cases

The contact tracing done for every TB case in children highlighted that 10 cases (17.54%) appeared in a *TB outbreak*.

Regarding *outcome evaluation* under treatment of the TB cases in children, 51 of them were included in the “complete treatment” category (89.5%) while 6 (10.5%) in the “cured” category. There weren’t cases of failure, default or death (figure 2).

IV.3. Analysis of the HIV-TB co-infection cases

From the total sample, 37 patients were registered with the *HIV/TB co-infection* diagnostic.

Sample analysis according to the *age* highlighted that most of the TB disease cases (active TB) at the HIV positive persons were registered in the 31 - 40 years of age group, representing 46% of the cases.

Gender distribution highlights the predominance of males (62.16%). For both genders, the most cases were recorded in the 31 – 40 years of age group (9 males, 8 females).

The structure of the sample of patients according to the *area of residence* is as follows:

- social-economically disadvantaged areas: 72.97% (27 cases);

- areas with average income: 21.62% (8 cases);
- area with above average income: 5.41% (2 cases).

The structure of the sample according to the *insured status* is as follows: 56.75% insured (20 cases) and 43.25% not insured (17 cases).

Cases classification at registration was dominated by the new TB cases, which were 91.89% (34 cases).

Group structure according to the *localization of the tuberculosis* is the following:

- pulmonary localization: 29 cases (78.37 %);
- extra-pulmonary localization: 8 cases (21.62%).

Analysis according to TB diagnostic criteria for the *main localization* divides the patients of the HIV-TB co-infection group as follows: cavitary TB, caseous – ulcerate TB and millitary TB 6 cases each, infiltrative TB – 5 cases, broncho-pneumonic TB form – 4 cases and bronchial TB – 2 cases. The presence of *extrapulmonary forms* of TB localization at HIV/TB cases is given by the ganglionar TB, TB pleuritis, TB meningitis, TB peritonitis and uro-genital TB. In 19 cases (51.35%) *bacteriological exam* was positive (at microscopy exam and culture).

Anti-TB treatment was initiated for all 37 HIV-TB co-infection cases, the average duration of treatment being of 9 – 12 months, except one case for which the duration was of 6 months. 29 treatment regimens were standard (included in the NTPSCP), while in 8 cases the regimens were individualized. Only 2 cases received intermittent treatment (3/7) in the consolidation phase.

No major *adverse reactions* were reported which would have required treatment interruption.

All HIV/TB co-infection cases were hospitalized in infectious diseases departments, where the HIV status was determined, including the antiretroviral therapy; following an interdisciplinary consult, the local pulmonologist initiated anti-TB treatment.

Regarding the evolution under treatment of the HIV/TB co-infection cases, a share of 64.86% were therapeutic successes (13 cured cases and 11 complete treatments), while in the other evaluation categories there were recorded 2 failures, 2 defaults and 9 deaths - figure 3.

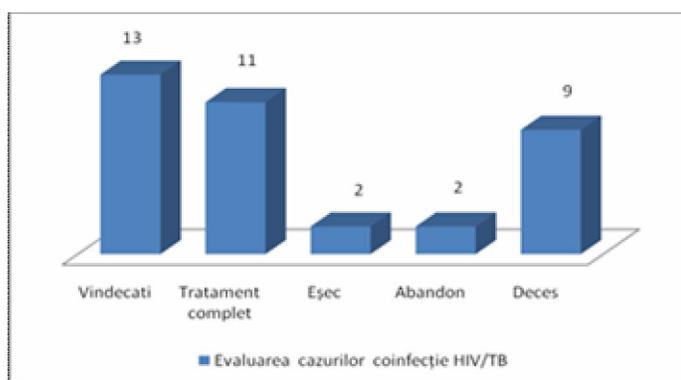


Figure 3. Treatment outcomes (evaluation categories) - HIV/TB co-infection

IV.4. Analysis of the MDR - TB

Out of the total number of TB cases recorded in the period of the analysis, 36 patients (2.5%) had *multidrug-resistance*.

All the MDR cases were bacteriologically confirmed, and the DST was validated in a National Reference Laboratory.

According to the *clinical type of chemo-resistance*, 6 presented primary MDR and 30 with acquired MDR.

The contact tracing highlighted that 7 patients were from families where other MDR TB cases were notified and thus the *infection source* was identified.

The age of these cases varied from 20 to 66 years, the distribution on age groups (except the 61-70 years age group) being almost equal.

Most cases were recorded at the *male gender* (27 versus 9).

According to the *place of residence*, 97% of the cases are from urban area and 3% from rural area. *Social category* of insured represented 67% of the cases, being dominant compared to the uninsured cases (33%). Proportion of *co-morbidities* was of 13.9% (5 patients).

Therapeutic regimens were correct according to the WHO guideline 33.3% of the cases. Only 27.7% of the MDR cases had injectable aminoglycoside in the intensive phase.

The treatment duration with the injectable aminoglycoside varied as follows:

- for 6 patients treated with kanamycin – 4 patients for 6 months and 2 patients for 10 months;
- for 2 patients treated with amikacin – 1 patient for 6 months and 1 patient for 10 months;
- for 2 patients treated with capreomycin – 1 patient for 1 month and 1 patient for 9 months;
- for 2 patients treated with streptomycin – 1 patient for 11 months and 1 patient for 24 months (according to the Management Guideline for the MDR-TB Cases, streptomycin was accepted as aminoglycoside with parenteral administration).

The quinolones were used in the MDR treatment at 31 cases during the entire duration, in daily administration, the most used one being ofloxacin (18 cases), followed by ciprofloxacin (13 cases), even if the available therapeutic guidelines recommended the exclusion of ciprofloxacin from the treatment regimens.

21 patients were tested in order to detect the HIV co-infection, out of which two were identified with HIV/MDR-TB co-infection; 15 patients weren't tested.

80% of the cases were bacteriologically monitored with microscopy exams and culture, done at 3 month.

During the treatment evolution follow-up, 11 patients were evaluated as “cured”, 13 as therapeutic failure, 7 patients defaulted and 5 patients died (figure 4).

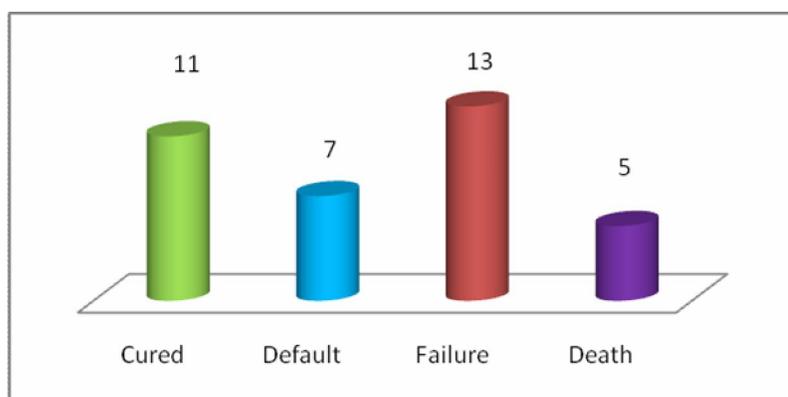


Figure 4. Evaluation of the MDR-TB cases after evolution under treatment

Therapeutic failure represented 36.1% of the MDR – TB group.

For the 13 patients declared as “failure” of treatment DST for extended series was repeated, identifying the same type of chemo-resistance as the one observed at the initiation of the individualized treatment. A new therapeutic regimen was initiated, but the maintenance of smear positive sputum determined the classification as “chronic case” without therapeutic resources, and for this reason a palliative regimen was established. At the final evaluation after 18 months, the patients presented extended lesions with a high destruction degree of the pulmonary parenchyma and severe functional respiratory amputation.

IV.5. Analysis of the mortality indicator

For the 34 deaths recorded, the analysis of age and gender criteria highlighted the predominance at ages over 60 years (35.3%), at the male gender (76.5%).

According to the *palce of residence*, 58.8% of the cases lived in an area with average economic level, and 41.2% came from a disadvantaged area.

Insured status creates the following categories: 27 patients were insured (79.4%), and 7 were not insured (20.6%).

Regarding to the *case classification* at the moment of notification, 20 (58.8%) were new cases, while 14 (41.2%) were relapses and retreatments. *TB localizations* were pulmonary in proportion of 94.1% (32 cases) and 5.9% extrapulmonary (2 cases).

Bacteriologic confirmation was done 100%, all the cases registered as death being confirmed with bK bacteriologic exam, 32 cases with smear exam and culture, and 2 cases with histopahtological exam.

Comorbidities association was present at 27 cases (79.4%); 9 HIV/AIDS cases (26.4%), 6 liver cirrhosis cases (17,6%), 6 neoplasm cases (17,6%), 4 cases of sugar diabetes (11.8%) and 2 toxicomania cases (5,9%).

Regarding the *therapeutic regimen*, 27 patients received the standard regimen, 7 had individualized treatment; 13 cases followed treatment for 1-3 months, 5 cases under 8 months, and 16 cases for more than 8 months. All the patients were in treatment at the moment of death.

V. Conclusions

Approaching TB based on the clinical, epidemiologic and therapeutic aspects represents the subject of the permanent, coherent and competent preoccupation of all responsible stakeholders for fighting this malady, at national, regional and world level, but the deficits registered time and again in optimization of the fight against TB prove the need to use some new concepts and specific methods.

With the study “Clinic-epidemiologic aspects of the tuberculosis endemic evolution in sector 5 of Bucharest municipality during 2007-2011”, I pursued to identify existing correlations between the main factors which are barrier in eliminating TB: HIV endemic, the phenomenon of myobacterian resistance to anti-TB drugs, combined with social-economic aspects and evaluation of the participation degree of each of the above-mentioned components, as reflected in the analyzed territory.

The research allowed the analysis of the demographic, economic, social parameters, the bacteriological and chemo-susceptibility testing criteria.

Territory with average density population and area, sector 5 of the Bucharest municipality recorded constant high values of the TB incidence, being on the first place among Bucharest's sectors. In the last 10 years, TB decrease rate was 32.3%, the annual average value being higher than the one at country level.

The illness cases were most frequent in the urban level, at males, with a gender ratio of 1.75/1, which corresponds to the higher risk of TB in males.

The most affected age group was 41-60 years, representing 59.1% of the total illness cases. The shifting of the patients share towards older ages took place in the context of diminishing the endemic level at national level.

In the analyzed sample, the illness cases were registered at insured persons in proportion of 68.4% compared to 31.6% at persons without insurance. The study allowed the identification of the higher incidence values at the self-employed persons, active in agriculture but in other fields also. Occupational categories most affected were the employees in the construction and industrial fields, with exposure to the coniotic emissions which acted as immune suppressors in the given conditions.

The poverty rate is maximum among youth aged 15 – 24 years, non-compliance being correlated with the risk to default. The marital status indicates that the divorced or single persons, together with poor material conditions, created the premises for the unfavorable evolution of the illness in the respective environment.

The social-economic analysis identified a number of illnesses 16.7 higher in the areas economically disadvantaged. The incidence in poor neighborhoods is similar to that recorded in India, Pakistan, while in financially prosper areas the value is similar to that in the Western countries (Hungary, Dutch, England). The assertion according to which the poverty represents a major individual or collective vulnerability for TB is verified for the analyzed sample.

Illnesses among Roma population, well represented in sector 5 of Bucharest, as shown by the 2011 Population Census, with 7800 persons declared Roma, were 2.5 more frequent than in the general population. The figure is similar to the values recorded at national level.

The social characteristic of the sector is the predominance of a highly polymorphic population: low fertility rate, low percentage of working persons, with average low education, a low declared unemployment rate (0.54%) and high number of the persons benefiting of social support. Insufficient budgetary contribution determines the lowest income compared to the other sectors. The spending for social protection, represented by social incentives and unemployment

allowance, are higher than the average value recorded at city level. The social canteens are in a larger number compared with the national average, and the poor population benefits frequently of these services.

The health services offer is average, if we take into account the participation at the health insurance fund. Nonetheless, 31.6% patients are not insured. There is a high number of iv drugs users, especially at the outskirts of the neighborhoods. The existing crime rate should also be taken into account.

Analysis of the sample shows that most of the cases were new cases, microscopy confirmed, proving late detection. The caseous-cavitary forms (913 cases) were bacteriologically confirmed in percent of 63.98%. pulmonary localization was predominant, compared to the extrapulmonary, which hystopathologic confirmation was of 18.2% (15.6% at national level).

The lesional score 3 met at 38.8% of the cavitary forms at the high percentage of the smear positive exams proves the lack of efficiency of the TB control measures.

The low number of culture confirmations and DST show a poor quality of the bacteriologic exam.

For the HIV-TB coinfection cases, even if the obtained values are inferior to those at national level (2.59% compared to 2.9%), they are statistically relevant because the testing percentage in Romania is much lower compared to the 80% value in developed countries.

The share of MDR-TB cases was under the values recorded at national level (2.52% compared to 3.63%), most of them being the cases with acquired MDR-TB (87.37%) compared to the primary MDR-TB (16.63%). Considering that only 38% of the positive cultures of the new cases and 64% of the retreatment had DST, the 2.52% value of MDR-TB out of the total TB cases remains arguably and requires chemo-susceptibility testing for all positive cultures

Treatment outcome evaluation with the analysis of the therapeutic success rate for sensible TB (as appears in the National Statistic Database in Romania from 2002 until 2013) was over 85%, higher than the 67% value recorded in the WHO European Region.

For the researched sample, the therapeutic success rate had an average of 88.9%, while the recorded values for the unfavorable treatment outcomes (failure, default, death) were lower than the national ones.

The real challenge for the National TB Control Program is the MDR-TB cases diagnostic and treatment, which are difficult because the lack of investigations for chemo-resistance rapid detection and proper funding, thus determining the late initiation of an efficient treatment.

The absence of some 2nd line TB drugs determined the impossibility to apply efficient therapeutic regimens. For the studied sample, only 30.8% of the cases had a treatment regimen according to the updated WHO guidelines.

The success rate of the MDR cases treated in Romania was among the lowest at world level (cohort 2008 – 19.6%, and 2009 – 20.2%), with little chances to increase in the near future.

The conditions underlying this low success rate are:

- long duration of the treatment and absence of the fixed drugs combinations;
- difficult tolerance associated with the lack of insuring the drugs for the adverse reactions and social support;
- reducing the number of hospitalization days and sending the patients at home, while some of them being still smear-positive;
- lack of implication of the family doctor in the DOT for the follow-up phase;
- stigmatization is another problem, being frequently a motive for the patient to require rapid reintegration in the community and increasing the risk of defaulting.

It's necessary to improve the information and education activities for the TB patients, in the initial phase (stationary phase) but especially their consolidation during the much longer period of ambulatory treatment, highlighting the knowledge about the disease, the duration and nature of the treatment, perception of the individual, family and community epidemiologic risks, the consequences of an inadequate treatment. With these aspects, treatment adherence and patients trust in the results obtained with the finalization of the treatment are more eloquent.

I consider that the innovative element of this work is represented by the analysis of the 31 patients group who were administered streptomycin as the 4th anti-TB drug. Follow-up of the bacteriological evolution allowed the perspective of changing the treatment regimen in the standard I regimen, having in view the increase of the curing rate.

Sputum conversion rate of 95.23% at 2 months after initiation of treatment for the above-mentioned group, compared to the value of 10% for the group who took ethambutol opens up the possibility of new research.

Mortality, as major impact indicator, recorded for the studied group values lower than the figures at national level, but still higher than those recorded at world level. In the future, the decrease of the TB mortality importance is based on the correct implementation of the disease control strategy, within which the cases' early detection methods and initiation of anti-TB under direct observation is the guarantee for meeting this objective.

The efficiency of a health program is illustrated also by the number of cases in the children population. The group of children with TB presents as a characteristic incidence with much higher values compared to the national ones, with variations from 32.7‰ in 2007 to 35.2‰ in 2011. 6 severe forms of TB (meningitis and miliary) were diagnosed, without HIV-TB co-infection and MDR. Out of the 57 registered cases, 10 came from TB outbreaks, the source of infection being identified. Their evolution under treatment was favorable, the success rate being 100% in all cases, without failures, defaults or deaths.

Recommendations

Proving the importance of the social-economic component gave me the possibility to formulate the following recommendations:

- gaining control over the TB endemic within a territory by strengthening the social protection measures: providing social allowances for the disadvantaged persons, insuring free adjuvant medication for the TB treatment, social vouchers for transport and food. Thus, increasing activities in order to determine the decision making organizations to resolve TB as priority public health problem, through social support of the TB patients shall lead to increased adherence to treatment and, as a consequence, higher curing rate;
- developing some different material, social and psychological support measure, in the hospitalization period as well as in the ambulatory period, in order to reduce the default risk, especially for the patients with TB-HIV co-infection and with MDR-TB;
- creating a legal framework for the involvement of the family doctor in the TB control activities, by including these obligations in the contract with the Health Insurance House regarding the identification of the TB suspect, contacts control, treatment monitoring and follow-up;
- organizing hospices to provide medium and long term care for the MDR-TB patients;

Initiative regarding the therapeutic regimen

Expanding at national level the studies for the improvement of the therapeutic success rate of the particular cases of active susceptible TB, new cases with extended lesions, with late diagnostic or progressing on a severe immune-suppressed background, by changing the standard I regimen.

Based on the obtained conclusions, subsequently there shall be requested the endorsement of the Pulmonology, Alergology, Immunology Committee of the Ministry of Health to review the

standard I regimen by replacing ethambutol with streptomycin at the mentioned particular cases and including the changes in the Methodological Norms for NTPSCP Implementation.

I consider there are extremely necessary more complex activities aiming at focusing the public opinion on the TB problem and identifying methods and social means to diminish the share of groups with high illness risk.