

## THE INCIDENCE OF *MYCOBACTERIUM TUBERCULOSIS* INFECTIONS AND RESISTANCE TO ANTITUBERCULOSIS DRUGS IN GIURGIU POPULATION

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### Abstract

*Tuberculosis due to Mycobacterium tuberculosis is an important health problem all over the world. Correct and early diagnosis of infection is a major condition for efficient treatment. Different localizations of mycobacterial infection are occurred but the most important is the pulmonary tuberculosis.*

*Pulmonary tuberculosis can be diagnosed by medical history and symptoms, laboratory and radiographic tests. Laboratory diagnosis of mycobacterial infections required microscopic examinations of sputum for acid – fast bacilli and a culture from sputum sample. Other techniques have provided a rapid and modern diagnosis but required high cost and special settings.*

*In 2012, 975 subjects are investigated for mycobacterial infection in Izvoru (Giurgiu) Pneumophthysiology Hospital, but just 34.8% from all were confirmed with M. tuberculosis infection. The micobacterial infections were related to other diseases and formulated in order to gender and age of patients. For these patients the antibiogram was performed. All bacterial strains from patients with initial infection were sensitive to antituberculosis drugs (Hydrazide and Rifampicin). Some bacterial strains from older infections were resistant to these drugs.*

*Keywords: Acid-fast bacilli, tuberculosis, drug resistance*

### 1. INTRODUCTION

Tuberculosis determined millions of deceased and morbidities worldwide and remains one of the major causes of death from a single infectious agent (Palomino, 2005). Tuberculosis seems to affects more than one third of the world's population, with 2 million deaths in a year or so (Eichbaum and Rubin, 2002; Nachegea and Chaisson, 2003).

In European Union, Romania take the first place, with 17694 new cases and reinfections in 2012, a higher disease incidence than other European countries reports; however, the incidence of tuberculosis in Giurgiu and in Romania was consistent decreased from 2002.

The most important micobacterial infection from the public health point of view is pulmonary tuberculosis. The common diagnosis is based on patient history and high clinical suspicions, symptoms, radiographs, microscopy of sputum and cultivation on solid medium (Palomino, 2005). *Mycobacterium tuberculosis* is a acid-fast bacillus, because of its cellular wall with high lipid

content. The sputum smear is hard colouring by usual technique and the growing on solid medium is quite slow.

Because the inadequate tools from laboratory for diagnosis of tuberculosis, sometime occur underdiagnosis or overdiagnosis of disease, or delayed diagnosis of drug resistance (Dorman, 2010). New methods are performed nowadays, like the molecular methods for understanding the molecular basis of antibiotic resistance of *M. tuberculosis* or detection of biochemical markers and immunological response. For example, Interferon- $\gamma$  release assays, BACTEC radiometric system (with radioisotopes for detection of *M. tuberculosis* in just a few days), the FASTPlaque TB assays (based on the ability of *M. tuberculosis* to support the growth of an infecting mycobacteriophage), the PCR tests, Nucleic Acid Sequencing, Gas-Liquid Chromatography and High-Performance Liquid Chromatography Methods (Eichbaum and Rubin, 2002; Palomino, 2005).

While certain methods are performed for detection of *M. tuberculosis* infection, other methods detect the drug resistance of bacteria.

The drug resistance of *Mycobacterium tuberculosis* has been a problem since early 1990s, because of multidrug-resistant tuberculosis. The substances and the mechanism of bacterial resistance are studied, while drug resistant tuberculosis seems to be a "potentially catastrophic challenge to global public health" (Nachega and Chaisson, 2003).

## 2. MATERIALS AND METHODS

This study was realised in 2012, in the Laboratory of Izvoru (Giurgiu) Pneumophtysiology Hospital. 975 patients were investigated because they had specific symptoms for pulmonary diseases. Among all, 791 were men and 184 were women, and their ages were from 18 to 83 years. Besides the clinical diagnosis, the laboratory tests were performed.

The sputum samples were gathered from patients. These were process by microscopic examination and by culturing *M. tuberculosis*.

*The microscopic examination* was performed after the sputum samples were used to obtain sputum smears. The dried smears were fixed by heat and staining by Ziehl Neelsen method. Microscopic observations were made and the results were explained according to standard values. This staining is used for acid – fast bacteria and it is the most frequently used microbiological test for detection of pulmonary tuberculosis. The sensitivity of sputum smear microscopy is poor (approximate 70%) and may be less in HIV co-infections (Dorman, 2010).

The sputum samples were used for culturing on solid media (Löwenstein-Jensen) after homogenization and decontamination with 4% NaOH. The inoculated tubes (three for each sputum sample) were incubated at 37°C for 60 days, with weekly observation and result registration (rather on 21, 30, 45 and 60 days). The results were correlated with microscopic observations. Culture of *M. tuberculosis* is more sensitive than smear microscopy and required biosafety practices and equipment to prevent infection of laboratory personnel (Dorman, 2010). *M. tuberculosis* grows slowly and diagnosis by culture is a long process (Eichbaum and Rubin, 2002).

All the isolated strains of acid - fast bacteria were tested for drug resistance. Because the multidrug-resistant strains of *Mycobacterium tuberculosis* estimated to increase up to 36% (in some countries), all isolates must be tested for this (Eichbaum and Rubin, 2002). The bacterial growth on the medium without antibiotic substances were compared with the bacterial growth on the test media (with Hydrazide and Rifampicin) after inoculation of a typical specimen of bacterial culture and incubation at 37°C. The results were interpreted as sensitive or resistant strain of *M. tuberculosis*.

### 3. RESULTS AND DISCUSSIONS

The results of this study are presented in next figures and tables. The percentage of positive cases from all 975 was 34.8%, according to microscopic and cultural tests (Figure 1), these means 340 patients (282 new cases of micobacterial infections and 58 old cases of tuberculosis). The cases of pulmonary tuberculosis were more frequent in men then in women.

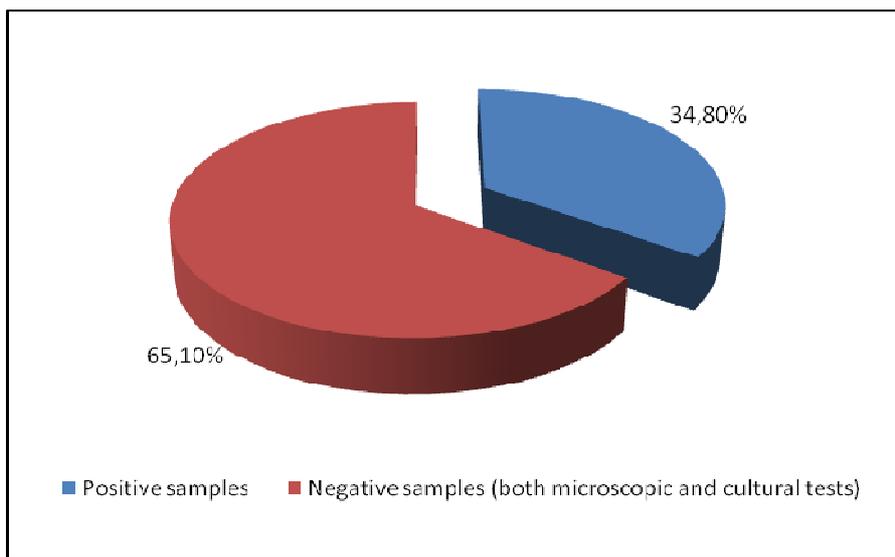


Figure 1. The frequency of positive samples

The gender and age of patients were appreciated for positive cases. These are presented in Figures 2 and 3. The most frequent cases were in women with age 18 – 30 years (43% from 68 cases of infection); the most men with tuberculosis attested by laboratory tests were from 31 – 50 years age group (48% from 272 positive cases) and from 51 – 70 years age group (30%, Figure 3).

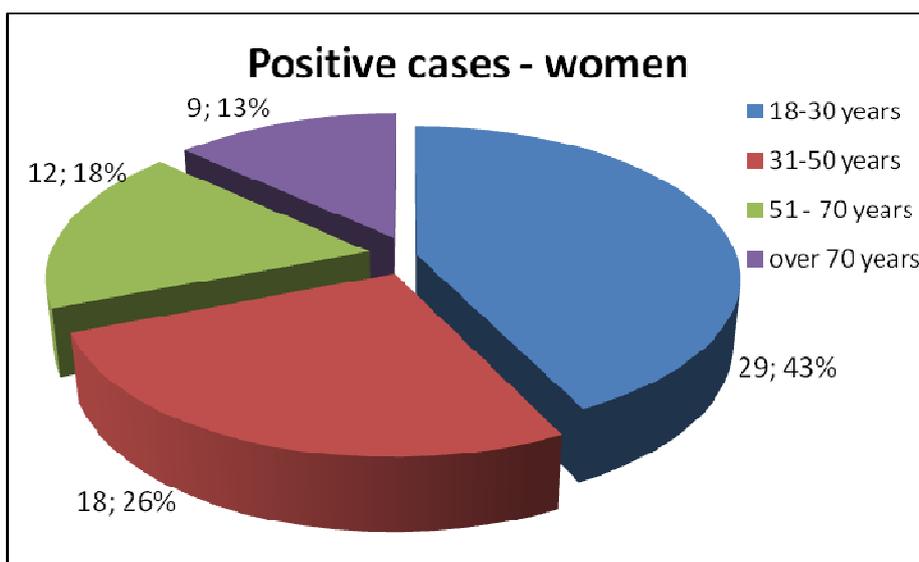


Figure 2. Positive samples in female patients

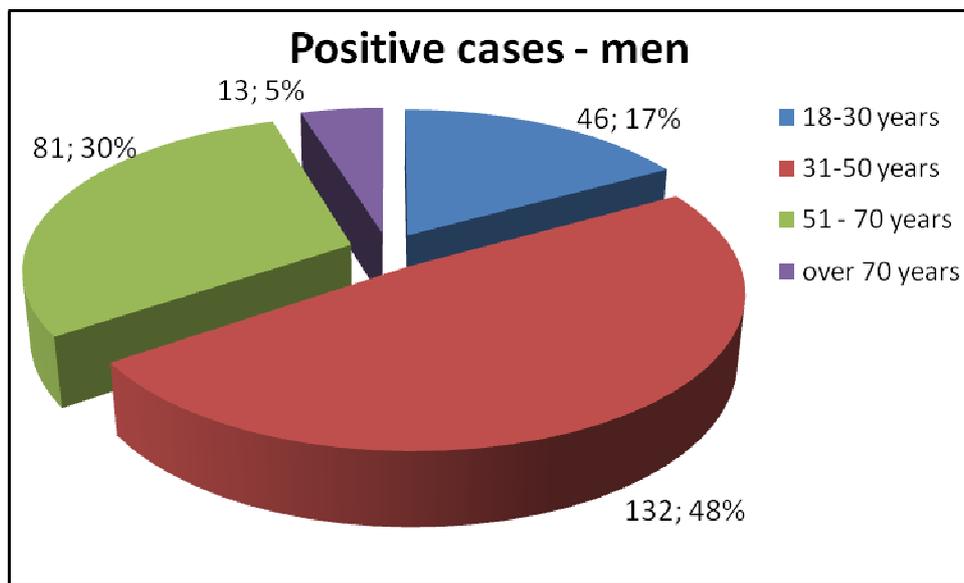


Figure 3. Positive samples in male patients

The aim of this study was to point out the incidence of micobacterial infection and other diseases in patients, too. In Table 1 the number of tuberculosis cases with associated diseases is presented (just some of the patients with tuberculosis had associated illness). The most cases were associated with respiratory and cardiovascular diseases in 51 – 70 years patients. These values define the opportunistic characteristics of infection with *Mycobacterium tuberculosis*.

Table 1. *Mycobacterium tuberculosis* infections and associated diseases

Associated diseases	Age group			
	18-30 years	31-50 years	51-70 years	over 70 years
Diabetes mellitus	0	2	5	1
Liver diseases	0	4	5	6
Cardiovascular diseases	0	5	12	2
Respiratory diseases	3	4	11	1
Anemia	4	2	2	0
HIV infections	2	4	1	0

The drug resistance tests were performed with first line antibiotics (Hydrazide and Rifampicin) used in micobacterial infection treatment. All the bacterial strains of *Mycobacterium tuberculosis* involved in new cases of pulmonary tuberculosis (282) were sensitive to these drugs. The strains involved in reinfections were resistant to Hydrazide (17 strains) or to Rifampicin (15 strains), just 45% from 58 cases were determined by sensitive bacterial strains (Figure 4). The resistant strains determined the relapse of infection and the new laboratory tests were necessary. Fortunately, the strains presented resistance just to one drug and the treatment was reviewed.

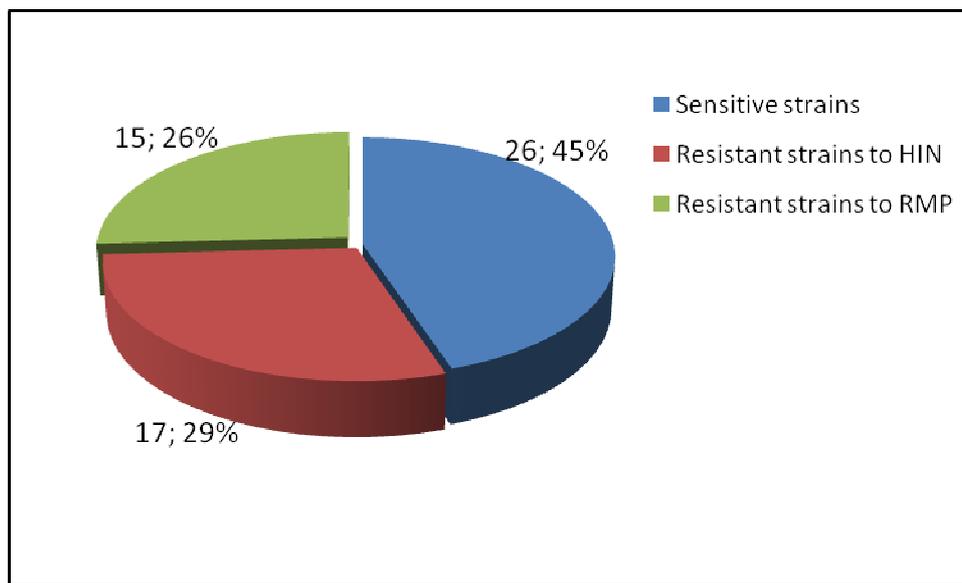


Figure 4. Bacterial strains involved in old cases – resistance to first line antibiotics (Hydrazide – HIN and Rifampicin - RMP)

#### 4. CONCLUSIONS

In this study, 34.8% of 975 patients were infected with *Mycobacterium tuberculosis*, 282 new cases of infection and 58 old cases with reinfections.

Among all positive cases, 80% were determined in male patients, the most affected age group was 31 – 50 years. The pulmonary tuberculosis was less frequent in female patients (just 20% of positive cases).

The associated diseases with micobacterial infections were mostly respiratory and cardiovascular diseases, especially in 51 – 70 years age group.

All the micobacterial strains involved in new cases of pulmonary tuberculosis were sensitive to first line antibiotics (Hydrazide and Rifampicin), but the strain involved in old cases were 55% resistant to one of the usually drugs.

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