NONTUBERCULOUS MYCOBACTERIA - CURRENT RISK IN BULGARIA

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ABSTRACT

Background: Nontuberculous mycobacteria (NTM) are generally free-living and have a potential to cause opportunistic infection. More than 190 species of NTM have been identified (http://www.bacterio.net/mycobacterium.html), up to 60 species of them are pathogenic. The distribution is geographically specific for some species and others are cosmopolitan. Understanding their diversity has practical value for the treatment and management of NTM disease. Except for isolated reports, there is no accurate information about NTM spread among Bulgarian patients.

Material and Methods: We retrospectively analysed the data on the NTM isolated from patients throughout the country for the period from January 2010 to December 2017. Identification to the species level was done by Line Probe Assay (LPA).

Results: 586 NTM strains were identified. We calculated the rate of NTM isolation in Bulgaria and NTM disease incidence by applying microbiological criteria defined by the American Thoracic Society (ATS). As a result, the laboratory isolation rate amounted to 1/100 000 people for the period, and when applying only the ATS criteria, the prevalence of NTM disease was 0,23 per 100 000 people. In both cases, we reported that NTM disease incidence remains low as compared to tuberculosis, though with an increasing trend. The prevalence of NTM varied significantly between northern and southern Bulgaria, as well as between the capital and the rest of the country's regions.

Conclusions: Slow growing NTM species predominate in Bulgaria but rapidly growing ones have isolation levels higher than the average for Europe and closer to that of Asian countries. Given the demographic situation in Bulgaria and the changing climatic factors, NTM infections need special attention.

Keywords: Nontuberculous Mycobacteria, geographical diversity, LPA.

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INTRODUCTION

An increasing isolation of NTM has been reported worldwide and is most pronounced in countries with declining incidence of tuberculosis . The trend for Bulgaria is owards a permanent decrease in the incidence of tuberculosis - from 35/100,000 in 2011 to 19.1/100,000 in 2019, 13.4/100,000 in 2020, 9.9/100 000 in 2021 [1]. Except for isolated reports, there is no accurate information about NTM situation on the territory of Bulgaria [2, 3]. An assessment of NTM isolation rates, as well as the geographic variation in isolation and disease severity is lacking.

NTM diseases will continue to aggravate in the country as a result of aging population, environmental exposure and the impact of climate change, geographical region, and migration flows that occurred in the recent years.

METHODS

The study was retrospective, conducted in the National reference laboratory of tuberculosis (NRL of TB). It is the only laboratory in Bulgaria performing molecular identification of NTM (GenoType© CM/AS; Hain Lifescience, Germany), and supplemented with phenotypic, biochemical and immunochromatographic methods. We used data of patients suspected of tuberculosis with an isolated NTM strain from all the TB network in country (30 laboratories for culture diagnostics). For the period from January 2010 to December 2017 inclusive, 586 strains of NTM were isolated. To determine the frequency of NTM over the period, each isolate corresponded to one patient. Each clinical material/

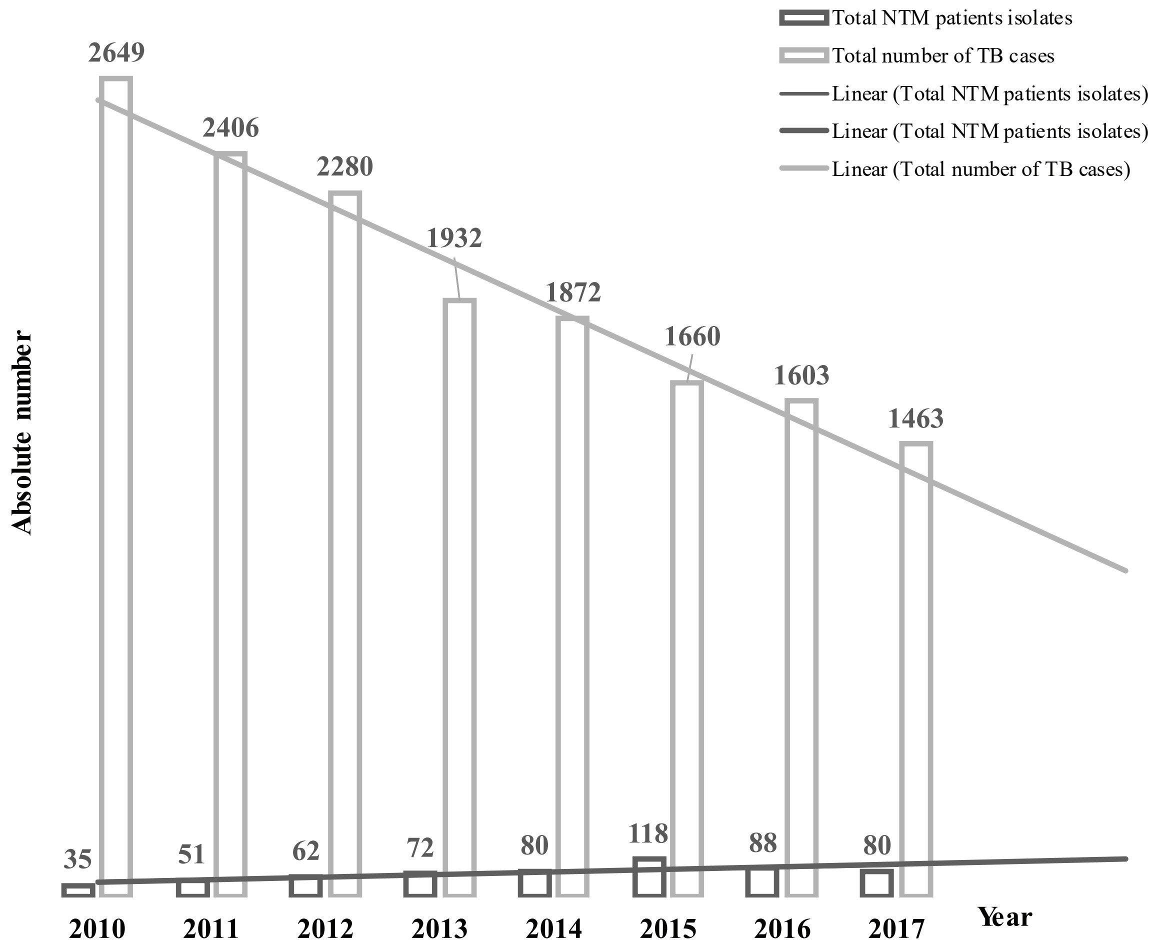

Figure 1. Distribution and trends for patients with isolated NTM strain and those registered with tuberculosis according to ECDC data for the study period.

strain was accompanied by information on the patient's age, sex, address, date and type of clinical material. We used demographic data reported by National Statistical Institute (NSI), which is a part of the Statistical Office of the European Communities (Eurostat).

Microsoft Excel (Microsoft, Redmond, Washington) was used to calculate prevalence rates, percentages, and mean ages.

RESULTS AND DISCUSSION

Patients and specimen data:

A total of 586 patients with identified NTM strain were confirmed in the NRL of TB. The trend was of increasing number of isolates over the eight-year period. There was a marked peak in 2015 (n=118, 20%). The reason was due to the accumulation of several factors including: introduction of the cycle for External Quality Assessment for cultural diagnostics of tuberculosis with differentiation between NTM and MBTC, modern laboratory equipment and regular supply of reagents and consumables, the professional qualification of the laboratory staff through periodic trainings.

According to our data, the global trend of increasing frequency of NTM isolation alongside with decreasing incidence of tuberculosis was observed in the country. (Fig 1).

Overall 97% (n=567) of the clinical materials were pulmonary (mostly sputum - 68% (n=392) and 11% BAL (n=67)). NTM strains from extrapulmonary sites (cerebrospinal fluid (CSF), urine, biopsy material) were 3% (n=19).

A positive result of smear microscopy had 8% (n=48) of all examined types of clinical material. Among them, smear positive sputum dominated - 85% (n=41). One of the two CSF included in the study was also positive.

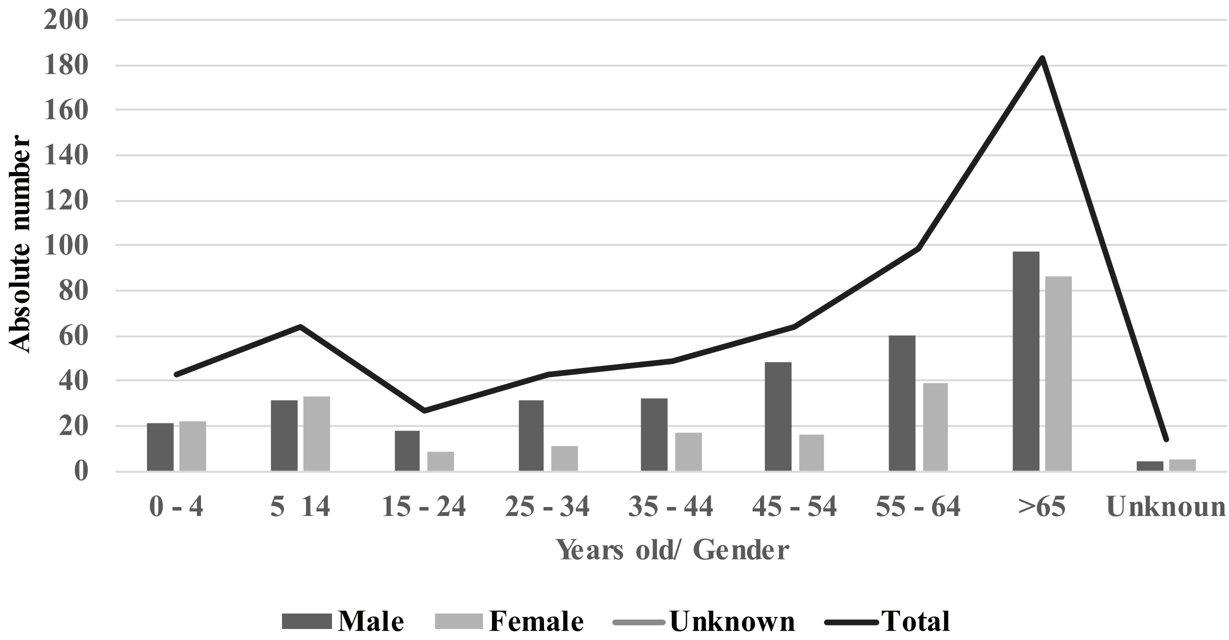

Figure 2. Gender and age distribution of patients with an isolated NTM strain, for the study period.

Demographic structure:

The gender ratio of patients with an isolated NTM strain followed the trend for tuberculosis patients in the country. NTM were isolated more often in males - 59% (n=343), 40% (n=237) were female and no gender information was available for six patients (1%). The biggest gender ratio difference was observed in the age group 45-54 years, where males were 75% (n=48) and females - 25% (n=16). Only in the age group 5 to 14 years old, a slight predominance of female gender was observed - 51,56% (n=33) (Fig. 2). This gender distribution, can be explained by the target group. We searched for and isolated NTM, mainly among suspected tuberculosis patients and those with pulmonary pathology as a differential diagnostic plan of TB. A more comprehensive national survey could change the situation.

The average age of the patients was 47 years.

Species structure of the isolated NTM in Bulgaria:

We performed species identification of isolated mycobacteria using conventional and molecular methods (LPA) and revealed valuable epidemiological information. The most frequently isolated in the country were the following species: *M. gordonae* (17%), *M lentiflavum* (15%), *M. forthuitum* (11%), *M. intracellulare* (9%), *M. chelonae* (8%), *M. avium* (7%). The total species diversity in the country, and their distribution according to the geographical regions and in the tree biggest cities: Sofia-city, Plovdiv and Varna are presented on Table 1. The species diversity of NTM was greater in Northern Bulgaria, but the frequency of their isolation was higher in Southern Bulgaria . In a few areas, only Slowly Growing Mycobacteria (SGM) were detected while Rapidly Growing Mycobacteria (RGM) were absent. That was valid in the regions of: Gabrovo, Smolyan, Targovishte, Shumen and Yambol. Montana and Haskovo were the regions dominated by RGM.

The influence of regional environmental factors could explain the situation, but this requires further investigation.

Slowly Growing Mycobacteria:

Slowly Growing Mycobacteria were dominant While *M. gordonae* prevailed all over the country, the reason for *M. lentiflavum* leading position was a peak in the children's ward of one hospital in 2015-2016. Possible contamination of gastric lavage at the stage of the clinical sampling procedure may explain this result.

As a total, the clinically significant isolates *M. avium - intracellulare* complex (MAC) were 16% (n=94) of NTM, and *M. kansasii* - 3% (n=17). While in EU *M. avium* is the dominant species in MAC, in Bulgaria *that* was *M. intracellulare*. It was more common in

Table 1. NTM isolation frequency in Bulgaria according to the geographic region.

NTM species	Total n (%)	Northern Bulgaria n (%)	Southern Bulgaria n (%)	Foreign residents n (%)	Sofia city n (%)	Plovdiv city n (%)	Varna city n (%)
M. abscessus	11 (1.9)	3 (27)	8 (73)		2 (18)	2 (18)	-
M. avium	42 (7.2)	11 (27)	30 (71)	1 (2)	22 (52)	-	4 (10)
M. celatum	1 (0.2)	1 (100)	-	-	-	0	1(100)
M. chelonae	47 (8)	12 (25)	35 (75)	-	6 (13)	21(45)	7(15)
M. forthuitum	62 (10.6)	15 (24)	47 (76)	-	19 (31)	17(24)	6 (10)
M. genavense/M. triplex	1 (0.2)	-	1 (100)	-	-	-	-
M. gordonae	102(17.4)	38 (37)	64 (63)	-	30 (29)	20 (20)	16 (16)
M. intracellulare	52 (8.9)	9 (17)	43 (83)	-	20 (38)	8 (15)	2 (4)
M. kansasii	17 (2.9)	5 (29)	12 (71)	-	7 (41)	2 (12)	4 (24)
M. lentiflavum	90 (15.2)	22 (25)	68 (75)	-	30 (33)	4 (4)	2 (2)
M. marinum	1 (0.2)	1 (100)	-	-	-	-	-
M. mucogenicum	10 (1.7)	6 (60)	4 (40)	-	1 (10)	2 (20)	3 (30)
M. peregrinum	19 (3.2)	8 (42)	11 (58)	-	6 (32)	2 (11)	3 (16)
M. scrofulaceum	2 (0.3)	1 (50)	-	1 (50)	-	-	1(50)
M. shimoidei	1 (0.2)	1 (100)	-	-	-	-	1 (100)
M. simae	1 (0.2)	1 (100)	-	-	-	-	-
M. szulgai	1 (0.2)	1 (100)	-	-	-	-	-
M. xenopi	2 (0.3)	2 (100)	-	-	-	-	-
genus *Mycobacrium*	123 (21)	43 (35)	80 (65)	-	34 (28)	9 (7)	19 (15)
mixed culture	2 (0.3)	1 (50)	1 (50)	-	-	-	1 (50)

males over 45 years old from Southern Bulgaria. *M. avium* was isolated with equal frequency in females and males over 25 years old, and predominated among the inhabitants of Southern Bulgaria. This was not surprising because *M. avium* is one of the species most frequently co-infecting the HIV-positive patients while in Bulgaria the rateof these patients is low. [4]. A possible risk factor for developing *M. intracellulare* lung infections is the exposure to domestic and natural water supplies [5]. This potential relationship may be the subject of a future study given the large volume of *M. intracellulare* lung infections (Table 1). At the same time, SGM (n=10) with single isolates in the country were identified: *M. celatum*, *M. genavense*, *M. marinum*, *M. shimoidei*, *M. simiae*, *M. szulgai* - 0.2% each. One of the most common NTM for European countries - *M. xenopi* was represented by only two isolates such as *M. scrofulaceum* (0.3%). One *M. scrofulaceum* was isolated from a foreigner - a citizen of Pakistan (Southeast Asia), where this species has a high isolation rate of 6.8% [6]. According to ATS we defined all species with single isolation as rare and geographically limited NTM species for our territory [7]. According to the address registration of those patients, predominated inhabitants of Northern Bulgaria& 90% (n=9). Notably, 40% (n=4) of them lived in the highly urbanized coastal region of Dobrich-Varna and only one (10%) lived in Southern Bulgaria, the district of St. Zagora. Some of the factors that could be considered include: the marine wetter and warm climate, karst rocks, characteristic of the area, as well as the incidence of tuberculosis [8]. It was shown that minerals widely found in clay soils such as kaolin facilitate the growth of NTM [9]. The frequency of tuberculosis for the region of Varna and Dobrich, is at the average level for the country (27.9/100 000), with a trend of increasing rate of MDR-TB [10].

When we compared our results to those of the other European countries, a unidirectional trend towards an increase of the overall NTM isolation rate was found. The most frequently reported species vary between the EU countries but always included: *M. avium*, *M. gordonae*, *M. intracellulare*, *M. fortuitum* and *M. xenopi* with an average of 7% unidentified isolates [11]. In the Netherlands, the increase was mainly due to isolation of *M. avium* and *M. gordonae* in pulmonary samples and was most pronounced in patients over 40 years of age [12]. In Italy, the most frequently isolated species were *M. avium* (29.2%), *M. intracellulare* (21.5%), *M. xenopi* (10.6%), *M. gordonae* (10.6%), *M. abscessus* (5.9%), *M. chimaera* (5.1%), and the most common patients - men over 75 [13].

***Rapidly Growing Mycobacteria*:** The isolation rate of 25% RGM in Bulgaria was closer to the Asian and higher than that for the EU countries. The dominant RGM - *M. fortuitum* (42%) prevailed in both Northern and Southern Bulgaria, followed by *M. chelonae* (32%), *M. peregrinum* (13%), *M. abscessus* (7%), and *M mucogenicum* (6%).

In the EU countries, the total of RGM strains represented between 10 and 20% of all NTM isolates. The most commonly identified were *M. abscessus* and *M. fortuitum* [7]. In Asia, RGM constituted 27% of NTM isolates compared to North America (17.9%), and South America (16%). Within Asia itself, there were differences in the levels of isolation between countries and regions. For example, in Tokyo (Japan) they were only 6,6% of all isolates, in contrast to South Korea (28,7%) and Taiwan (50%) [14]. In Taiwan, *M. fortuitum* and *M. abscessus* were the second and third most frequently isolated NTM species after MAC, while in South Korea *M. abscessus* was the second most frequently isolated NTM after MAC [14]. Serious problems due to the very high levels of antibiotic resistance of RGM were reported in the East Asian countries, including Japan, Korea and Taiwan [14]. The reason for the high rate of isolation of RGM in the Asian countries remains unclear, although geographical, climatic or racial ones have been discussed. Some studies focus on ecological niches and their subsequent transmission to humans [15]. Except that geographical reasons for distribution, RGM themselves (primarily *M. abscessus*) are often associated with patients with cystic fibrosis all over the world [16].

Without the possibility of more accurate identification we defined 21% of all NTM as genus *Mycobacterium*. The reason for this high percentage should be sought in the identification methods we used.

This was the first study that showed the frequency of isolation of NTM species from patient samples and estimated the nationwide NTM prevalence for Bulgaria.

We determined the rate of NTM isolation in Bulgaria as 1/100 000. The number of NTM patients' isolates (one NTM per patient if it duplicated during the years) was divided by the average annual population of the country, according to the National statistical institute data for the relevant year, and was multiplied by 100 000 [18]. Our results were comparable to EU countries with a population close to ours. Based on data from Croatia, Estonia, Greece, Luxembourg and Slovenia, NTM isolate rate were between 0.4-2% of samples [17]. Because of the often insufficient clinical and radiological data accompanying the samples, this approach was useful and predictive of disease. It had its limitations. In some cases, we would overestimate the severity of isolation (e.g., *M. gordonae*, *M. lentiflavum*) or underestimate it, if only one sample was sent for laboratory identification without additional conformation material or the patient was tested in a time interval ot in accordance with the study.

Only 23% (n=132) of the patients included in the study met the ATS criteria for diagnosis of nontuberculous mycobacterial disease [19]. Applying these, we obtained NTM disease incidence rates of 0,23/100 000 population.

CONCLUSION

Geography plays an important role in NTM species distribution in our country. The overall burden of NTM disease in Bulgaria is still low as compared to that of tuberculosis. It is higher in the most urbanized settlements. We are facing an emerging problem. The incidence of current disease cases is strongly underestimated.

COMPLIANCE WITH ETHICAL STANDARDS

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DISCLOSURE OF CONFLICT OF INTEREST

There is no conflict of interest to declare.

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