DISTRIBUTION OF MYCOBACTERIUM AVIUM COMPLEX IN BULGARIA

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ABSTRACT

The members of Mycobacterium avium complex (MAC) are opportunistic microorganisms, common in the environment (soil and water) and cause infections in birds, mammals and humans. The objective of the present study was to determine the prevalence of MAC representatives among suspected for tuberculosis patients in Bulgaria over an eight-year period – from 2010 to 2017. The exact epidemiology of pulmonary non-tuberculous mycobacterial (NTM) disease was difficult to determine because unlike cases of infection with M. tuberculosis complex, notification of NTM and MAC in particular, is not mandatory in terms of Ordinance 21 of the Ministry of Health (1) and there is no any accurate information about their prevalence in Bulgaria. The survey was conducted in the National Reference Laboratory of Tuberculosis at the National Centre of Infectious and Parasitic Diseases. MAC was identified using phenotypic methods, biochemical features and molecular genetic assay (LPA).

A total of 596 NTM strains were identified during the study period and 16.8% (n=100) of them were MAC. In EU countries, MAC representatives are prevalent among NTM isolates, but in Bulgaria they are significantly less in number.

KEYWORDS:

LPA, NTM, MAC

INTRODUCTION

Non-tuberculous mycobacteria (NTM) are all Mycobacterium species other than Mycobacterium tuberculosis complex (MTC) and Mycobacterium leprae. There are currently more than 170 species of these environmental, mostly opportunistic pathogens (2). Recently, NTM organisms have gained attention due to increased isolation frequency (3-5), considered to be most pronounced in countries like Bulgaria, where the incidence of tuberculosis (TB) is declining (6).

Mycobacterium avium complex (MAC) consists of two main species: M avium and M intracellulare. M. avium can be further classified in subsp. avium, subsp. silvaticum and subsp. paratuberculosis. M. avium subsp. paratuberculosis is an obligate pathogen in ruminants and is associated with Crohn disease in humans (3). It is very slow-growing – primary isolation requires several months of incubation. Sequence analysis of 16S rRNA or hsp65 gene identified new species and subspecies within MAC. For example, M. avium subsp. hominissuis was separated from M. avium subsp. avium based on several differences determined with IS1245 RFLP, 16S-23S rDNA ITS and growth temperature (2, 3, 15).

Furthermore, recent reports describe serious infections in patients who had undergone open cardiac surgery using contaminated heater-cooler device during extracorporeal circulation. The infections occurred in patients in Europe, the United States, Australia, and were caused by Mycobacterium chimaera. This organism is a slow-growing NTM species ubiquitous in soil and water, and included in the MAC as it is closely related to M. intracellulare. M. yongonense associated with pulmonary infections is another newly identified species also closely related to M. intracellulare (2, 7).

MAC may be found both in natural and man-made environment. Routes of infection with MAC are pulmonary by inhalation of aerosols, gastrointestinal by ingestion of contaminated water/food, and direct inoculation through trauma or existing wounds. MAC bacteria can cause chronic pulmonary infection in patients with pre-existing pulmonary disease (e.g. COPD), cervical lymphadenitis in immunocompetent children, localised infections after traumatic inoculation, and disseminated...
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disease in the severely immunocompromised, e.g. HIV-infected, transplant recipients and those on immunosuppressive chemotherapy. *M. avium* infection is more often associated with the last listed patients. Worldwide, *M. avium* is the causative agent in more than 95% of MAC infections in patients with AIDS but rarely causes lung diseases among them (8). On the contrary, *M. intracellulare* is a pathogen responsible for pulmonary diseases in immunocompetent individuals.

Worldwide, pulmonary disease caused by MAC appears to be increasing (9). There is no evidence of human-to-human transmission (3). The organisms are common in the environment and there is substantial evidence showing that the environmental niche is in biofilms lining suburban water pipes. Many MAC have been isolated from drinking water (9).

Currently, in the United States MAC isolates represent 26% of the total mycobacterial isolates (9). In the European Union (EU) and the European Economic Area (EEA) Finland is the only country with mandatory notification of NTM. Several countries have published reports on the nationwide prevalence (10). According to a recently published snapshot of NTM in pulmonary samples collected in 2008 from 30 different countries, MAC predominated in most of them, followed by *M. gordonae* and *M. xenopi* (10).

**OBJECTIVE:**
To determine the prevalence of MAC representatives among patients in Bulgaria suspected for tuberculosis for the period from 2010 to 2017.

**MATERIAL AND METHODS**
The study included patient isolates processed throughout the whole TB laboratory network in Bulgaria. Species identification was performed only in the National Reference Laboratory of Tuberculosis at the National Centre of Infectious and Parasitic Diseases (NRL TB, NCIPD). The quality of microbiological diagnosis was ensured through external quality assessment (EQA) at national level by NRL TB and by INSTAND e.V. on supranational level.

A total of 596 strains were specified as NTM. For the purpose of the study each patient was represented by a single strain. Strains were isolated from the following clinical specimens: sputum, bronchoalveolar lavage (BAL), gastric washes (in children), cerebrospinal fluid (CSF), throat swabs, surgery material and urine of patients suspected for tuberculosis. The laboratories use methods optimised for cultivation of *M. tuberculosis* complex: on a solid and/or liquid media, respectively, Löwenstein-Jensen and/or MGIT (BACTEC MGIT 960 TB system) according to the relevant standard operating procedures in national and international guidelines (11, 12). The main methods used for isolation and identification of NTM at the NRL TB were: Ziehl-Neelsen staining, culture on Löwenstein-Jensen and MGIT media with determination of colony morphology, pigmentation and photoreactivity, temperature dependence and speed of growth; resistance to the p-nitrobenzoic acid; absence of MRT64 antigen by BD MGIT TBc Identification Test and line probe assay (LPA) Geno Type® Mycobacterium CM and AS (14). LPA are PCR tests designed to identify the most common and relevant to human pathology NTM. Species identification was performed using LPA after DNA isolation from pure culture with subsequent amplification and reverse hybridisation to specific oligonucleotides immobilised on a membrane strip. Geno Type® Mycobacterium CM and Geno Type® Mycobacterium AS can be performed independently and also complement each other. Isolates defined as genus *Mycobacterium* by GenoType® CM, were tested with GenoType® AS.

**RESULTS AND DISCUSSION**
In the beginning of the study period there is a gradually increasing trend in the number of NTM/ MAC isolates until 2015. After that the number of isolates starts to decrease and reaches a plateau around 85 NTM per year. The total number of MAC isolates between 2010 and 2017 is 100 with a clear trend towards increase of NTM as a whole. The rise in NTM/MAC isolation could be a result of improved laboratory diagnosis. During the 2nd cycle of the External Quality Control evaluating the diagnostic proficiency of laboratories, NRL TB, NCIPD introduced quality assessment of culture-based diagnosis of tuberculosis, and in particular the differentiation between MTC and NTM strains. That may have made impact towards more
precise laboratory diagnosis. However, the ageing population with increasing prevalence of some forms of chronic lung diseases probably is another important factor as well.

**Figure 1.** Distribution of NTM/MAC strains during the study period.

NTM were isolated in 24 out of 30 microbiology laboratories performing culture-based diagnosis of tuberculosis in Bulgaria. In 9 (30%) of them MAC was not reported.

We observed a marked contrast in the number of NTM/MAC isolates between the capital and other country districts. Most of the patients are residents of Sofia and cities with large population like Plovdiv, Varna and Burgas. One of the reasons for the prevalence in urban areas may be the plumbing system and biofilm formation. NTM in biofilms are more resistant than those in natural water and can serve to repopulate plumbing pipes and instruments after disinfection.

**Figure 2.** Geographic distribution of NTM/MAC strains.
The processed specimens in which MAC were detected are presented in Fig. 3. About a quarter of them – 23% (n = 23, all sputum) were with positive smear microscopy.

Figure 3. Clinical specimen types in which MAC were detected.

The distribution of MAC by gender was similar to that of TB infection with slight male prevalence – 55% (n=55), female – 45% (n=45). However, most studies identify preponderance of NTM among females. A large population-based estimate of trends in NTM in the USA was conducted by Prevots et al. from 2004 to 2006. Their data showed that NTM surpassed TB with an increasing prevalence. They also found that the prevalence of NTM was 1.1-1.6-fold higher among women relative to men across the different states (13).

A study in Europe conducted by the NTM-NET collaborative group showed predominance of NTM diseases in female patients (14).

Patient age structure is presented in Fig. 4. Our results indicate that the overall prevalence of MAC is relatively steady among the age groups of up to 14 years (about 1%). Thereafter, it increases considerably and reaches about 15% for people at the age of 25-44 years. Most frequently MAC were isolated from population aged over 45 years – 82% (n = 82). In accordance with worldwide reports, our results demonstrate a clear age-related prevalence.

Figure 4. Age structure of patients with MAC isolates for the period 2010-2016.
During the eight-year study period in Bulgaria *M. intracellulare* showed prevalence rate of 56% (n=56) and *M. avium* ssp. representatives – 44% (n=44). Our results are dissimilar to those in other European countries where *M. avium* ssp. are predominant. Such ratio is proven to be typical of the Australian continent where its occurrence is explained with changes in the age and gender ratio of the population. One of the reasons for the observed results in this study may be the lack of detailed clinical information in our country. Therefore, differentiation between contamination of specimens and disease is difficult. *M. intracellulare* are ubiquitous in the environment and the environmental niche is in biofilms lining water pipes. Another reason may be the low incidence of *M. avium* ssp. among immunosuppressed individuals in Bulgaria such as patients with HIV, cystic fibrosis and transplant recipients.

According to a study conducted by the NTM-Network, the relative frequency of *M. intracellulare* versus *M. avium* in different parts of the world is diverse. A noteworthy difference is the relative prevalence of *M. avium* in North and South America, whereas *M. intracellulare* is most common in Australia (57% of all cultured mycobacteria and 80% of MAC) and South Africa (40% of all cultured mycobacteria and 77.5% of MAC). In Europe MAC was found with the largest share among NTM species – 37%, and 47% of them were confirmed as *M. avium* ssp., 31% *M. intracellulare* and only 22% were identified as part of MAC (14).

In contrast to the growing body of literature supporting the distribution of MAC among immunosuppressed patients and in particular those with HIV, in Bulgaria for a period of eight years (2010-2017) only one patient was confirmed to be HIV-positive and infected with *M. avium* ssp. In Bulgaria *M. intracellulare* isolates predominated. It should be noted that *M. intracellulare* was detected in 35% (n=5) of BAL specimens included in our study, all of them originating from one hospital. In these cases the possibility of contamination should be considered.

**CONCLUSION**

Although the present study is not population-based, it clearly shows a tendency towards increase in the number of NTM/MAC isolates in Bulgaria, which is in line with results observed elsewhere, and provides a snapshot of the predominant types of MAC in our country. A significant number of MAC isolates are *M. intracellulare*. The clinical significance of the increased MAC isolation from human samples observed in this study makes it difficult to interpret whether these isolates are associated with colonisation or disease. Additional studies,
including detailed clinical data, are needed to better understand the changes in MAC epidemiology. NRL TB, NCIPD has the capacity and is actively looking for identification of *M. chimaera*, but so far this species has not been registered.

**REFERENCES**