Preliminary Data Regarding the Nontuberculous Mycobacteria Isolation from Clinical Specimens, from Human Patients, in Iași County

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Abstract

The nontuberculous mycobacteria (NTM) represents a large group of ubiquitous environmental mycobacterial species, with more than 160 members that can pose a threat for human health, especially for the immunocompromised hosts. The aim of the current study was the collection of data regarding the frequency of NTM isolation from human clinical specimens, thus providing preliminary data regarding the infection prevalence in Iași County, Romania. Between May 2015 and March 2017 epidemiological data regarding NTM isolates in the Bacteriology Laboratory at the Clinical Hospital of Pneumology in Iași were analyzed. A total of 63 mycobacteria strains were isolated and identified as nontuberculous (by smear microscopy, culture on solid and liquid media and immunochromatographic assay). Out of the total number of samples that were submitted in the laboratory for the diagnosis of tuberculosis, during the studied period, 0.25% of them were identified as NTM. The prevalence for 2015 was estimated at 0.18%, and 0.34% for 2016. Given the challenging diagnostic procedures and the absence of notifiable status of NTM in most European Union countries, the epidemiological situation is broadly unknown. This emphasizes the need of regular recording and reporting of NTM isolation frequency for awareness of the risk of associated mycobacterial infections.

Keywords: *Nontuberculous mycobacteria, NTM, Mycobacterium*

Introduction

Related to the bacteria that causes human and animal tuberculosis (*Mycobacterium tuberculosis* complex-MTC) and leprosy (*M. leprae*), the nontuberculous mycobacteria (NTM) represents a large heterogenic group, with over 160 species officially recognized, less than a quarter being familiar to clinicians or microbiologists (Panagiotou *et al.*, 2014). Also referred to by other terms, such as atypical mycobacteria or MOTT- mycobacteria other than tuberculosis, their existence was recognized shortly after the discovery of tuberculous bacillus, at the end of the 1800s (Johnson and Odell, 2014).

The mycobacterial diseases have gradually gained importance and have come to the attention of researchers, veterinarians and human doctors, reported as etiologic agent of various diseases in a wide host range, from humans to numerous animal species, such as livestock, pets or wildlife. NTM are often isolated from the environment, mainly in water, soil or vegetation. Despite the fact that NTMs are characterized by moderate pathogenicity, the incidence of infections caused by it has currently registered an increase, especially in developed countries (Rindi and Garzelli, 2016). A rise in NTM isolation rates from patients with cystic fibrosis was recorded in Europe, in the

last years (Qvist *et al.*, 2015). *M. avium* complex (MAC), *M. goodnae*, *M. xenopi*, *M. intracellulare* and *M. fortuitum* are amongst the most commonly isolated NTM species, with implications in human pathology (van der Werf *et al.*, 2014; Hoefsloot, van Ingen *et al.*, 2013). Clinical manifestations include pulmonary disease, disseminated disease, lymphatic, skin, soft tissue or bone disease. Given the fact that person to person transmission is considered extremely rare, NTM disease is not reportable in most countries (Griffith *et al.*, 2007).

In our country, there is a significant lack of knowledge concerning the diversity and the incidence of the main NTM species involved in human and veterinary pathology. Thus, the aim of the current study was the assessment of preliminary data regarding NTM isolation from clinical specimens, in the Bacteriology Laboratory of the Clinical Hospital of Pneumology in Iasi, from June 2015 to March 2017.

Materials and Methods

The study was conducted over a period of 21 months, from June 2015 to March 2017, a total of 63 mycobacterial isolates were identified as NTM in the Bacteriology Laboratory at the Clinical Hospital of Pneumology, in Iasi. Aspects regarding

primary isolation, specimen diversity, patients' age range distribution and gender were examined.

The isolation procedure starts with the digestion and the decontamination of pathological products using modified Petroff's method, with 4% NaOH and HCl, in order to remove any undesirable contaminant. The obtained sediment is inoculated into Löwenstein Jensen (LJ) solid media (the current "gold standard") and on liquid media - a Middlebrook 7H9 Broth base with a fluorescent indicator, used by the automated system present in the laboratory: BACTEC MGIT 960 (Becton Dickinson and Company), in this way shortening the incubation time. In general, the incubation period is 2 month, at a temperature of 37°C. Each positive culture is then morphologically examined. Ziehl-Neelsen and fluorescent Auramine-Rhodamine stains are used for the microscopic examination of each clinical specimen.

NTM differentiation from the members of the *Mycobacterium tuberculosis* Complex was accomplished by using a simple and rapid immunochromatographic test - SD Bioline TB Ag MPT64 RAPID® (Standard Diagnostics, Inc., Yongin, South Korea), based on the identification of the MPT64 protein, secreted in the growth

Figure 1. Isolation rate of NTM

Figure 2. Isolation rate of NTM

Table 1. NTM primary isolation on media

Lowenstein-Jensen Medium				Liquid medium BBL-MGIT
21 days	30 days	45 days	60 days	
5	7	14	16	21

Table 2. Age range for positive NTM patients

Patients age range	<20	20-40	40-60	60-80	>80
No. of cases	0	5	18	30	10

phase and specific to the MTC members (Toihir *et al.*, 2011).

Results and discussions

For the considered period, the overall isolation rate of NTM out of the total samples recorded (n=25271) in the laboratory for diagnostic was 0.25%. There were 14 (0.18%) NTM isolates in 2015, 48 (0.34%) in 2016 and only one in the first three months of 2017. The values match the findings mentioned in the literature (van der Werf *et al.*, 2014). The monthly isolation rates vary from 0 to 1.33%, the last in May 2016 (Figure 1 and 2).

The majority of the samples (approx. 97%) were isolated from respiratory tract specimens, mainly sputum (70%), only two being extrapulmonary samples, pus and tissue biopsy. No acid fast bacilli were observed at the microscopic examination. Twenty one samples registered growth only in the automated system, on liquid medium, BBL-MGIT, and 37 only on LJ solid medium, only 5 of the samples presented growth on both media (Table 1.). More often, the growth on solid media was observed after 45-60 days of incubation, 38% of the NTM isolates were registered positive after two months at 37°C.

Approximately 83% of the samples originated from patients from Iasi County and the other 17%, from neighboring regions: Bacău (n=2), Vaslui (n=2) Neamț (n=4), Botoșani (n=1), Suceava (n=2). Most of the records were new cases (87%), 4 being registered in treatment monitoring and 4 as relapses.

The majority of the samples came from patients with age over 60 years old, 30% with age between 60 and 70 and the median age was 62.3 years (Table 2). From female patients were isolated 43% of the NTM and 57% from males.

Conclusions

NTM infections are not systematically reported by public health authorities and therefore epidemiological and surveillance data are not available, despite their importance in the diagnosis and management of these conditions. In view of the increasing incidence of infections caused by these bacteria, recorded in many countries, there is a need for evaluation of the NTM species involved in both human and veterinary pathology. The clinical significance of NTM isolation from human specimens is to be carefully evaluated by the clinicians, given the difficult interpretation whether the isolated species are the etiology of the disease or just due to colonization.

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