




# Epidemiology of Non-Tuberculous Mycobacteria isolated from clinical specimens in Madrid, Spain, from 2013 to 2017

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## Abstract

The epidemiology of non-tuberculous mycobacteria (NTM) in Spain is largely unknown because systematic reporting is not compulsory. The aim of our study was to describe the frequency and diversity of NTM species in our region and their distribution according to the source sample, gender, and age of the patients. We performed a multicenter study of all NTM isolated in 24 public hospitals in Madrid from 2013 to 2017. A total of 6,923 mycobacteria were isolated: 4535 (65.5%) NTM, and 2,388 (34.5%) *Mycobacterium tuberculosis* complex (MTB). Overall, 61 different NTM species were identified. The most frequently isolated species were *Mycobacterium avium* complex (47.7%), *M. lentiflavum* (12.2%), *M. goodii* (9.2%), *M. fortuitum* (8.9%), and *M. abscessus* (3.9%). Whereas MTB cases were stable during the study period, the number of NTM isolates increased considerably from 930 isolates in 2013 to 1012 in 2017; a sharp increase occurred in the last year. The rise in NTM isolates was mostly due to *M. lentiflavum*, *M. kansasii*, and *M. abscessus* mainly isolated from respiratory specimens in patients older than 60. The increase in isolation rate of NTM in our region is consistent with the increasing rates reported worldwide in the last decades. The rise in NTM isolates was mainly attributed to *M. lentiflavum* but it also should be noted the increasing of species with high pathogenic potential such as *M. kansasii* and *M. abscessus*.

**Keywords** Non-tuberculous mycobacteria · Epidemiology · Isolation rates · *Mycobacterium lentiflavum* · *Mycobacterium abscessus* · *Mycobacterium*

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## Introduction

Non-tuberculous mycobacteria (NTM) include all *Mycobacterium* species other than *Mycobacterium tuberculosis* complex and *Mycobacterium leprae*. These environmental bacteria are considered opportunistic pathogens, and several species are frequently associated with human disease. Although there are significant differences in geographic distribution of NTM, the most commonly isolated species are *Mycobacterium avium* complex (MAC), *M. goodii*, *M. kansasii*, *M. marinum*, *M. xenopi*, *M. fortuitum*, *M. chelonae*, and *M. abscessus* [1, 2].

In recent years, a considerable increase in NTM isolation rates has been observed worldwide [3–5]. In fact, NTM account for almost half of the total number of isolated mycobacteria [6]. The reasons for this increase are not entirely clear; it may be due to development of more sensitive molecular assays for NTM identification, but also to a true increase of NTM disease cases, or even to environmental changes in the habitats where these organisms are usually found [7].

Reporting NTM infection to public health authorities is not mandatory in most countries. Therefore, precise epidemiological data are lacking. Some European countries have published studies on the epidemiology of NTM showing an increase in NTM isolation [3, 5, 8, 9]. However, in Spain, studies about MNT human infections are scarce [10–12], and therefore the epidemiology of MNT infections remains unknown.

The aim of this study was to determine the frequency and diversity of NTM species in our region and their distribution according to the source sample, gender, and age of the patients.

## Methods

We retrospectively reviewed all laboratory reports from 15 microbiology laboratories that perform the laboratory diagnosis of mycobacterial diseases from 24 public hospitals in Madrid (mainly an urban area with a mean population of 6.640.705 inhabitants). All patients with at least 1 isolate of NTM obtained from clinical specimens during a 5-year study period from 1st of January 2013 to 31st of December 2017 were included in the study. MTB cases from the same hospitals and period were also registered for comparison purposes.

In the case of multiple consecutive positive cultures from the same patient, only the first isolate was included. Patients' information, including age, gender, and sample, was obtained from laboratory records.

All strains were isolated using liquid culture systems (BACTEC™ MGIT™ 960, BD, USA, and/or Bact/ALERT® MP, Biomérieux, France), and/or solid culture media (Löwenstein-Jensen). Species level identification was performed using matrix-assisted laser desorption ionization–

time of flight (MALDI-TOF) and/or molecular probes (Genotype CM/AS [Hain Lifescience, Germany]). In some cases, the strain was submitted to the National Microbiology Center for a detailed identification at species level.

Research ethics approval was not necessary for retrospective studies in our Institutions when clinical charts are not reviewed, and informed consent was not required as the data were analyzed anonymously.

## Results

During the study period, we identified 6.923 mycobacterial isolates from the same number of patients, 4535 (65.5%) were NTM and 2.388 (34.5%) were MTB. NTM isolates were obtained 2.061 for men and 2.474 for women (45.4 vs. 54.5%,  $p < 0.001$ ) with a mean age of 61.5 years (64.4 for men and 62.3 for women).

The distribution over time of the NTM and MTB isolates is showed in Fig. 1. Whereas MTB cases were stable during the study period, the number of NTM isolates increased considerably from 930 isolates in 2013 to 1012 in 2017; a sharp increase occurred in the last year.

Table 1 shows the isolation rates of the different NTM species identified in the study. A total of 61 different NTM species were isolated. The most frequently isolated species were MAC (47.7%), *M. lentiflavum* (12.2%), *M. goodii* (9.2%), *M. fortuitum* (8.9%), and *M. abscessus* (3.9%).

Figure 2 shows the distribution over time of the eight most prevalent species excluding MAC isolates that represented almost 50% of all MNT and which isolation rate was stable during the study period. Most species showed stable isolation rates; however, we observed that some species increased considerably during the study period. Particularly, the isolation rate of *M. lentiflavum*, the second most prevalent species, increased from 73 isolates in 2014 to 161 in 2017. Although *M. kansasii* only represented 1.5% of total MNT isolates, it should be noted that the number of isolates increased from 5 in 2013 to 22 in 2017. Regarding rapidly growing mycobacteria (RGM), *M. abscessus* also increased more than 2.5 times between 2013 and 2017.

NTM isolates were obtained mainly from respiratory samples (89.8%); sputum was the most common respiratory sample, followed by bronchial aspirate (BAS), bronchoalveolar lavage (BAL), and pleural effusion. Most extrapulmonary strains were isolated from urine, followed by lymph node biopsies, skin biopsies, and blood (Table 2).

When the data were analyzed by sex and age range, we observed that NTM infections were more common in women than in men (54.5 vs. 45.4%,  $p < 0.001$ ). Regarding to age distribution, NTM isolates were largely obtained from patients

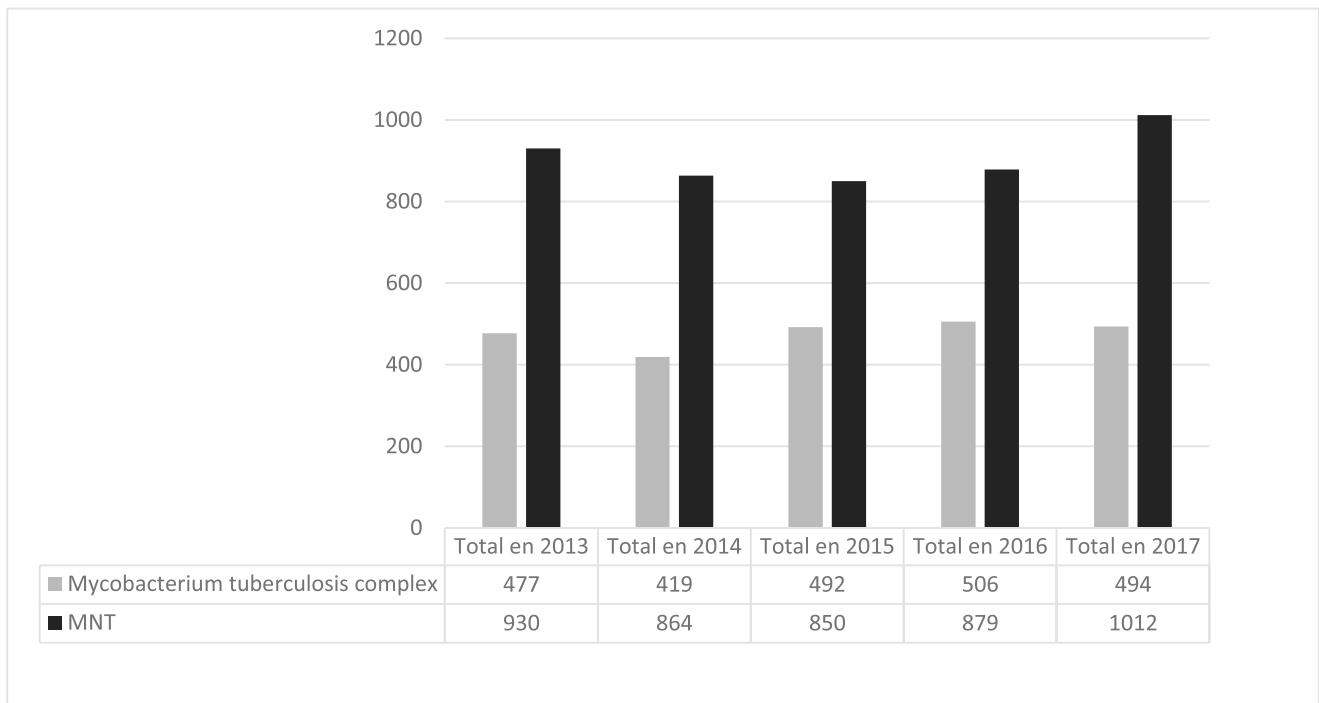


Fig. 1 NTM and TBC isolated during years 2013–2017

Table 1 Isolation rates

Species	2013	2014	2015	2016	2017	Total, (%)
MAC	453	429	408	417	457	2164 (47.7)
<i>M. lentiflavum</i>	112	73	101	108	161	555 (12.2)
<i>M. goodnae</i>	100	64	100	66	89	419 (9.2)
<i>M. fortuitum</i>	73	90	76	79	87	405 (8.9)
<i>M. abscessus</i> **	20	32	40	37	50	179 (3.9)
<i>M. xenopi</i>	23	31	28	22	26	130 (2.9)
<i>M. chelonae</i>	26	23	18	31	16	114 (2.5)
<i>M. mucogenicum</i>	14	17	21	20	18	90 (2)
<i>M. kansasii</i>	5	12	10	16	24	67 (1.5)
<i>M. celatum</i>	0	5	6	6	3	20 (0.4)
<i>M. simiae</i>	4	2	3	5	3	17 (0.4)
<i>M. malmoense</i>	2	3	0	2	8	15
<i>M. szulgai</i>	1	1	0	1	1	4
<i>M. genavense</i>	0	0	1	1	2	4
<i>M. interjectum</i>	0	2	1	1	0	4
<i>Mycobacterium</i> spp.	51	29	5	12	4	101
Other species*	46	51	32	55	63	247

\*Other species included: *M. mageritense*, *M. peregrinum*, *M. parascrofulaceum*, *M. cosmeticum*, *M. marinum*, *M. scrofulaceum*, *M. senegalense*, *M. rodhesiae*, *M. interjectum*, *M. porcinum*, *M. palustre*, *M. septicum*, *M. simiae*, *M. arupense*, *M. brisbanense*, *M. neoaurum*, *M. shinoidae*, *M. smegmatis*, *M. agri*, *M. austroafricanum*, *M. elephantis*, *M. gastri*, *M. holsaticum*, *M. kumamotoense*, *M. obuense*, *M. phocaicum*, *M. terrae*, *M. bohemicum*, *M. brumae*, *M. celeriflavum*, *M. chlorophenolicum*, *M. chobuense*, *M. colombiense*, *M. flavescens*, *M. fluoranthenvorans*, *M. hassiacum*, *M. immunogenum*, *M. moriokaense*, *M. nonchromogenicum*, *M. novocastrense*, *M. phlei*, *M. szulgai*, *M. thermoresistibile*, *M. triplex*, and *M. triviale*

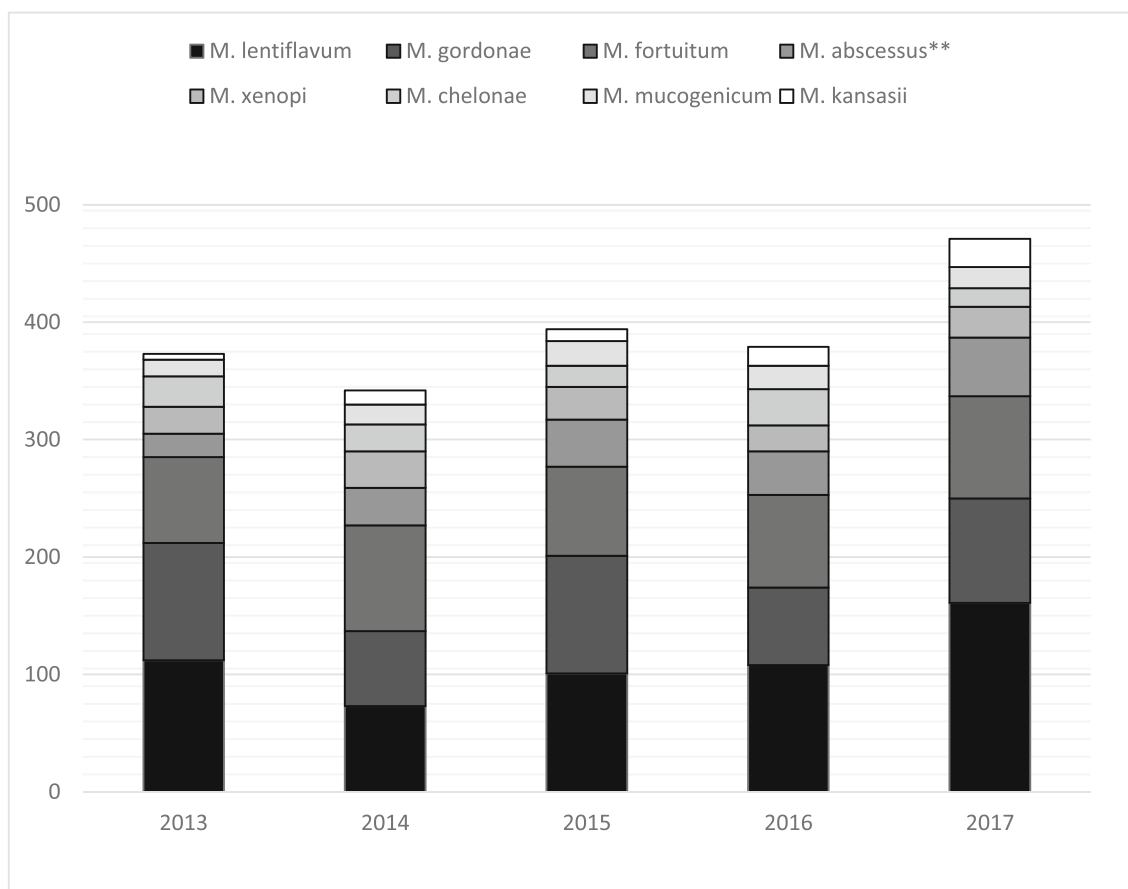
older than 60 years ( $n = 3151$ , 69.5%). The most commonly isolated species from patients aged 1–10 years old were MAC and *M. lentiflavum*.

### Discussion

NTM diseases detection and communication are not compulsory in Spain; therefore, the overall epidemiological situation of NTM in our setting is largely unknown. To the best of our knowledge, this is the largest study of NTM epidemiology conducted in Madrid (one of the most populated regions in Spain) during several years when the same microbiological protocols were used. Our data show that whereas the number of MTB cases was stable during the study period, an increase in isolation rate of NTM in our setting was detected, especially in the last year of the study.

More isolates of NTM were obtained from woman than from men, a data different from those of other series [3, 6, 13–14], but similar to other reports [4]. According to other previous studies, the highest number of isolates (approximately 70%) was obtained from respiratory samples of patients older than 60 years [14].

A total of 61 different NTM species were identified, and only 2% of the isolates could not be identified as valid species. MAC, *M. lentiflavum*, *M. goodnae*, *M. fortuitum*, and *M. abscessus* were the most frequently isolated species. The distribution was similar to that reported in previous European



**Fig. 2** NTM isolated during years 2013–2017. Only the eight most prevalent species (excluding MAC that showed stable rate of isolation) are individually reported with different column fillings

studies [3, 9, 10, 13], but differently to those reports, we found a high rate of *M. lentiflavum* detection. In fact, it was the second prevalent species, comprising 12% of all the NTM in our setting. In Europe, Portugal has also recorded high rates of *M. lentiflavum* isolates (6%) [8]. We have found that

*M. lentiflavum* isolates were obtained mainly from respiratory specimens where these mycobacteria are commonly considered a colonizer or contaminant. However, it should be noted that 11% of the isolates of this species were from lymph node biopsies, mostly in patients aged 1–10 years old. This observation is in agreement with recent studies [11, 15, 16] that describe *M. lentiflavum* as an important etiologic agent of cervical lymphadenitis in children.

Remarkably, *M. kansasii*, which is usually considered a pulmonary pathogen, increased considerably from 5 isolates in 2013 to 24 in 2017. Despite this increase, it only represents 1.5% of all MNT isolates, a difference with other geographical areas where this species is frequently isolated [8].

According to previous reports, we observed that *M. fortuitum* and *M. abscessus* were the most frequently isolated RGM [3, 9, 13]. Whereas the isolation rates of *M. fortuitum* remained stable during the study, the number of *M. abscessus* isolates gradually increased from 20 isolates in 2013 to 50 in 2017. This fact must be analyzed in the context of considering *M. abscessus* as an emerging pathogen [17], especially among some specific populations like cystic fibrosis patients, as many reports in the literature has shown in

**Table 2** NTM isolates according to source of sample

Source of sample	N° of isolates
Respiratory samples	4072
Sputum	3847
BAS	214
BAL	15
Pleural effusion	10
Extra respiratory samples	463
Urine	255
Lymph node	133
Skin biopsy	42
Blood	18
Other	15

the recent years [18, 19], even with the detection of hyperpathogenic clones that have been described worldwide [20, 21]. Because this is a retrospective microbiological study, we are not able to analyze neither the strains nor the clinical data, but the increase in the number of isolates of this pathogenic species could be a matter of concern for the next years.

It must be noted that Madrid is mainly an urban region with a continental climate. This must be taken into account for comparison purposes of MNT isolation rates with other regions of a rural environment that have wetter climates. Since NTM are environmental organisms, these data are of importance, because it has been speculated that changes in climate or in the water system disinfection could affect the number or frequency of the different isolated species [2, 7].

In conclusion, our data show an increase in NTM isolation rate in our region that is according to the general increase in NTM infections reported worldwide in the last decades. Unexpectedly, *M. lentiflavum*, which is considered a nonpathogenic environmental contaminant, was the second most commonly isolated species. The increase in NTM isolation frequency was mainly attributed to *M. lentiflavum*, but it also should be noted the increasing of *M. kansasii* and *M. abscessus*, common pathogens that could be of great importance in the next future.

Further studies involving the clinical significance of increased isolation rate of NTM from clinical samples to better understand changes in the epidemiology of NTM and its impact on clinical practice are needed.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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