

# Nontuberculous mycobacteria isolated from pulmonary specimens between 2004 and 2009: causative agent or not?

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## SUMMARY

Nontuberculous mycobacteria were identified from 45891 samples of 19553 patients with a prediagnosis of pulmonary tuberculosis between November 2004 and January 2009. Among 10041 (21.9%) culture positive samples, 208 (2.1%) pulmonary samples recovered from 77 individual patients were differentiated as mycobacteria other than tuberculosis (MOTT). Proportion of mycobacteria evaluated as causative agent for clinical infection were found as 0.16% (n=31), mostly *M. avium complex*, *M. abscessus* and *M. kansasii*. Additionally, *M. fortuitum-peregrinum complex*, *M. simiae*, *M. szulgai / intermedium* and *M. scrofulaceum* were found as causative agent in 2, 2, 2 and 1 patient, respectively. Identification of infections caused by environmental or opportunistic pathogen mycobacteria is required in rapid and accurate diagnosis, infection control and treatment planning of infections caused by *M. tuberculosis complex* and/or MOTT.

**KEY WORDS:** Atypical mycobacterium, LiPA, PCR based reverse hybridization, nontuberculous mycobacteria, mycobacteria other than tuberculosis

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Mycobacteria are found in soil, water, dust granules, domestic and wild animals, milk or other food materials. There are more than 120 identified mycobacterium species known to cause disease in humans (Tortoli, 2006; Lettieri, 2007). Atypical (nontuberculous) mycobacteria have been classified by Runyon (Runyon, 1959) as group I-photochromogens, group II-scotochromogens, group III-nonphotochromogens and group IV-fast growers. Humans can be infected from environment and colonization may occur on skin surface or in body secretions. *Mycobacterium tuberculosis* (MTB) complex strains are still the most common cause of my-

cobacterial infections around the world and opportunistic infections due to mycobacteria other than tuberculosis (MOTT) have been increasing as a result of a suppression in immunity such as AIDS or other systemic failures (Tortoli, 2006; Lettieri, 2007; Piersimoni and Scarparo, 2008). Pulmonary disease is the most common site of infection. Bronchial cultures should be obtained or patients should have at least 3 sputum specimens collected on separate days, and nontuberculous mycobacteria (NTM) should be confirmed by positive results in at least two of these three specimens. Among mycobacterial species, *M. avium-intracellulare complex*, *M. chelonae*, *M. abscessus*, *M. kansasii* and *M. xenopi* are the most common NTM infections reported (Martín-Casabona *et al.*, 2004; Tortoli, 2006; Griffith *et al.*, 2007; Lettieri, 2007). *M. gordonae* generally does not cause infections in human and it has been isolated as contaminant and differentiation from pathogenic species is important for diagnosis

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laceum (n=2) (2.6%), *M. lentiflavum* (n=2) (2.6%) (Table 1). Among the specimens, 14 isolates which couldn't be identified by INNO-LiPA and/or GenoType MTBC were taken to the further analysis by GenoType Mycobacterium AS (Additional species) and identified as *M. simiae* (n=6) (7.8%), *M. szulgai/intermedium* (n=3) (3.9%) and *M. lentiflavum* (n=2) (2.6%) and remaining three isolates (3.9%) were found as unidentified atypical mycobacteria (*Mycobacterium spp.*).

In the present study, 31 (0.16%) (27 male, 4 female, mean age: 53.90 SD: 12.28) patients were evaluated to be infected by an atypical mycobacterium as causative agent according to the

ATS/IDSA statement of diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases (Griffith *et al.*, 2007), while seven patients were considered as suspicious atypical infection (presumptive causative or contaminant) and taken to the further follow-up procedure clinically. The causative agents (n=31) isolated from the patients were *M. avium* complex (n=13) (41.9%) [*M. intracellulare* (n=10) and *M. avium* (n=3)], *M. abscessus* (n=5) (16.1%), *M. kansasii* (n=5) (16.1%), *M. fortuitum-peregrinum* complex (n=2) (6.5%), *M. simiae* (n=2) (6.5%), *M. szulgai / intermedium* (n=2) (6.5%) and *M. scrofulaceum* (n=1) (3.2%). Additionally, an isolate (3.2%) which couldn't be

TABLE 1 - Distribution of mycobacteria isolated from the patients evaluated as infection by a causative agent or contamination between 2004 and 2009<sup>1</sup>.

Mycobacterial Species	No. of isolates (n=77)			Routine susceptibility testing <sup>3</sup>
	Causative (n=31)	Contaminant (n=39)	Causative or Contaminant <sup>2</sup> (n=7)	
<i>M. gordonae</i> (n=17)	none	17	none	not applicable
<i>M. avium</i> complex (n=16)				
<i>M. intracellulare</i> (n=12)	10	1	1	1) CLA 2) MOX, LZD4
<i>M. avium</i> (n=4)	3	1	None	
<i>M. fortuitum-peregrinum</i> complex (n=11)	2	7	2	AK, IPM, DO, FQ, S / SXT, FOX, CLA, LZD
<i>M. abscessus</i> (n=7)	5	2	None	AK, DO, FQ, S / SXT, FOX, CLA, LZD
<i>M. kansasii</i> (n=7)	5	1	1	1) RIF 2) AK, CIP, CLA, ETB, RBT, SM, S/SXT, INH, MOX <sup>4</sup>
<i>M. simiae</i> (n=6)	2	2	2	1) RIF 2) AK, CIP, CLA, ETB, RBT, SM, S/SXT, INH, MOX <sup>4</sup>
<i>M. chelonae</i> complex (n=3)	None	3	None	AK, DO, FQ, S/SXT, FOX, CLA, LZD, TOB
<i>M. szulgai/intermedium</i> (n=3)	2	None	1	not applicable
<i>M. scrofulaceum</i> (n=2)	1	1	None	not applicable
<i>M. lentiflavum</i> (n=2)	0	2	None	not applicable
MOTT <sup>5</sup> (n=3)	1	2	None	not identified

<sup>1</sup>The patients were evaluated as pulmonary disease which meets the clinical and microbiologic criteria based on ATS/IDSA guideline (Griffith *et al.*, 2007).

<sup>2</sup>Number of isolates recovered from suspicious patients which have been taken to the follow-up procedure clinically. <sup>3</sup>Drugs recommended for in vitro susceptibility testing by ATS/IDSA official statement (Griffith *et al.*, 2007). <sup>4</sup>The first choice of the drug is shown in rank one and if resistance occurs against the first drug, routine susceptibility testing can be applied for the drugs expressed in the second rank. <sup>5</sup>Mycobacteria other than tuberculosis which couldn't be identified in species-level by means of commercial LiPA kit. Abbreviations used for drugs; AK: amikacin, CIP: ciprofloxacin CLA: clarithromycin DO: doxycycline, ETB: ethambutol, FOX: cefoxitin, FQ: fluoroquinolones, IPM: imipenem, INH: isoniazid, LZD: linezolid, MOX: moxifloxacin, RIF: rifampicin, RBT: rifabutin, SM: streptomycin, S/SXT: sulphonamide or trimethoprim-sulfamethoxazole, TOB: tobramycin.

identified by LiPA was reported as causative agent. The samples obtained from the other 39 patients were finalized as laboratory or environmental contamination. Besides these, we observed mixed mycobacterial patterns in culture media of several samples which were not taken to the study and confirmed as contamination acquired from laboratory and/or environment. All *M. gordonae* (the most common species identified) isolates were reported as contamination in accordance with the previous literature. Distribution of mycobacteria isolated from the patients evaluated as infection by a causative agent or contamination between 2004 and 2009 has been shown in Table 1 and LiPA patterns of atypical mycobacteria in Figure 1.

Infections caused by NTM species comprise a wide range of 0.5-35.0% among all mycobacterial infections (Ergin A and Hascelik G, 2004; Martín-Casabona *et al.*, 2004; Tortoli, 2006; Bicmen *et al.*, 2007; Griffith *et al.*, 2007; Lettieri, 2007). According to the previous data reported by Griffith *et al.* (Griffith *et al.*, 2007), *M. gordonae*

*ae* is the most common contaminant, *M. avium* complex is the most common causative in USA and both are the most frequent species found worldwide (and in our study in accordance with this data). Among the common species in our study, *M. abscessus* and *M. kansasii* are also widespread in the world. However, some rare mycobacteria such as; *M. fortuitum-peregrinum* complex, *M. simiae* (mostly in Southwest U.S. and associated with pseudo-outbreaks), *M. chelonae* complex, *M. szulgai/intermedium*, *M. scrofulaceum* (mostly in South Africa and uncommon in other areas) and *M. lentiflavum* have been isolated in our study as well.

Atypical mycobacterial infections cannot only be diagnosed by acid-fast staining and using classical anti-TB drugs causes ineffective treatment of such patients. Therefore, unnecessary usage of drugs and longer time of hospitalization may occur. In addition to the recovery of the same mycobacterium species from the repeating cultures of the patients, clinical data, chest x-ray and histopathologic findings also play an important

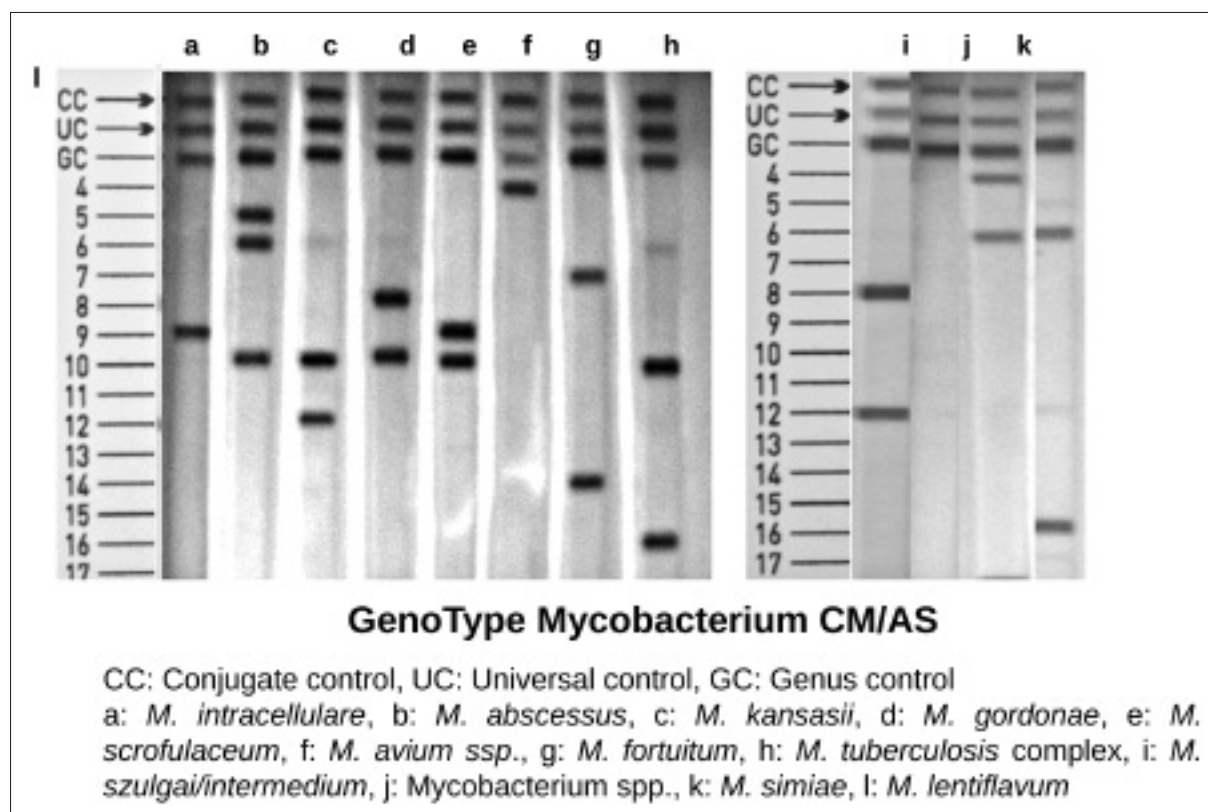


FIGURE 1 - LiPA patterns of atypical mycobacteria.

role in diagnosis of atypical mycobacterial infections.

The present study indicates that the frequency of atypical mycobacterial infections in West Anatolian Region (Aegean Region) is very low (0.16%) and mostly caused by *M. avium complex*, *M. abscessus* and *M. kansasii*. After NAP test, commercially available LiPA kits are useful in microbiological identification of the most NTM (>95%) in species level. However, newer versions of molecular assays in routine use may be necessary to detect and identify some rare atypical mycobacteria emerging. It is thought that direct molecular detection of atypical mycobacteria from patients' samples is not necessary for our region and the algorithm for laboratory diagnosis can be applied in two steps such as; molecular identification from positive culture by LiPA after an initial discrimination by BACTEC 460 NAP test. Thus, a molecular detection method which meets the cost effectiveness and high sensitivity for direct detection of *M. tuberculosis complex* from clinical samples would be better in routine use as well. A reliable diagnosis must be based on both a highly suspicious clinical picture and trustworthy microbiologic studies. In addition to these, all positive cultures should be highly investigated, especially with less common species or in species known to be common contaminants (*M. goodii*, *M. mucogenicum*, *M. terrae*, *M. kansasii*, *M. abscessus*). Therefore, identification of such environmental and opportunistic pathogen mycobacteria is essential in rapid, accurate diagnosis and treatment planning of infections caused by *M. tuberculosis complex* and/or MOTT.

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