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 mycobacterial 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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(Tortoli, 2006; Griffith *et al.*, 2007; Lettieri, 2007). However, surveillance data is limited and NTM infections are noncommunicable and therefore not reportable. Thus, the true prevalence is not fully known. Nevertheless, NTM infections have increased by 8% to 9% per year in several studies (Tortoli, 2006; Lettieri, 2007; Piersimoni and Scarparo, 2008). In a previous multicenter study (Martín-Casabona *et al.*, 2004), including 14 countries in different periods between 1976 and 1996, nonchromogenic mycobacteria were found to be predominant among 53 isolates from a laboratory in Turkey in 1995 and 1996 [i.e.; *M. fortuitum* (33.9%), *M. gordonae* (22.6%) and *M. avium* complex (9.4%)] as well as in Atlantic coast of Brazil, United Kingdom, Finland and Denmark. In another study reported from Turkey (Ergin A and Hascelik G, 2004), 63 (17.3%) of 364 mycobacterial species between 1997 and 2002 were identified as MOTT. However, only seven (1.9%) of 63 NTM patients were regarded as having clinical mycobacteriosis. As a part of the present report, identification of atypical mycobacteria isolated from suspicious tuberculosis (TB) patients in our hospital between November 2004 and June 2006 was reported previously (Bicmen *et al.*, 2007).

In the present study, it was intended to survey and identify causative or contaminant atypical mycobacteria to provide useful information about surveillance data for improvement of further diagnostic and/or medical strategies in our region. A total of 45891 samples obtained from 19553 patients were analyzed between November 2004 and January 2009. The study was undertaken in the Microbiology Laboratory of Training and Research Hospital for Chest Diseases and Chest Surgery, a regional reference hospital for tuberculosis and chest diseases in the Aegean (West Anatolian) Region of Turkey. All patients were evaluated as suspicious TB infection according to the clinical aspects and samples were submitted to the laboratory for isolation and identification of causative agent. After decontamination process (for sputum and nonsterile specimens), acid-fast staining and cultivation by BACTEC MGIT 960 system and in Lowenstein-Jensen medium were performed in all samples. When positive, the strains were evaluated for identification by conventional methods (such as pigment production and colony appearance on Lowenstein-Jensen slant, growth time and cord formation in microscopic examination). BACTEC 460 p-nitro- -acetylamino- - hydroxypropiophenone (NAP) test was used for differentiation of atypical mycobacteria from MTB complex in positive cultures. Additionally, commercially available PCR based reverse hybridization (Line Probe Assay=LiPA) kits were used for further identification of atypical mycobacteria in species level. Briefly, the assays included DNA isolation, mycobacterial 16S-23S rRNA spacer amplification and hybridization with specific probes. Results were evaluated visually on a reading chart according to the hybridization bands corresponding to the patterns of atypical mycobacteria on the strips. Interpretation and evaluation of test results and quality controls were performed according to the recommendation of the manufacturer. A conjugate control, a universal control and a *Mycobacterium* genus probe line were evaluated for quality control and validation of the assay. A blank amplified control sample was used as negative control for each test. The performance evaluation of these test systems was carried out by manufacturers in comparison with the reference method by using *Mycobacterium* strains (Innogenetics, 2003; Hain Lifescience, 2006). Overall diagnostic sensitivity, accuracies after initial and discrepancy testing and specificity were reported as 98.8%, 91.6%, 97.6% and 100%, respectively (Innogenetics, 2003).

During the study period, mycobacterial growth was positive in 10041 (21.9%) of the samples and 11 different mycobacterium species were identified from 208 (2.1%) samples [206 pulmonary (sputum, bronchial aspiration, bronchoalveolar lavage), 1 pleural fluid and 1 joint fluid)] of 77 patients (63 male and 14 female, mean age: 55.09 SD: 15.07). While repeated atypical mycobacterial growth was found in 36 (46.8%) patients on different days, 41 (53.2%) patients showed atypical mycobacterial growth only in one sample or in separate multiple samples obtained within the same day. Distribution of atypical mycobacteria among patients was found to be as follows: *M. gordonae* (n=17) (22.1%), *M. avium* complex (n=16) (20.8) [*M. intracellulare* (n=12) (15.6%), *M. avium* (n=4) (5.2%)], *M. fortuitum-peregrinum* complex (n=11) (14.3%), *M. abscessus* (n=7) (9.1%), *M. kansasii* (n=7) (9.1%), *M. simiae* (n=6) (7.8%), *M. chelonae* complex (n=3) (3.9%), *M. szulgai/intermedium* (n=3) (3.9%), *M. scrofu-*

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*[1].

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

[1]The patients were evaluated as pulmonary disease which meets the clinical and microbiologic criteria based on ATS/IDSA guideline (Griffith et al., 2007). [2]Number of isolates recovered from suspicious patients which have been taken to the follow-up procedure clinically. [3]Drugs recommended for in vitro susceptibility testing by ATS/IDSA official statement (Griffith et al., 2007). [4]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. [5]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 trimetophrim-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* 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

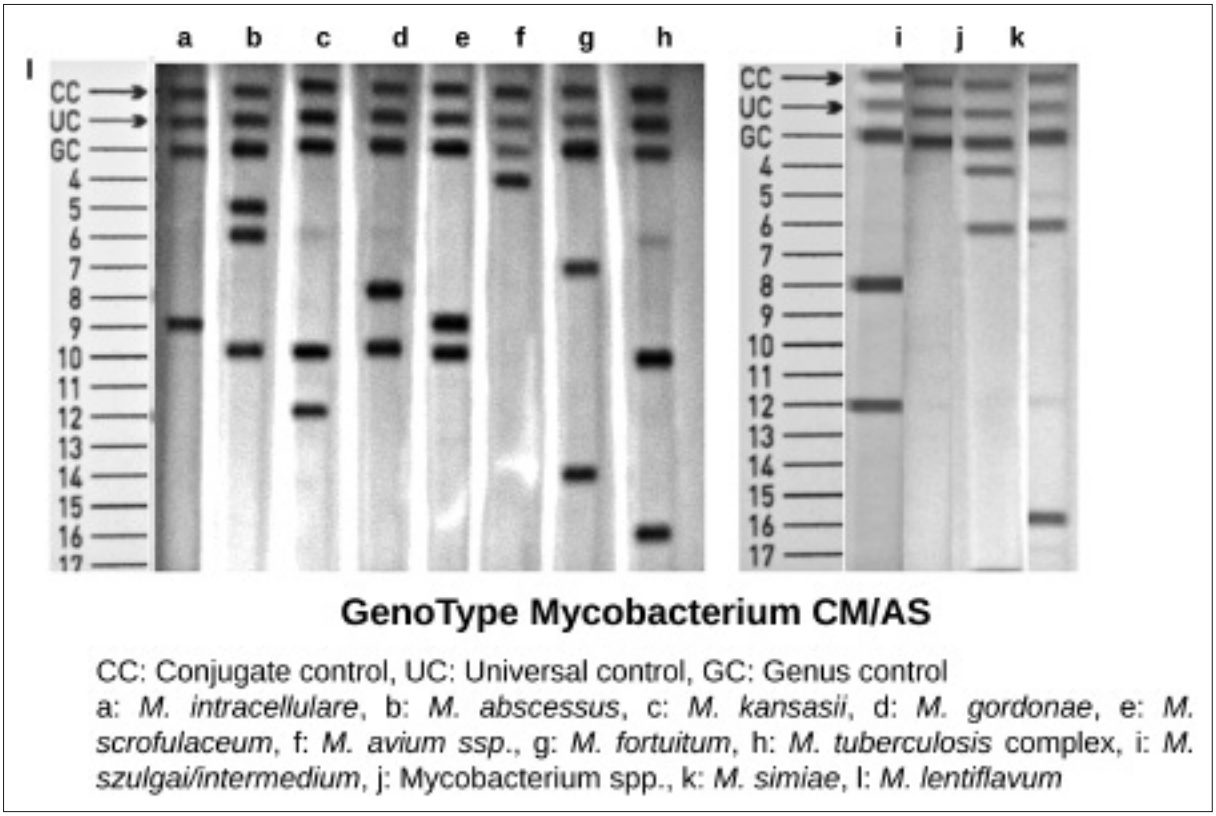

FIGURE 1 - *LiPA patterns of atypical mycobacteria.*

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. gordonae, 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.

REFERENCES

Bicmen C., Coskun M., Gunduz A.T., Senol G., Cirak A.K. and Tibet G. (2007). Identification of atypical mycobacteria isolated from clinical specimens by line probe assay (LiPA). *Mikrobiyol. Bul* **41**, 503-510. (Turkish).

Ergin A., Hascelik G. (2004). Non tuberculous mycobacteria (NTM) in patients with underlying diseases: results obtained by using polymerase chain reactionrestriction enzyme analysis between 1997-2002. *New Microbiol.* **27**, 49-53.

Griffith D.E., Aksamit T., Brown-Elliott B.A., Catanzaro A., Daley C., Gordin F., Holland S.M., Horsburgh R., Huitt G., Iademarco M.F., Iseman M., Olivier K., Ruoss S., von Reyn C.F., Wallace R.J. Jr, Winthrop K.; ATS Mycobacterial Diseases Subcommittee; American Thoracic Society; Infectious Disease Society of America. (2007). An Official ATS/IDSA Statement: Diagnosis, Treatment, and Prevention of Nontuberculous Mycobacterial Diseases. (2007). *Am. J. Respir. Crit. Care Med.* **175**, 367-416.

Hain Lifescience. (2006). GenoType Mycobacterium CM v1.0: instruction manual. Hain Lifescience GmbH, Nehren, Germany.

Innogenetics. (2003). Inno-LiPA Mycobacteria v2: instruction manual. Innogenetics N.V., Ghent, Belgium.

Lettieri C.J. (2007). Nontuberculous mycobacteria: update on diagnosis and treatment. CHEST 2007: *American College of Chest Physicians 73rd Annual Scientific Assembly*, Chicago, Illinois. (http://www.medscape.com/viewprogram/8523).

Martin-Casabona N., Bahrmand A.R., Bennedsen J., Thomsen V.O., Curcio M., Fauville-Dufaux M., Feldman K., Havelkova M., Katila M.L., Koksalan K., Pereira, MF., Rodrigues F., Pfyffer G.E., Portaels F., Urgell, J.R., Rusch-Gerdes S., Tortoli E., Vincent V., Watt B.; Spanish Group for Non-Tuberculosis Mycobacteria. (2004). Non-tuberculous mycobacteria: patterns of isolation. A multicountry retrospective survey. *Int. J. Tuberc. Lung Dis.* **8**, 1186-1193.

Piersimoni C., Scarparo C. (2008). Pulmonary infections associated with non-tuberculous mycobacteria in immunocompetent patients. *Lancet Infect. Dis* **8**, 323-334.

Runyon E.H. (1959). Anonymous mycobacteria in pulmonary disease. *Med. Clin. North. Am.* **43**, 273-290.

Tortoli E. (2006). The new mycobacteria: an update. *FEMS Immunol. Med. Microbiol.* **48**, 159-78.