Identification of non-tuberculous mycobacteria from clinical samples

Paola Molicotti[1§], Alessandra Bua[1§], Sara Cannas[1], Marina Cubeddu[1], Melania Ruggeri[1], Piero Pirina[2], Stefania Zanetti[1]

[1]Department of Biomedical Sciences University of Sassari;
[2]Clinic of Respiratory Diseases, University of Sassari, Italy;
[§]P.M. and A.B. contributed equally to this work

SUMMARY

Non-tuberculous mycobacterial infections cause morbidity worldwide. NTM are considered opportunistic pathogens, and several species have been associated with human disease which has typically pulmonary, skin and soft tissue, lymphatic or disseminated presentation.
This study evaluated the distribution of non-tuberculous mycobacteria in Sardinia. *Mycobacterium avium*, *Mycobacterium gordonae* and *Mycobacterium xenopi* were frequently found. Our results agreed with literature data both for the frequent isolation of *M. avium*, *M. xenopi* and *M. gordonae*, and the symptoms and radiological evidence of the patients analysed.

KEY WORDS: Disease, *M. avium*, *M. gordonae.*

Received January 14, 2013 *Accepted* June 19, 2013

Non-tuberculous mycobacteria (NTM) have been recovered in many parts of the world and from a variety of environmental reservoirs (Falkinham, 2002). The isolation of NTM and diagnosis of clinical diseases appear to be increasing owing to the availability of precise diagnostic standards and the growing numbers of persons at risk for NTM including immunocompromised hosts and elderly adults.
Considering the increasing importance of NTM in the clinical laboratory, this study evaluated the distribution of NTM in Northern Sardinia. From 2011 to 2012, 3000 patients were analyzed in the Laboratory of Mycobacteriology, University of Sassari, and the presence of NTM was confirmed in 21. In these subjects, evidence of the disease caused by NTM was confirmed according to all the criteria defined by the American Thoracic Society (Griffith *et al.,* 2007). Microscopic, nested-IS6110 PCR, *hsp65*PCR-restriction enzyme analysis and culture tests were carried out according to standard protocols (Ardito *et al.,* 2001; Devallois *et al.,* 1997).
9 (43%) NTM species were collected from females and 12 (57%) from males in an age range of 10-80 years. Twenty NTM were isolated from a total of 20 clinical samples collected from hospitalized Italian patients and 1 from a Chinese subject. Siteen patients had a pulmonary infection and NTM were isolated from 13 sputa (62%) and 3 gastric aspirates (14%). Five patients (24%) had an extra-pulmonary infection: one with mycobacterial skin infection (5%), 3 with cervical lymph node infection (14%) and one patient had a gastrointestinal infection (5%). Microscopic examination of specimens was negative, but it was positive when performed on positive cultures. Nested-IS6110 PCR, specific for *M. tuberculosis* identification, was negative in all positive cultures. Molecular identification with the hsp65 PCR-RFLP method indicated that three species of NTM were found prevalently: 5 *Mycobacterium avium* (24%), 5 *Mycobacterium gordonae* (24%)

Corresponding author
Alessandra Bua
Department of Biomedical Sciences
University of Sassari
Viale San Pietro, 43/b - 07100 Sassari, Italy
E-mail: ale.b76@email.it

and 5 strains of *Mycobacterium xenopi* (24%). Moreover we identified 2 strains of *Mycobacterium chelonae* (9%), 1 of *Mycobacterium alvei* (5%), 1 of *Mycobacterium marinum* (5%), 1 of *Mycobacterium szulgai* (5%) and 1 strain of *Mycobacterium intracellulare* (5%). Three strains of *M. avium* were isolated from lymph node biopsies collected from paediatric patients and 2 strains were isolated from sputum. *M. intracellulare* was isolated from sputum, *M. xenopi* was isolated from 2 sputum samples and 2 gastric aspirates and one stool sample. 4 strains of *M. gordonae* were isolated from sputum and one from gastric aspirate sample. *M. chelonae* was isolated from sputum such as *M. alvei* and *M. szulgai*, finally *M. marinum* was isolated from a skin biopsy (Table 1). For the patients with pulmonary infection clinical and radiological data were collected. Productive cough was the most frequently observed symptom (100%), followed by dyspnea (50%), haemoptysis particularly in patients with *M. xenopi* and *M. avium* infection (40%), fever (30%) and, to a lesser extent, fatigue and weight loss. All patients had a clinical history of long-term exposure to tobacco smoke and in one case previous pulmonary TB. A CT scan pattern suggestive of pulmonary emphysema was present in all patients, two also had a diagnosis of lung cancer, four a typical pattern of bilateral bronchiectasis and in five patients mono or bilateral pulmonary infiltrates were detected.

In this study 76% of the NTM were the cause of pulmonary infection. Patients with lung disease due to NTM are generally elderly adults (Piersimoni and Scarparo, 2008). In our study most cases of pulmonary infection were detected in patients aged between 67-80 years, except for a Chinese patient of 35 years who had a pulmonary infection due to *M. intracellulare*. In young subjects NTM lung disease may coexist or be facilitated by comorbidities such as cystic fibrosis (Hoiby and Pressler, 2006) or chest wall disorders (Iseman *et al.,* 1991). Moreover, other studies have emphasized the occurrence of infection in the setting of bronchiectasis (Fowler *et al.,* 2006) as found in our study where the younger patients with pulmonary NTM infection had bilateral bronchiectasis. Symptoms of NTM pulmonary infection include chronic cough and sputum production as observed in most cases of pulmonary disease in our study. Moreover most of our patients also showed haemoptysis in the infection due to *M. xenopi* and *M. avium*, and dyspnea in the infection caused by *M. gordonae*. These signs were less commonly observed and generally were associated with advanced NTM disease (Griffith *et al.,* 2007). Molecular identification indicated that three species of NTM were prevalently diffuse: *M. avium*, *M. xenopi* and *M. gordonae*. *M. avium* was isolated from two sputum samples of elderly adults and from three cervical lymph node biopsies of children. Lymph nodal infection is a typically infantile disease and recently the aetiology of cervical lymphadenitis has changed: *Mycobacterium scrofulaceum*, previously considered the prevalent cause of the dis-

TABLE 1 - *Number of NTM strains isolated in different clinical samples.*

NTM strains	*Sputum*	*Gastric Aspirate*	*Lymph nodes biopsies*	*Skin*	*Stool*
M. avium	2		3		
M. intracellulare	1				
M. xenopi	2	2			1
M. gordonae	4	1			
M. alvei	1				
M. marinum				1	
M. szulgai	1				
M. chelonae	2				

ease, has become quite rare, whereas *M. avium* has been isolated more frequently. We isolated only *M. avium* from cervical lymph nodes of children according to literature data. The pathogenicity of *M. xenopi* is low and host immune impairment is generally required for the onset of disease (Piersimoni and Scarparo, 2008). In our study patients affected by pulmonary infection by *M. xenopi* had emphysema and haemoptysis that is an uncommon event in the course of a general disease. In another patient *M. xenopi* was isolated from a stool sample. *M. gordonae* causes disease particularly in immunosuppressed individuals and there have been only rare reports of immunocompetent individuals developing disease (Mazumder *et al.,* 2010). All our patients with *M. gordonae* infection had a pulmonary abnormality including a history of infection with *M. tuberculosis* in one case and chronic bronchitis in the others. Moreover, patients had a common symptomatic manifestation including cough and dyspnea and the radiographic findings indicated consolidation and pulmonary infiltrates. Isolations of *M. gordonae* from cultures of sputum are more likely to represent a contamination (Griffith *et al.*, 2007); the microbiological, clinical and radiological evidence in our patients, as defined by the American Thoracic Society, demonstrated the disease (Griffith *et al.,* 2007). *M. chelonae* is a rare respiratory pathogen and more commonly causes skin and soft tissue infections. We isolated two strains of *M. chelonae* from sputum samples of two patients who had cough and sputum production and whose chest radiograph indicated parenchymal infiltrates. Three NTM species were found less frequently: *M. szulgai*, *M. alvei* and *M. marinum*. Rarely *M. alvei* is responsible for lung infection (Lee *et al.,* 2011), the patient with *M. alvei* infection was an elderly man with pleural effusion and heteroplasia.

The results of our work agreed with literature data both for the prevalent isolation of *M. avium*, *M. xenopi* and *M. gordonae* and for the symptoms and radiological evidence of the patients analysed. Interesting findings were the isolation of the two strains of *M. chelonae* from sputum samples and of a strain of *M. alvei* that rarely has been responsible of lung infection. Our data indicated that correct NTM identification is very important to avoid the under or over-evaluation of the role of a clinical isolate and not to miss significant pathogens.

REFERENCES

Ardito F., Posteraro B., Sanguinetti M., Zanetti S., Fadda G. (2001). Evaluation of BACTEC Mycobacteria Growth Indicator Tube (MGIT 960) automated system for drug susceptibility testing of Mycobacterium tuberculosis. *J. Clin. Microbiol.* **39**, 4440-4444.

Devallois A., Goh K.S., Rastogi N. (1997). Rapid identification of mycobacteria to species level by PCR-restriction fragment length polymorphism analysis of the hsp65 gene and proposition of an algorithm to differentiate 34 mycobacterial species. *J. Clin. Microbiol.* **35**, 2969-2973.

Falkinham J.O., III. (2002). Nontuberculous mycobacteria in the environment. *Clin. Chest. Med.* **23**, 529-551.

Fowler S.J., French J., Screaton N.Y., et al. (2006). Nontuberculous mycobacteria in bronchiectasis: prevalence and patient characteristics. *Eur. Respir. J.* 1204-1210.

Griffith D.E., Askamit T., Brown-Elliot B.A., et al. (2007). An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacteria diseases. *Am. J. Respir. Crit. Care Med.* **175**, 367-416.

Iseman M.O., Buschman D.L., Ackerson L.M. (1991). Pectus excavatum and scoliosis: thoracic abnormalities associated with pulmonary disease caused by *Mycobacterium avium* complex. *Am. Rev. Respir. Dis.* **144**, 914-916.

Hoiby N, Pressler T. (2006). Emerging pathogens in cystic fibrosis. *Eur. Respir. Mon.* **35**, 66-78.

Lee C.H., You H.L., Wang J.W., Tang Y.F., Liu J.W. (2011). Prosthetic joint infection caused by *Mycobacterium alvei* in an elderly patient. *J. Clin. Microbiol.* 3096-3098.

Mazumder S.A., Hicks A., Norwood J. (2010). *Mycobacterium gordonae* pulmonary infection in an immunocompetent adult. *North Am. J. Med. Sci.* **2**, 205-207.

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