Case Report

Pulmonary Disease due to *Mycobacterium massiliense*

Subhra Mitra[1], S. Roy Tapadar[1], D. Banerjee[1], S. Bhattacharjee[2], Sunanda Dey[3] and S. Kundu[2]

Department of Respiratory Medicine, Midnapore Medical College and Hospital[1], Institute of Postgraduate Medical Education and Research[2] and Super Religare Laboratories Limited[3], Kolkata, West Bengal, India

ABSTRACT

We report a case of a patient suffering from multidrug-resistant pulmonary tuberculosis (MDR-PTB) who later developed an invasive infection of the respiratory tract with a rapidly growing non-tuberculous mycobacteria (NTM), recently identified as *Mycobacterium massiliense*, closely related to *M. abscessus*. *To the best of our knowledge*, this is the first case of *M. massiliense* infection being reported from India. **[Indian J Chest Dis Allied Sci 2011;53:53-57]**

Key words: *Mycobacterium massiliense*, Non-tuberculous mycobacteria, Rapidly growing mycobacteria.

INTRODUCTION

Mycobacterium massiliense, a newly-described species of rapidly growing mycobacteria (RGM), is closely related to the *Mycobacterium chelonae–Mycobacterium abscessus* group.[1] First identified by Adekambi *et al*,[1] the name pertaining to Massilia, the Latin name of Marseille, where the organism was isolated. Since its first recovery from lung secretions in 2004,[1] it has been isolated from blood,[2,3] intramuscular injection sites[4] and surgical wounds.[2,5] It has been implicated as an invasive pathogen, a claim also supported by its close relation to *M. abscessus*, a known pulmonary pathogen.

CASE REPORT

A 60-year-old woman presented with a 10-year history of cough and mucoid/ mucopurulent sputum along with occasional haemoptysis. Shortness of breath had developed over the last two years. She received anti-tuberculosis treatment (ATT) in various courses in 2000, 2002 and 2003. She stayed in a Sanatorium from November 2004 to November 2006 where she was empirically treated with second-line anti-tuberculosis drugs.

Past history showed sputum-smear positive for acid-fast bacilli (AFB) in 2000, 2002, 2003 and 2004 and culture for *Mycobacterium tuberculosis* positive in 2002 and 2003. Details of mycobaterial isolation and treatments are presented in table 1. From January 2007 onwards, she was managed in our hospital.

This time, she was again found to be sputum-smear positive and culture positive for *M. tuberculosis* by BACTEC 460 TB method. Drug sensitivity testing showed the growth to be resistant to rifampicin, isoniazid and pyrazinamide. She was prescribed injection kanamycin, together with ofloxacin, ethionamide, ethambutol, cycloserine and PAS according to the drug susceptibility report and was admitted for the initial period of therapy. She was found culture negative for *M. tuberculosis* in September 2007. Kanamycin was stopped after eight months of therapy and oral anti-tuberculosis drugs were continued. She remained sputum-smear negative for AFB, attended the outpatients less regularly but reportedly continued her anti-tuberculosis drugs.

A re-evaluation in April 2009, prompted by recurrent symptoms, found her to be sputum-smear positive for AFB on two occasions. Culture for *M. tuberculosis* by the BACTEC 460 radioactive method grew non-tuberculous mycobacteria (NTM). *Mycobacterium* speciation by deoxyribonucleic acid (DNA) sequencing yielded *Mycobacterium massiliense*. Anti-tuberculosis drugs were stopped (her last anti-tuberculosis drugs course ran for two years and two months) and she was kept under observation. She remained sputum-smear positive for AFB and a sputum culture for mycobacteria grew NTM again in November 2009.

A repeat culture in April 2010 by BACTEC MGIT 960 (Fluorescent technology) grew RGM and *Mycobacterium* speciation yielded *M. massiliense*. This time a drug sensitivity study was done (Table 2). BACTEC drug susceptibility for *M. tuberculosis* was determined by following the modified proportion method. The critical proportion for resistance was taken as 1% for all the anti-tuberculosis drugs.

[Received: May 18, 2011; accepted after revision: September 8, 2011]

Correspondence and reprint requests: Dr Subhra Mitra, 603 Auroville, 10 Mandeville Gardens, Kolkata-700 019 (West Bengal), India; Phone: 91-033-24604465; E-mail: susami2@gmail.com

Table 1. Summary of isolations of acid-fast bacilli and treatments given

Period	Positive Smears*	Positive Cultures**		NTM Identification	Treatment		
		MTB	NTM		Drugs	Start of Treatment	Duration
2000–2001	2/3	ND			R, H, E		~6 months
2002	2/3	1/1			H,E,Ofx	April 2002	~10 months
2003	2/3	1/1			S,R,H,Z,E, R,H,Z,E	March 2003	~ 2 months ~ 6 months
2004–2006 (sanatorium)	18/24	0/1			Am, Z, E, Ofx, Eto Ofx, E, Eto	November 2004	~6 months ~18 months
2007–2008 (our department)	12/26	1/2 (DST done)			Km, Ofx, Eto, E, PAS, Cs	February 2007	~8 months
					Ofx, Cs E, Eto, PAS	November 2007	~18 months
April 2009	3/3		1/1	*M. massiliense*	None		
November 2009	3/3		1/1	ND	None		
April 2010	2/2		1/1	*M. massiliense*	None		
June–July 2010	2/2		DST done		Am, Do, Ofx, Clr	July 2010	~1 month
August 2010 March 2011	0/10				Do, Ofx, Clr	August 2010	~ 8 month

*=Positive smears/smears done; **=positive culture / cultures done; ND=Not done
MTB=*Mycobacterium tuberculosis*, NTM=Non-tuberculosis mycobacteria; DST=Drug sensitivity testing; R=Rifampicin; H=Isoniazid; E=Ethambutol; Ofx=Ofloxacin; S=Streptomycin; Z=Pyrazinamide; Am=Amikacin; Eto=Ethionamide; Km=Kanamycin; PAS=Para-aminosalycylic acid; Cs=Cycloserine; Do=Doxycycline; Clr=Clarithromycin

Table 2. Antimicrobial drug resistance pattern of the isolated strain of *Mycobacterium massiliense*

Drug	MIC (mcg/mL)	Pattern
Trimethoprin/sulfamethoxazole	0.25/4.75	Susceptible
Ciprofloxacin	0.12	Susceptible
Moxifloxacin	0.25	Susceptible
Cefoxitin	4	Susceptible
Amikacin	1	Susceptible
Doxycycline	0.12	Susceptible
Clarithromycin	0.06	Susceptible
Linezolid	1	Susceptible
Imipenem	2	Susceptible
Cefepime	1	Susceptible
Amoxicillin/clavulanic acid 2:1 ratio	2/1	Susceptible
Ceftriaxone	4	Susceptible
Minocycline	1	Susceptible
Tobramycin	1	Susceptible

The identification of isolate as NTM in April 2009 was done by BACTEC 460 and PNB method (sensitive/resistant to p-nitrobenzoic acid). Speciation of NTM was done by DNA sequencing utilising 16S rRNA[6,7] and hsp65[8] gene targets, and the bacteria was identified as *Mycobacterium massiliense*. Routine susceptibility testing for treatment of RGM is recommended.[9]

Drug susceptibility of NTM (Table 2) was done by broth microdilution method (MIC testing). Susceptibility testing and subsequent result interpretations are as per CLSI guidelines M24-Aa.[10]

There was a weight loss of 10 Kg since the start of her illness. Her nutritional status was poor with a body mass index of 10 (weight 24 Kg). She was pale and had clubbing with bilateral, scattered, coarse, mid-to-late inspiratory crackles on chest examination. On investigation, haemoglobin was 10.9 g/dL and erythrocyte sedimentation rate was 22mm in the first hour and white blood cell count was 7900/mm^3. Blood sugar, urea, serum creatinine, liver function and thyroid function tests were in the normal range. Serology for human immunodeficiency virus (HIV) was negative and CD4+ count was 253/mL.

A recent chest radiograph (postero-anterior view, Figure 1) (May 2010) showed fibrotic lesions with thin-walled cavities in both the lung fields. Compared with the radiograph done a year earlier, there was some increase in the size of the cavities. High resolution computed tomography of the thorax (Figure 2) in November 2009 showed bilateral tubular bronchiectasis and scattered fibrosis with thin-walled cavities in the right upper and middle and left lower lobes.

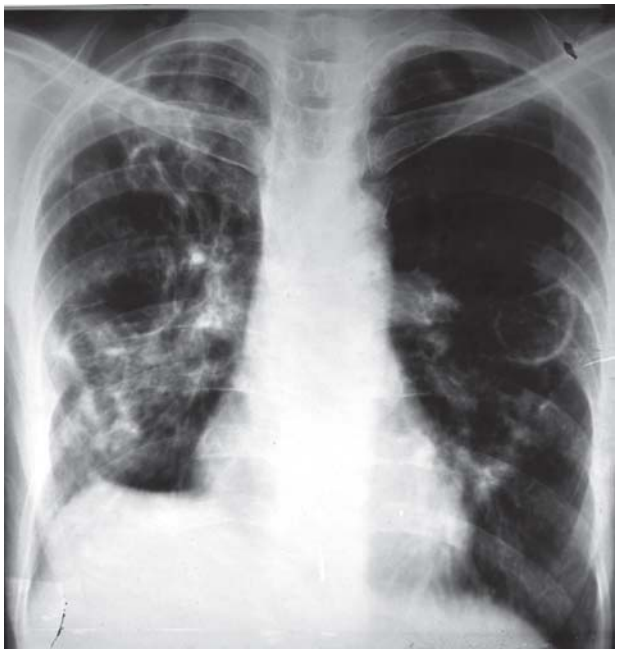

Figure 1. Chest radiograph (postero-anterior view, May 2010) showing fibrotic lesions with thin-walled cavitary lesions in both the lung fields.

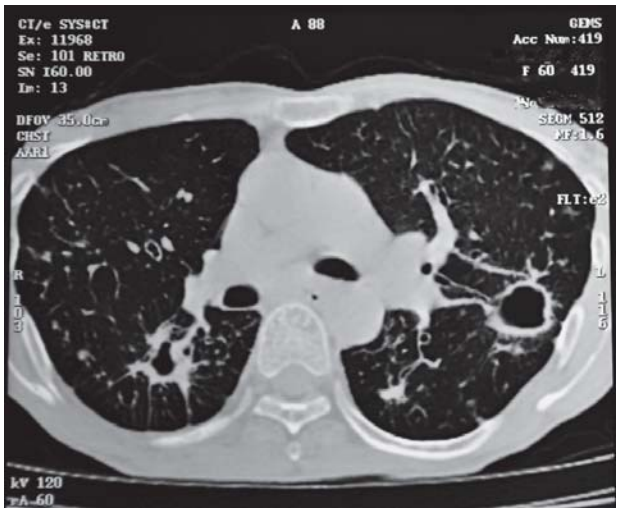

Figure 2. Computed tomography of the chest (November 2009) showing bilateral tubular bronchiectasis and scattered fibrosis with thin-walled cavities in the right upper and left lower lobes.

From July 2010 onwards, the patient was treated with injection amikacin and oral doxycycline, ofloxacin, clarithromycin (based on drug susceptibility report) on a body weight basis. After institution of therapy, there was a decrease in the volume, purulence and blood streaking of the sputum. Temperature remained in normal range but she developed tinnitus and vertigo one month later, requiring withdrawal of amikacin. Sputum-smear for AFB was negative at the end of the first and second months of therapy. On a review in March 2011, after 9 months of therapy, she had minimal symptoms, an increase in weight of 7 Kg, and was sputum-smear negative for AFB. Chest radiograph (PA view, Figure 3) showed fewer infiltrations and diminished size of the cavities in both the lung fields.

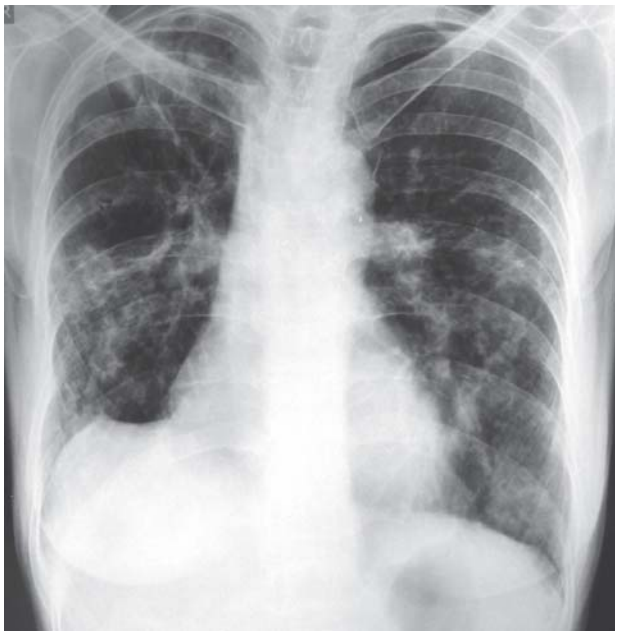

Figure 3. Chest radiograph (PA view, March 2011) showing reduced infiltrations and diminished cavity size in both the lung fields.

DISCUSSION

Currently, more than 125 NTM species have been catalogued,[9] an increase from approximately 50 in 1997. The partial 16S rRNA gene sequence analysis is the most widely-used method for identification of NTM and has led to the description of 40 new species since 1992.[1]

In a developing country like India, tuberculosis (TB) is a major health problem though NTMs are also being increasingly reported as causative agents for human infections.[11] While Jesudason and Gladstone[11] from south India reported an NTM isolation rate of 3.9%, Chakrabarthi *et al*[12] from Chandigarh documented an NTM isolation rate of 7.4% from various clinical specimens. *M. fortuitum* and

M. chelonae were the commonest isolates in these studies.

Chronic pulmonary disease is the most common clinical manifestation of NTM and it reflects the form of disease and co-morbidities rather than the species of NTM involved.[13] Our patient, an elderly thin-built woman, had prior TB, bronchiectasis and possibly gastro-oesophageal reflux disease as facilitating co-morbidities and presented clinico-radiologically as chronic pulmonary TB.

The radiographic features of NTM may be primarily fibrocavitary, similar to pulmonary TB, or characterised by nodules and bronchiectasis.[8] The cavities are thin-walled with greater pleural reaction and lesser surrounding parenchymal opacities than that seen in classic pulmonary TB.[9,13]

Isolation of NTM in culture is essential for the diagnosis of NTM lung disease. However, since the NTM are ubiquitous and may contaminate respiratory specimens, two or more positive culture results for NTM from separate expectorated sputum samples are required to make the microbiologic diagnosis clinically significant as per the American Thoracic Society guidelines.[9] In our patient, sputum culture was thrice positive for NTM and DNA speciation done twice yielded *M. massiliense*.

Colonisation without infection (i.e., no tissue invasion) is an unproven condition for NTM,[9] some preferring the term 'indolent disease' in that it may not be evident that NTM are causing progressive disease unless the patient is followed up for several years. The significance of NTM isolation from a patient during therapy for pulmonary TB is uncertain.[9] Co-existence of *M. tuberculosis* and NTM has been reported in both HIV-1 infected and non-infected patients.[14] In these situations NTM may be present only as a saprophyte (coloniser),[14] but both may also simultaneously cause disease requiring specific treatments for the two mycobacteria.[15]

Persistent symptoms with repeated isolation of the same pathogenic NTM species from respiratory specimens and progressive radiologic changes suggested disease rather than colonisation,[16] prompting us to treat our patient after a year of observation.

Rapidly growing mycobacteria are uniformly resistant to the "standard" anti-tuberculosis drugs and antibiotic susceptibility testing is, therefore, recommended for all clinically significant isolates. The treatment of the present patient was based on the drug-susceptibility report.

Koh *et al*[17] compared the clinical features and treatment outcomes between patients with *M. abscessus* and those with *M. massiliense* lung disease. The clinical and radiographic manifestations of disease caused by both species were similar. Response rates to combination antibiotic therapy including clarithromycin were higher in patients with *M. massiliense* than in those with *M. abscessus* lung disease.[17] Inducible resistance to clarithromycin found in *M. abscessus* isolates may explain this lack of efficacy.[17]

In our patient, sputum-smear conversion occurred after one month of therapy and has remained negative at eight months follow-up with a significant clinico-radiologic improvement.

Non-tuberculosis mycobacteria may be an under-recognised cause of AFB sputum positivity, especially in patients who have received treatment several times or have developed MDR-TB.

ACKNOWLEDGEMENTS

Authors are thankful for the assistance provided by Dr B.R. Das, Executive Director, Research and Development, and Head Molecular Diagnostics, Religare SRL Diagnostics, Mumbai and Dr Vidya Nerurkar, Senior Microbiologist, Religare SRL Diagnostics, Mumbai.

REFERENCES

1. Adékambi T, Reynaud-Gaubert M, Greub G, Gevaudan MJ, La Scola B, Raoult D, *et al*. Amoebal coculture of "Mycobacterium massiliense" sp. nov. from the sputum of a patient with hemoptoic pneumonia. *J Clin Microbiol* 2004;42:5493-501.
2. Simmon KE, Pounder JI, Greene JN, Walsh F, Anderson CM, Cohen S, *et al*. Identification of an emerging pathogen, Mycobacterium massiliense, by rpoB sequencing of clinical isolates collected in the United States. *J Clin Microbiol* 2007;45:1978-80.
3. Tortoli E, Gabini R, Galanti I, Mariottini A. Lethal Mycobacterium massiliense sepsis, Italy *(Letter to the Editor). Emerg Infect Dis* 2008;14:984-5.
4. Kim HY, Yun YJ, Park CG, Lee BH, Cho YK, Park BJ, *et al*. Outbreak of Mycobacterium massiliense infection associated with intramuscular injections. *J Clin Microbiol* 2007;45:3127-30.
5. Viana-Niero C, Lima KV, Lopes ML, da Silva Rabello MC, Marsola LR, Brilhante VC, *et al*. Molecular characterization of Mycobacterium massiliense and Mycobacterium bolletii in outbreaks of infections after laparoscopic surgeries and cosmetic procedures. *J Clin Microbiol* 2008;46:850-5.
6. Shrestha NK, Tuohy MJ, Hall GS, Reischl U, Gordon SM, Gary W, *et al*. Detection and differentiation of mycobacterium tuberculosis and nontuberculous mycobacterial isolates by real-time PCR. *J Clin Microbiol* 2003;41:5121-6.
7. Park H, Jang H, Song E, Chang CL, Lee M, Jeong S, *et al*. Microbiology, detection and genotyping of mycobacterium species from clinical isolates and specimens by oligonucleotide array. *J Clin Microbiol* 2005; 43:1782-8.
8. Telenti A, Marchesi F, Balz M, Bally F, Bottger E, Bodmer T. Rapidly growing mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis. *J Clin Microbiol* 1993;31:175-8.
9. American Thoracic Society/Infectious Disease Society of America. An official ATS/IDSA statement: diagnosis,

treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med* 2007;175:367-416.
10. National Committee for Clinical Laboratory Standards. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes. Approved Standard. Wayne, PA: NCCLS; 2003. Document No. M24-A.
11. Jesudason MV, Gladstone P. Nontuberculous mycobacteria isolated from clinical specimens at a tertiary care hospital in south India. *Indian J Med Microlbiol* 2005;23:172-5.
12. Chakrabarthi A, Sharma M, Dubey ML. Isolation rates of different microbial species from Chandigarh. *Indian J Med Res* 1990;91:111-4.
13. Glassroth J. Pulmonary disease due to nontuberculous mycobacteria. *Chest* 2008;133:243-51.
14. Epstein MD, Aranda CP, Rom WN. The significance of Mycobacterium avium complex cultivation in the sputum of patients with pulmonary tuberculosis. *Chest* 1997;111; 142-7.
15. Davidson PT. The diagnosis and management of disease caused by M avium complex, M kansasii, and other mycobacteria. *Clin Chest Med* 1989;10:431-43.
16. Field SK, Cowie RL. Lung disease due to the more common nontuberculous mycobacteria. *Chest* 2006;129: 1653-72.
17. Koh WJ, Jeon K, Lee NY, Kim BJ, Kook YH, Lee SH, *et al*. Clinical significance of differentiation of Mycobacterium massiliense from Mycobacterium abscessus. *Am J Respir Crit Care Med* 2011;183:405-10.

THE INDIAN JOURNAL OF CHEST DISEASES AND ALLIED SCIENCES

Vallabhbhai Patel Chest Institute, University of Delhi, Delhi-110 007

Director and Editor-in-Chief	:	**Prof. S.N. Gaur**
Place of Publication	:	Vallabhbhai Patel Chest Institute, University of Delhi, Delhi
Web Address	:	**http://www.vpci.org.in**
ISSN No.	:	0377-9343
Language	:	English
Periodicity	:	Quarterly (Published in the months of January, April, July and October)
Method of Printing	:	Offset Process
Overall Size	:	27.5 cm x 20.5 cm
Print Area	**:**	**24.0 cm x 17.0 cm (Advertisement materials required in Print Area size only)**
Spectrum of Readership	:	The readership comprises faculty members of medical colleges/research institutions, chest physicians, cardiologists, medical practitioners, postgraduate students, research scholars, biomedical scientists and libraries of medical colleges/hospitals; including 800 fellows/members of the National College of Chest Physicians (India)
Countries where it is Circulated	:	India, USA, Canada, South American countries, USSR, UK, The Netherlands, Germany, Italy, African countries, Middle East, Japan, China, Australia and New Zealand
International Standing	:	Abstracted and indexed in the globally reputed abstracting periodicals such as Index Medicus, Medline, IndMed, INSEAR, and Ulrich's Directory, etc

ADVERTISEMENT RATES*(w.e.f. 1st January 2011)*

	Per Issue	*Per Volume (4 issues)*
Ordinary Full Page	₹ 8,000.00	₹ 25,000.00
Back Cover	₹ 15,000.00	₹ 55,000.00
Inside Cover	₹ 12,000.00	₹ 45,000.00
Facing Contents	₹ 15,000.00	₹ 55,000.00
Colour Ads	**50% extra on above rates**	

Note:* 1. ***The above rates are not subject to any Tax Deduction at Source.*

2. *Advertisement materials (four positives in case of colour ads) are to be provided by the advertiser in a CD with a print out (hard copy) of the advertisement.*
3. *A discount of 10% will be offered to Advertising Agencies only.*
4. ***Advertisement Payments** (along with the space order and advertisement material) should be made in **advance** through **Banker's Cheque / Bank Drafts** only and should be drawn in favour of the **DIRECTOR, V. P. CHEST INSTITUTE, DELHI.***

E-mail: publication@vpci.org.in and ijcdas@yahoo.co.in; Fax: 91-11-27666549; Phone: 91-11-27667102, 27667667, 27667441 Extn 135