Original Article

Prevalence of Nontuberculous Mycobacteria in Patients Suspected of Tuberculosis and Drug Resistant Tuberculosis

Megha Sharma[1], Gaurav Sharma[2], Ravi Prakash Shaliwal[3], Shipra Bhargava[4], Bharti Malhotra[5]

[1]PhD Scholar, [2,4]Microbiologist FIND, [3]Technical Officer FIND, [5]Professor, Department of Microbiology, SMS Medical College, Jaipur, Rajasthan, India

DOI:10.37821/ruhsjhs.5.4.2020.326

ABSTRACT

Introduction: Nontuberculous mycobacteria (NTM) are being increasingly recognized as pathogens of importance. NTM cause disease similar to tuberculosis (TB) and are resistant to anti-tubercular drugs, so may present as drug resistant TB (DRTB). There is a lack of data on prevalence of NTM. The present study was aimed to detect the prevalence of NTM in pulmonary specimens of patients suspected of TB/DRTB.

Methodology: A total of 18,334 pulmonary samples received in Mycobacteriology laboratory during January 2018 to December 2018 from TB/DRTB suspect patients were cultured on Mycobacteria growth indicator tube (MGIT) 960 and Lowenstein Jenson (LJ) media. The culture and acid fast bacilli (AFB) smear positive samples were subjected to SD Bioline TB-Ag MPT 64 rapid test to differentiate between *Mycobacterium tuberculosis* (*MTB*) and NTM.

Results: Among 18,334 samples, Mycobacteria were grown in 6653 (36.2%) samples among which 6593 (99.1%) were *M tuberculosis* and 65 (0.9 %) were NTM, co-infection of *MTB* and NTM was found in 7.6%(5/65) samples. LJ isolated 6 (9.2%) additional NTM than MGIT while MGIT isolated 23 (35.3%) additional NTM than LJ.

Conclusion: Yield for NTM was higher in MGIT than LJ but on LJ separate colonies of NTM and *MTB* could be seen and helped in identifying co-infection which was missed on MGIT. It is important to repeatedly identify same species from these patients to establish pathogenicity. However, there was very poor follow up from clinician/ patients to submit new sample, which should be stressed upon them.

Keywords: Drug resistant tuberculosis (DRTB), *Mycobacterium tuberculosis* (*MTB*), Nontuberculous mycobacteria (NTM).

INTRODUCTION

Nontuberculous mycobacteria (NTM) are a group of Mycobacterium species other than *Mycobacterium tuberculosis* (*MTB*). These mycobacteria can be found in soil and aquatic environment.[1] NTM were considered mostly as colonizers or ignored as environmental contaminants in the past, but are now increasingly recognized as important pathogens in both immuno-compromised and immuno-competent persons.[2] Till 18th August 2020, 233 species of NTM have been identified worldwide.[3] It has been reported that NTM has been isolated from various medical equipment and environment such as drinking water pipelines, water tanks, hospital faucets and ice machines, diagnostic laboratories, hospital ice, potting soil, house dust, shower aerosols, and hot-tub aerosols.[4] These are also reported to cause surgical-site infections, post-injection abscesses, osteomyelitis, catheter-related blood-stream infections, and central nervous system infections.[5] The detection of NTM is very important since the failure to detect NTM infection has led to mistaken treatment for tuberculosis.[6] With the increase in DRTB, there is a growing concern that NTM infections could be misdiagnosed as TB/DRTB, as disease caused by NTM usually does not respond to anti-tubercular medication which may be species specific.[7,8]

Studies have shown that the prevalence of NTM has increased in not only high burden but other low burden countries of tuberculosis also.[6,7] Over the past decades, the pulmonary NTM are being increasingly reported

worldwide including India.[9] The isolation rate of NTM from India has been reported to be ranging from 0.5% to 8.6%.[10] Multidrug resistant tuberculosis has been reported to be confused with disease caused by NTM in upto 11% of the cases.[11,12] There is very limited information about the prevalence of NTM infection in India as NTM disease is not a reportable condition and there is lack of awareness among clinicians coupled with lack of laboratory capacity to diagnose these infections.[6] Therefore, investigating the prevalence of NTM infection is need of the day. Therefore, the objective of the present study was to determine the prevalence of NTM among TB and DRTB suspects in Rajasthan.

METHODS

This observational study was conducted in Mycobacteriology Lab, SMS Medical College, Jaipur and carried out during January 2018 to December 2018. All sputum samples of TB and DRTB suspects received in Mycobacteriology lab were included in the study. This study was approved by the Institutional Ethics Committee.

All the samples were subjected to smear microscopy by Ziehl Neelsen (ZN) staining method, decontamination by N-acetyl-L-cysteine-sodium hydroxide (NaALC-NaOH) method as per Revised National Tuberculosis Control Program (RNTCP) guidelines. Briefly, an equal volume of 0.5% N-acetyl-cysteine (NALC) solution was added to each sputum sample, tube was tightened and vortexed for 15-30 seconds until the sample was liquefied. Specimens were then incubated at room temperature (20-25°C) for 15 minutes followed by addition of 50 ml of phosphate buffered saline (PBS) pH 6.8 for neutralization and subsequently centrifuged at 3000 × g for 20 minutes at 4-12°C. The sediments were resuspended in 2.5 ml PBS after discarding the supernatant.[13] 2-3 drops of the sediment were inoculated onto LJ, which was incubated for 8 weeks at 37 °C and read weekly. If there was no growth after 8 weeks on LJ it was considered as negative, 0.5 ml of the sediment was inoculated into MGIT tube as per protocol.[14] MGIT culture not showing any growth after 6 weeks of incubation was considered negative. The positive MGIT was processed for ZN staining to confirm presence of acid fast bacilli (AFB). Culture positive samples from LJ/MGIT were subjected to SD Bioline TB Ag MPT 64 Rapid test.

SD Bioline TB Ag MPT 64 Test: Culture positive growth from MGIT and LJ were subjected to SD Bioline TB Ag MPT 64 Rapid test to discriminate between *MTB* and NTM. MPT 64 negative were con-firmed as NTM.[15] Briefly, 100 µl of positive liquid culture / 2 or 3 colonies dissolved in 200 µl extraction buffer were added in sample well. Results were obtained within 15-20 minutes. Presence of control band indicates the validity of the test as shown in figure.

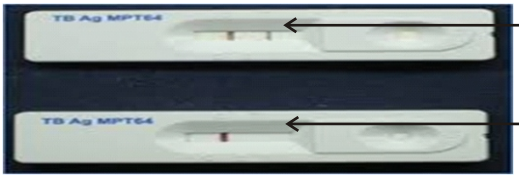

Figure: SD Bioline TB Ag MPT 64 Test.

RESULTS

Out of the total 18334 samples, growth of Mycobacteria was obtained in 6653 (36.2%) cases among which 6593 (99.1%) were *M tuberculosis* and 65 (0.9%) were NTM. Co-infection of *MTB* and NTM was found in 7.6% (5/65) samples. LJ isolated 6 (9.2%) NTM additional to MGIT while MGIT isolated 23 (35.3%) NTM additional to LJ (Table 1). Among 65 NTM, 56.9% (37/65) were rapid grower and 43% (28/65) were slow growers; 12/65(18.4%) had pigmented and 16/65 (24.6%) non pigmented colonies.

Table 1: Total sample processed in MGIT/LJ

	MGIT	LJ	Total
MTB Positive	6580 (35.8%)	5960 (32.5%)	6593 (35.9%)
MTB Negative	11299 (61.6%)	12048 (65.7%)	11392 (62.1%)
NTM	59 (0.32%)	42 (0.22%)	65 (0.35%)
Contamination	396 (2.1%)	284 (1.5%)	284 (1.5%)
Total	18334	18334	18334

Among 65 NTM isolated, 8 (12.3%) were isolated from smear negative TB suspects, 26 (40%) from smear negative DRTB suspect cases, and 31(47.6%) from smear positive DRTB suspects (Table 2).

DISCUSSION

The epidemiology and prevalence of TB is well documented but prevalence of NTM is not well established

Table 2: Profile of patients enrolled in the study

	MTB Positive	NTM	Culture Negative	Contamination	Total
Smear positive	88	0	3	2	93
TB suspect	(94.6%)	(0%)	(3.2%)	(2.1%)	
Smear negative	161	8	4094	67	4330
TB suspect	(3.7%)	(0.18%)	(94.5%)	(1.5%)	
Smear positive	5296	31	946	103	6376
DRTB suspect	(83.1%)	(0.48%)	(14.8%)	(1.6%)	
Smear negative	1048	26	6349	112	7535
DRTB suspect	(13.9%)	(0.3%)	(84.3%)	(1.5%)	
Total	6593 (35.9%)	65 (0.35%)	11392 (62.1%)	284 (1.5%)	18334

due to scarcity of NTM reports from the developing countries with high TB burden. Only a handful of studies have been published from India.[16-25] In the present study, the prevalence of NTM among TB suspects was low (0.9%). Variable prevalence of NTM (0.38-27.4%) has been reported from India.[16-25] Four large TB centers in India have also reported low NTM prevalence among TB/DRTB suspects, 0.38% at Pondicherry,[18] 0.8% at Mumbai,[20] 0.38% at Delhi[21], and 0.77% in North India.[25] A possible explanation for the low NTM disease prevalence in these studies and in the present study can be that the samples were collected from a larger population as these healthcare facilities cover large number of districts in their respective states. Whereas, two Indian studies reported higher NTM prevalence (3.9% and 9.9%).[19,22] A study from Delhi[26] isolated 1.4% NTM from presumptive tuberculosis patients among which 1.8% and 1.3% NTM were isolated from MGIT and LJ culture, respectively and most of them (37%) were from sputum. Variable prevalence of NTM has been reported worldwide, Zambia reported 15% for NTM and 0.2 % were *MTB*/NTM co-infected among these.[27] Iran reported 15.1% NTM among TB suspects.[28] Culture positivity may be affected by other factors; in smear positive cases mycobacterial growth was not 100% as the growth may be affected due to harsh decontamination, previously treated cases may be smear positive but growth is not obtained as the dead bacteria remain smear positive for some time, moreover the incubation in the present study was at 37^0C only while some NTM have special temperature requirements; *M ulcerans* and *M marinum* requires incubation at 29°C and 33°C but *M ulcerans* requires low (2.5%) oxygen concentration, *M xenopi* requires 42°C to 45°C. Similarly NTM require specialized media and culture only on LJ or MGIT medium may affect positivity rate adversely. *M haemophilum* grows best at 28°C to 30°C on solid medium with hemin or hemoglobin as an iron source. *M genavense* grows best in liquid medium supplemented with Mycobactin J, at an acidic pH (pH 5.5). Specialized media like Paraffin slide culture, Rapidly growing mycobacteria (RGM) medium, Middle- brook 7H11 agar, Burkholderia Cepacia selective agar (BCSA) have been reported to enhance recovery rate of NTM.

In the present study, we found co-infection of NTM and *MTB* in 7.6% cases. USA reported 35% co-infection with *MTB*.[29] Case of co-infection was also reported from Chandigarh, in a case with subretinal granuloma having infection with *Mycobacterium tuberculosis complex, Mycobacterium fortuitum,* and *Mycobacterium bovis* in an immuno-suppressed, non-HIV patient.[30] A study from sub-Himalayan region of North India reported two patients with multidrug-resistant (MDR)-TB and NTM detected by real-time polymerase chain reaction (PCR).[31]

In the present study, we isolated 9.2% NTM from LJ and 35.3% from MGIT. MGIT has been reported to give better yield for mycobacteria than LJ.[13] Moreover NTM MTB co-infection could be identified on LJ but not on MGIT. In TB treatment program, patients are managed mainly on the basis of smear microscopy which does not differentiate between *MTB* and NTM, however the radiology signs and symptoms of TB and NTM disease are similar and it is difficult to differentiate unless culture and identification is done.[27] In the present study, 56.9% were rapid growers and 43% were slow growers; 18.4% pigment producer and 24.6% were non chromogens. Rapid growers like *M*

fortuitum have been known to cause cutaneous infections, soft tissue infection, pulmonary disease, post traumatic or post-surgical infections and are the most frequently isolated species worldwide; Belgium (2.1%), Denmark (5.3%), United Kingdom (6.0%), France (6.5%), Finland (6.7%), Germany (12.2%), and the Czech Republic (17.5%).[6] Studies from Delhi and North India have also reported it as predominant species.[32,33] Non chromogenic, slow growers like *Mycobacterium avium intracellulare* (MAC) are known to cause chronic pulmonary disease, local lymphadenitis, and disseminated opportunistic infections in AIDS patients.[34] MAC was the commonest species reported from Japan at 33-65/100,000 cases in 2005[22] also reported as predominant species from North America, East Asia, China and India.[6,35] *M ulcerans,* a non chromogenic species is another commonly found mycobacteria worldwide.[36] Pigmented NTM like *M flavescense* (both rapid and slow grower Sctochromogen) is reported to cause bone and joint infection, osteomyelitis, lung disease, and disseminated post-injection infection.[37,38]

In the present study, we isolated NTM in only 0.1% TB suspect cases while isolation was higher from DRTB suspects (0.5% in smear positive and 0.3% in smear negative). Similarly 34.8% and 30% NTM were reported in MDR TB suspects in Iran and China, respectively.[39,40] A study from Delhi also reported 17.6% NTM in suspected MDR pulmonary TB cases.[6] NTM infection may be misdiagnosed as DRTB and patient may continue anti-tuberculosis therapy to no effect, NTM infections may cause lung disease in co-infection cases and hence, impacting management of TB in endemic settings like India. Moreover NTM are known to localize in preexisting lesions of lung which are common in known TB patients. To prove pathogenicity, follow up 2nd sample is essential from cases in whom NTM is isolated and if same species is isolated it is likely to be pathogenic, but major drawback in our study was that 2nd sample was obtained in only 20% cases.

In TB control program settings, if growth is AFB positive on MGIT, it is processed for differentiation for NTM and *MTB* by rapid antigen detection test like SD Bioline test. If this test is positive for *MTB*, it is processed accordingly, no effort is made to speciate the NTM nor does the clinician send 2nd sample to confirm, no effort is made to rule out presence of NTM by either subculture on LJ or molecular test to differentiate NTM, and *MTB*. As a result co-infection is missed if culture is done only on MGIT. When LJ is also used to separate colony characters of NTM then *MTB* could be appreciated and co-infection could be identified and confirmed. There is a need to identify not only NTM but the NTM *MTB* co-infection too. Moreover many studies on NTM lack information on patients' data and in most instances ATS definition criteria for NTM disease have not been applied. Therefore, it is difficult to conclude whether these NTM isolates were just commensals, contaminants, or causative agent of disease. Systematic large scale studies involving different regions of India should be planned to know the prevalence and diversity of NTM species and patient outcome to truly understand the role of NTM and NTM *MTB* co-infection in causing morbidity and mortality in India.

In addition, limitations such as follow up details including drug treatment regimens and disease outcomes are not available in most of these studies including ours.

CONCLUSION

Yield for NTM was higher in MGIT than LJ but on LJ, separate colonies of NTM and MTB could be seen and helped in identifying co-infection which was missed on MGIT. Though NTM prevalence is low but it is important to repeatedly identify same species from these patients to establish pathogenicity. Follow up from clinician/ patients to submit 2nd sample, should be stressed upon. Long term follow up of such patients is important to know outcome of diseases in case of NTM and NTM-*MTB* co-infection.

Acknowledgment: We acknowledge the support provided by FIND, India and thankful to Dr Dilip Kala, state TB officer.

REFERENCES

1. Claeys TA, Robinson RT. The many lives of non tuberculous *Mycobacteria. J Bacteriol.* 2018;200: (11): e00739-11.
2. Johansen MD, Herrmann JL, Kremer L. Non-tuberculous mycobacteria and the rise of *Mycobacterium abscessus*. *Nat Rev Microbiol.*2020; 18:(7):392- 407.
3. http://www.bacterio.net/mycobacterium.html.accessed on 18th August 2020.
4. Halstrom S, Price P, Thomson R. Review: Environmental

mycobacteria as a cause of human infection. *Int J Mycobacteriol*. 2015;4(2):81-91.

5. Set R, Shastri J. Laboratory aspects of clinically significant rapidly growing mycobacteria. *Ind J Med Microbiol*. 2011; 29:343-52.
6. Gopinath K, Singh S. Non-tuberculous *Mycobacteria* in TB endemic countries: Are we neglecting the danger? *PLoS Negl Trop Dis*. 2010;4:e615.
7. Aksamit TR, Philley JV, Griffith DE. Non-tuberculous *mycobacterial* (NTM) lung disease: The top ten essentials. *Respir Med*. 2014;108(3):417-25.
8. Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F et al. An official ATS/IDSA statement: Diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med.* 2007; 75:367-416.
9. Jain S, Sankar MM, Sharma N, Singh S. High prevalence of non-tuberculous mycobacterial disease among non-HIV infected individuals in a TB endemic country-experience from a tertiary center in Delhi, India. *Pathog Glob Health.* 2014;108(2):118-22.
10. Patwardhan V, Chakravarti A. Non tubercular *Mycobacteria* (NTM) with special emphasis on drug resistance mechanisms, susceptibility testing and therapy. *Jeevanu Times*.2014;14(1): 22-25.
11. Xu K, Bi S, Ji Z, Hu H, Hu F, Zheng B et al. Distinguishing non tuberculous mycobacteria from multidrug-resistant *Mycobacterium tuberculosis*, China. *Emer inf Dis.* 2014; 20:1060-62.
12. Tabarsi P, Baghaei P, Farnia P, Mansouri N, Chitsaz E, Sheikholeslam F et al. Non tuberculous mycobacteria among patients who are suspected for multidrug-resistant tuberculosis-need for earlier identification of non tuberculosis mycobacteria. *The Amer J of the Med Sciences.* 2009;337:182-84.
13. Kent PT, Kubica GP. *Public Health Mycobacteriology: A Guide for the Level III Laboratory.* Atlanta, GA: US Department of Health and Human Services, Centers for Disease Control; 1985.
14. Rishi S, Sinha P, Malhotra B, Pal. A comparative study for the detection of Mycobacteria by BACTEC MGIT 960, Lowenstein Jensen media and direct AFB smear examination. *Ind J Med Microbiol.* 2007;25:383-86.
15. Maurya AK, Nag VL, Kant S. Evaluation of an immunochromatographic test for discrimination between *Mycobacterium tuberculosis* complex and non tuberculous mycobacteria in clinical isolates from extra-pulmonary tuberculosis. *Ind J of Med Res*. 2012;6(135):90-106.
16. Paramasivan CN, Govindan D, Prabhakar R, Somasundaram PR, Subbammal S, Tripathy SP. Species level identification of non-tuberculous mycobacteria from South Indian BCG trial area during 1981. *Tubercle.*1985; 66:9-15.
17. Narang P, Narang R, Mendiratta DK, Roy D, Deotale V, Yakrus MA et al. Isolation of *Mycobacterium avium complex* and *M. simiae* from blood of AIDS patients from Sevagram, Maharashtra. *Indian J Tuberc.* 2005;52:21-26.
18. Sivasankari P, Khyriem AB, Venkatesh K, Parija SC. Atypical mycobacterial infection among HIV seronegative patients in Pondicherry. *Indian J Chest Dis Allied Sci.* 2006;48: 107-09.
19. Jesudason MV, Gladstone P. Non tuberculous mycobacteria isolated from clinical specimens at a tertiary care hospital in South India. *Indian J Med Microbiol.* 2005;23: 172-75.
20. Shenai S, Rodrigues C, Mehta A. Time to identify and define non-tuberculous mycobacteria in a tuberculosis-endemic region. *Int J Tuberc Lung Dis.* 2010;14:1001-08.
21. Myneedu VP, Verma AK, Bhalla M, Arora J, Reza S, Sah GC et al. Occurrence of non-tuberculous Mycobacterium in clinical samples-a potential pathogen. *Ind J Tuberc.* 2013; 60:71-76.
22. Jain S, Sankar MM, Sharma N, Singh S, Chugh TD. High prevalence of non-tuberculous mycobacterial disease among non-HIV infected individuals in a TB endemic country - Experience from a tertiary center in Delhi, India. *Pathog Glob Health.* 2014;108:118-22.
23. Maurya AK, Nag VL, Kant S, Kushwaha RA, Kumar M, Singh AK et al. Prevalence of nontuberculous mycobacteria among extra-pulmonary tuberculosis cases in tertiary care centers in Northern India. *Biomed Res Int.* 2015;465403:1-5.
24. Umrao J, Singh D, Zia A, Saxena S, Sarsaiya S, Singh S et al. Prevalence and species spectrum of both pulmonary and extra-pulmonary non tuberculous mycobacteria isolates at a tertiary care center. *Int J Mycobacteriol.* 2016; 5:288-93.
25. Sharma SK, Sharma R, Singh BK, Upadhyay V, Mani I, Tripathi M et al. A prospective study of non-tuberculous mycobacterial disease among *tuberculosis* suspects at a tertiary care centre in north India. *Indian J Med Res*. 2019; 150(5):458-67.
26. Verma AK, Arora VK. Isolation and identification non-tuberculous Mycobacteria from presumptive tuberculosis patients. *Int J CurrMicrobiol App Sci*. 2018; 7(11): 2929-38.

27. Chanda-Kapata P, Kapata N, Klinkenberg E, Mulenga L, Tembo M, Katemangwe P et al. Non-tuberculous mycobacteria (NTM) in Zambia: Prevalence, clinical, radiological and microbiological characteristics. *BMC Infect Dis*. 2015;15:1264-66.
28. Nasiri MJ, Dabiri H, Fooladi AAI, Amini S, Hamzehloo G, Feizabadi MM. High rates of nontuberculous mycobacteria isolation from patients with presumptive tuberculosis in Iran. *New Microbes New Infect*. 2017;21:12-17.
29. Maiga M, Siddiqui S, Diallo S, Diarra B, Traoré B, Shea YR et al. Failure to recognize non tuberculous mycobacteria leads to misdiagnosis of chronic pulmonary *tuberculosis*. *PLoS One.* 2012;7:17.
30. Sharma K, Gautam N, Sharma M, Dogra M, Bajgai P, Tigari B et al. Ocular mycobacteriosis-dual infection of *M. tuberculosis complex* with *M. fortuitum* and *M. bovis. J Ophthalmic Inflamm Infect.* 2017;7:2.
31. Kotwal A, Raghuvanshi S, Sindhwani G, Khanduri R. Mycobacterium *tuberculosis* and non tuberculosis mycobacteria co-infection: Two cases from the sub-Himalayan region of North India in a year. *Lung India*. 2017;34(5):494-96.
32. Chakrabarti A, Sharma M, Dubey M**.** Isolation rates of different mycobacterial species from Chandigarh (North India)**.** *Ind J Med Res*. 1990; 91:111-14.
33. Das BK, Sharma VK, Bhau LN, Saxena SN, Bhardwaj BK.Characterization of mycobacterial strains from clinical specimens**.** *Ind J PatholMicrobiol***.** 1982;25:19-27.
34. Greene JB, Sidhu GS, Lewin S, Levine JF, Masur H, Simberkoff MS et al. *Mycobacterium avium intracellulare*: A cause of disseminated life threatening infection in homosexuals and drug abusers. *Ann Intern Med*. 1982;97:53946.
35. Hoefsloot W, Van Ingen J, Andrejak C, Angeby K, Bauriaud R, Bemer P et al. The geographic diversity of non tuberculous mycobacteria isolated from pulmonary samples: an NTM-NET collaborative study. *EurRespir J.* 2013; 42:1604-13.
36. Walsh DS, Portaels F, Meyers WM. Buruli ulcer (*Mycobacterium ulcerans* infection). *Trans R Soc Trop Med Hyg.* 2008;102:969-78.
37. Brown BA, Springer B, Steingrube VA, Wilson RW, Pfyffer GE, Garcia MJ et al. *Mycobacterium wolinskyi* sp nov and *Mycobacterium goodie* sp nov rapidly growing species related to *Mycobacterium smegmatis* and asso-ciated with human wound infections: A cooperative study from the International working group on Mycobacterial Taxonomy. *Int J SystBacteriol.* 1999;49: 1493-1511.
38. Mastroianni A. *Mycobacterium flavescens* vertebral osteomyelitis in an immunocompetent host. *Le Infezioni in Medicina*. 2003;11:97-101.
39. Kahkouee S, Esmi E, Moghadam A, Karam BM, Mosadegh L, Salek S et al. Multidrug resistant tuberculosis versus nontuberculous mycobacterial infections: A CT-scan challenge. *Brazj Infect Dis*. 2013;17(2):137-42.
40. Jing H, Wang H, Wang Y, Deng Y, Li X, Liu Z et al. Prevalence of nontuberculous mycobacteria infection, China, 20042009. *Emerg Infect Dis*. 2012;18:527-28.

Corresponding Author

Dr Bharti Malhotra, Senior Professor, Department of Microbiology, SMS Medical College, Jaipur, Rajasthan, 302004.

email: drbhartimalhotra@gmail.com