Short Communication

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

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Non-tuberculous mycobacteria, MGIT, Multiplex PCRIntroduction

Non-tuberculous mycobacteria (NTM) considered mostly as colonizers or ignored as environmental contaminants in the past, are now increasingly recognized as important pulmonary pathogens in both immunocompromised and immunocompetent population.[1] Specific risk factors identified are HIV infection, cystic fibrosis, underlying chronic lung disease, previous tuberculosis (TB), and work in the mining industry.[1] These are also reported to cause surgical-site infections, post-injection abscesses, osteomyelitis, catheter-related blood-stream infections, and central nervous system infections.[2] Non-tuberculous mycobacteria rates of infection and disease has significantly increased in recent years and rates vary widely depending on population and geographic location.[1] Most reports are from developed countries that have low rates of TB. However, in countries with high burden of TB, including India, NTM pulmonary disease often goes unrecognized and is misdiagnosed as pulmonary TB because clinical presentation of NTM and *Mycobacterium tuberculosis* (MTB) diseases are indistinguishable from each other. Prevalence of NTM is unknown 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.[3] Among few reports available, NTM isolation rates are reported to range from 0.5 to 8.6% in India.[4] A recent study from central India reported prevalence of NTM increased from 1.0% in 2005 to 3.5% in 2008 and 88.6% of the NTM isolated were clinically relevant.[4] Identification of NTM is of clinical relevance as most of the NTM are notably resistant or only partially susceptible to the standard anti-tubercular drugs and the treatment strategies and the duration of these infections differ from MTB.

We conducted retrospective review of isolation rates of *Mycobacterium tuberculosis* complex (MTC) and NTM, the species of NTM characterized and their clinical significance during January 2011–June 2012 at our tertiary care hospital (700 bedded) in Delhi, India. The study was approved by the ethical committee of our institute. A total of 436 clinical specimens were processed for mycobacteria culture, 237 from pulmonary (sputum, bronchoalveolar lavage, bronchial wash, and endotracheal aspirates) and 199 from extrapulmonary sites (urine, pus, peritoneal fluid, lymph node aspirate, synovial fluid, endometrial biopsy, CSF). Specimens were digested and decontaminated by the standard *N*-acetyl-L-cysteine–NaOH method and inoculated into BACTEC mycobacteria growth indicator tube (MGIT) 960 vials (BD Diagnostics, Sparks, MD, USA) and Löwenstein–Jensen (LJ) medium slants (Hi Media laboratories, Mumbai, India). Smear microscopic examination was performed using Zeihl Neelsen (ZN) and fluorochrome stains. All positive MGIT vials were subjected to identification of MTC, by *p*-nitrobenzoic acid (PNBA) assay on MGIT 960 as described elsewhere[5] and SD TB MPT64 antigen rapid assay by immunochromatographic method (Standard Diagnostics, Seoul, South Korea). *p*-Nitrobenzoic acid resistant and/or MPB 64 antigen

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negative isolates (suspected to be NTM) were subjected to a multiplex PCR using primers for mycobacterium genus (targeting hsp65), MTC (targeting ESAT6), and *Mycobacterium avium* complex (targeting MAC) specific genes.[3] Confirmed NTM isolates were further speciated by pigment production, growth on MacConkey agar, rate of growth, nitrate reduction test, tellurite reduction, urease production, tween-80 hydrolysis, arylsulfatase, heat-stable catalase, iron uptake, and tolerance to 5% NaCl.[6] Drug-susceptibility testing was performed for MTC for PSIRE (pyrazinamide, streptomycin, isoniazid, rifampicin, and ethambutol) by BACTEC MGIT 960 (1% proportional method).

Of 436 specimens, 109 (25.6%) were positive for acid-fast bacilli by smear microscopy (ZN and/or fluorescent) and 131 (30.7%) specimens were culture positive. Of 131 positive cultures, 118 (90.1%) isolates were identified as MTC (71.2% from pulmonary and 28.8% from extrapulmonary samples) and 13 (9.9%) isolates as NTM species. The NTM were isolated from pulmonary specimens in nine (69.2%) patients, and four (30.8%) were isolated from non-pulmonary specimens, one each from urine, ascitic fluid, bone, and blood specimens. The most common NTM species characterized was *Mycobacterium kansasii* (4) followed by *Mycobacterium chelonae* (3), *Mycobacterium xenopi* (2), *Mycobacterium scrofulaceum* (1), *M. avium* (1), *Mycobacterium asiaticum* (1), and *Mycobacterium fortuitum* (1). Clinical details and speciation of the NTMs isolated are shown in Table 1. Fluorescent staining was more sensitive as it detected 12 additional culture positive cases that were negative by ZN stain (of these, four were NTM). As NTM are ubiquitous in nature, NTM pulmonary infections consistent with the American thoracic society (ATS) and British thoracic society (BTS) guidelines (defined in Table 1) were considered clinically relevant.[7,8] Of nine patients with pulmonary NTM isolated, seven (77.9%) had definite NTM disease and two (15.4%) had probable NTM disease; among four patients with extrapulmonary specimens, two (50%) had definite NTM disease, one (25%) had probable NTM disease, and one (25%) had possible disease or colonization (Table 1). Majority of patients were males (69.2%) and 46.1% (6/13) were older in age ($\geq$60 years). The main underlying risk factors found to be associated with NTM infections were pre-existing pulmonary disease (54%), chronic obstructive pulmonary disease (COPD; 23.1%), past and/or present history of TB (30.8%, two pulmonary TB and one Pott's spine cases were diagnosed by both smear for AFB and culture positivity for MTB and a case of osteomyelitis was smear positive for AFB), chronic smoking (15.4%), diabetes (23.1%), steroids (7.6%), and malignancy (7.6%). No predisposing condition was detected in three patients (23.1%). All 13 patients with NTM disease were HIV seronegative (ARCHITECT® HIV Ag/Ab Combo Assay, Abbott diagnostics).

Between PNBA and MPB64 antigen assay results, 100% concordance was observed. All mycobacteria grew both in MGIT and LJ medium; however mean isolation time was much shorter with MGIT than with LJ (11 vs 28 days). Furthermore, mean recovery time of MTC from pulmonary specimens was lesser than that of non-pulmonary specimens (10 vs 16 days) by MGIT culture. Among NTMs, slow-growing mycobacteria grew in mean time of 12.9 days. Rapidly growing mycobacteria (RGM) bloodstream infection due to *M. chelonae* in one of the patient could be detected by MGIT in 2.2 days. Of the MTC, 67% were sensitive to all tested drugs, 14.7% were multidrug-resistant (MDR), and 17.6% were mono-resistant. Treatment details of the patients were not retrieved.

While clinical diagnosis of NTM pulmonary disease is challenging, reliance only on positive smear microscopy for the initiation of treatment for suspected TB in most TB endemic countries and not on *Mycobacteria* culture, species identification, and drug-resistance testing results, is unfortunate as acid-fast bacilli visualized in smears may not necessarily be MTB. Moreover, NTM co-infections with MTB disease are not infrequent, though rarely diagnosed.[10] As NTM strains exhibits high drug-resistance to first-line anti-tubercular drugs, many of NTM infections would be considered TB treatment failures, and subsequently treated for multidrug-resistant TB (MDR-TB) disease. The same was highlighted in a recent report from China, where NTM accounted for 30% of suspected MDR-TB cases and 4% of retreatment TB cases.[11] In another study from New Delhi, India, detected NTM were detected by multiplex PCR in 17.6% of the suspected MDR-pulmonary TB cases and in 12.4% of the suspected extrapulmonary TB cases.[3] These findings highlight the necessity of laboratory speciation of mycobacteria and initiation of treatment for mycobacterial infections based on *in vitro* susceptibility testing. In the present study, 12 of the 13 NTM infections (92.3%) were provisionally diagnosed as pulmonary or extrapulmonary TB based on clinical presentation and smear microscopy findings. Of these, three patients (23.1%) having past history of TB were considered for retreatment (MDR-TB suspected) and one patient was found to be co-infected with MTB (pulmonary NTM and Pott's spine). None of the patient in this series was HIV seropositive. In a recent 3 years study from India, 42% of AFB cultures were positive and approximately 2% of these were NTM. Pulmonary infections represented 81% of all NTM cases and the major predisposing condition was underlying lung disease (54%) including 40% patients with a past or present

Table 1 Clinical and microbiological features of non-tuberculous mycobacteria (NTM) isolated

NTM species	Age (years) /sex	Site of infection	Other underlying disease	Specimen (number of culture positive)	Radiological findings	Smear microscopy		Growth on MGIT culture (days)	Multiplex PCR	Clinical significance of NTM isolated (as per ATS)
						ZN Stain	Fluorescent stain			
Pulmonary (n = 9)										
Mycobacterium xenopi (n = 2)	71/M	Pulmonary	COPD, past history of pulmonary TB, chronic smoker	Sputum (2)	Fibrocalcific (old TB) lesions in upper lobe, consolidation in right middle zone	Negative	5 fluorescing bacilli in 200 fields	7	hsp65 + ESAT6-MAC-	Definite NTM disease
	84/M	Pulmonary	COPD	Sputum (2)	Bilateral pulmonary infiltrates	Negative	Negative	9	hsp65 + ESAT6-MAC-	Definite NTM disease
Mycobacterium kansasii (n = 2)	59/M	Pulmonary	Ankylosing spondylitis, on steroids	Sputum (2)	Bilateral pulmonary infiltrates	2+	20 bacilli/HPF	10	hsp65-* ESAT6 + MAC-	Definite NTM disease
	76/F	Pulmonary	Diabetes mellitus	Sputum (2)	Consolidation in left middle and lower zone	1+	2–4 fluorescing bacilli/HPF	16.5	hsp65-* ESAT6 + MAC-	Probable NTM disease
Mycobacterium scrofulaceum (n = 1)	65/M	Pulmonary	COPD, chronic smoker	Sputum (1)	Consolidation in bilateral upper and middle lobe	Negative	0–2 fluorescing bacilli/HPF	15	hsp65 + ESAT6-MAC-	Probable NTM disease
Mycobacterium fortuitum (n = 1)	56/M	Pulmonary	Diabetes mellitus, concurrent Pott's spine	Bronchoalveolar lavage (1)	Consolidation in right upper and middle zone	Negative	6 fluorescing bacilli in 200 fields	16 and 14 (2 sputum samples)	hsp65 + ESAT6-MAC-	Definite NTM disease
Mycobacterium avium (n = 1)	60/M	Pulmonary	None	Sputum (2)	Right hilar and mediastinal lymphadenopathy	2+	>50 fluorescing bacilli/HPF	5	hsp65 + ESAT6-MAC+	Definite NTM disease
Mycobacterium asiaticum (n = 1)	53/M	Pulmonary	Diabetes mellitus with neuropathy, pulmonary TB defaulter	Sputum (2)	Consolidation in left middle and lower zone	3+	>100 fluorescing bacilli/HPF	18	hsp65 + ESAT6-MAC-	Definite NTM disease
Mycobacterium chelonae (n = 1)	3/M	Pulmonary	Post-operative case of tracheoesophageal fistula	Bronchoalveolar lavage (1)	Diffuse bilateral nodular lesions and large nodule in upper right lobe	Scanty	7 fluorescing bacilli in 200 fields	5	hsp65 + ESAT6-MAC-	Definite NTM disease
Extrapulmonary (n = 4)										
M. kansasii (n = 2)	61/M	Osteomyelitis	Past history of pulmonary TB	Synovial fluid, granulation tissue (1)**	Lytic lesion in medial condyle of right knee	Scanty	2–3 fluorescing bacilli/HPF	13	hsp65- *ESAT6 + MAC-	Definite NTM disease
	51/F	Abdomen	None	Ascitic fluid‡	ND	Negative	7 fluorescing bacilli in 200 fields	23	hsp65- *ESAT6 + MAC-	Probable NTM disease

Table 1 Continued

NTM species	Age (years) /sex	Site of infection	Other underlying disease	Specimen (number of culture positive)	Radiological findings	Smear microscopy		Growth on MGIT culture (days)	Multiplex PCR	Clinical significance of NTM isolated (as per ATS)
						ZN Stain	Fluorescent stain			
M. chelonae ($n = 2$)	29/F	Urinary tract	None	Midstream urine (1 of 3 samples)	ND	Negative	Negative	6	hsp65 + ESAT6-MAC-	Possible NTM disease/ contamination
	30/F	Central venous catheter-related mycobacteremia[9]	Ovarian carcinoma, on chemotherapy	Blood from catheter (4), 1peripheral (1), catheter tip	ND	ND	ND	2.2	hsp65 + ESAT6-MAC-	Definite NTM disease

Case Definitions:
Definite NTM lung disease (criteria by American Thoracic Society):[7] at least two sputum samples positive for culture with NTM (or a single isolate in the case of bronchoscopy specimens) coexistent with appropriate radiographic findings and clinical symptoms.
Definite Extrapulmonary NTM disease (British thoracic Society, BTS guidelines):[8] one isolation of NTM from sterile site, repeated isolation (>2 times) from a non-sterile source, and histological evidence of tissue invasion with compatible radiological and/or clinical features.
Catheter-related blood-stream infections: typical symptoms (exit site or tunnel site erythema, purulence, fever) and positive blood culture.
We defined probable NTM disease as one positive culture for NTM from sterile or non-sterile sources with compatible radiological and/or clinical findings but no evidence of tissue invasion or positive blood culture.
Possible NTM disease/colonization: patients who did not fulfill the criteria for definite or probable NTM disease with single isolation of NTM from clinical specimens were considered to have possible NTM disease or colonization.
* *M. kansasii* produces double band for ESAT6 primer, so it hinders the amplification of hsp65.
**Histopathological examination of biopsy showed granulomatous inflammation with necrosis and chronic inflammatory changes.
‡Ascitic fluid cytology showed lymphocytic infiltrate with macrophages, leukocyte count: 200 cells/mm^3 (12% neutrophils, 83% lymphocytes).
TB: tuberculosis; ZN: Zeihl Neelsen; ATS: American thoracic society; COPD: chronic obstructive pulmonary disease; MGIT: mycobacteria growth indicator tube; HPF: high-power field.

history of pulmonary TB while only 2% patients were known HIV-positive.[12]

High prevalence of NTM infections observed among non-HIV seropositive individuals in a TB endemic country in this report, underscores the need for increased awareness of these emerging human pathogens and importance of mycobacteria speciation to reduce morbidity and mortality resulting from these diseases. Use of liquid culture medium and molecular methods in clinical laboratories can significantly reduce turn-around time particularly for diagnosis of infections due to slow-growing NTM and MDR-TB. However, with improved facilities for recovery of NTMs that may result in increased frequency of isolation of these organisms, NTM disease requires clinical correlation and differentiation from colonization.

Disclaimer Statements

Contributors Sarika Jain contributed to concept and designing of the study, searching literature, analysing data and preparing the manuscript. Manimuthu Sankar performed conventional and molecular tests for identification of NTM isolates and contributed in analysing the data. Navneet Sharma contributed in data acquisition and data analysis. Sarman Singh contributed in manuscript preparation and editing. T. D. Chugh contributed in the concept of study, searching literature and manuscript editing.

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