Comparison of clinico–radiological features of patients with positive cultures of nontuberculous mycobacteria and patients with tuberculosis

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ABSTRACT

Objectives: To identify the clinico-radiological features of patients with positive cultures for nontuberculous mycobacteria (NTM) and compare those to a sample of patients with tuberculosis (MTB).

Methods: A laboratory database was used to retrieve all specimens submitted to King Khalid University Hospital, Riyadh, mycobacteriology laboratory for mycobacterial smears and cultures during the period from October 1999-April 2002. Using this database, the original records of the mycobacteriology laboratory and a review of the patient's health records, a standard proforma was completed that included demographic, clinical, radiological and laboratory information on patients included in this study. The patients were divided into 2 groups; the NTM group, which included all patients with positive cultures for NTM and the MTB group, which included a sample of patients with documented tuberculosis.

Results: During the study period, 286 patients had positive mycobacterial cultures. Seventy patients (24.5%) grew NTM and 216 (75.5%) grew MTB. For patients with MTB, 54 patients were included as per the selection protocol of the study. There was no difference between the 2 groups in all measured demographic variables. The presence of weight loss and fever was significantly more in the MTB group. Radiologically, the presence of hilar adenopathy was more significant among patients with MTB than those with NTM (17% versus 4%, p=0.02). However, bronchiectatic changes were seen significantly more among NTM patients compared to patients with MTB (26% versus 11%, p=0.03).

Conclusion: The isolation of NTM in the mycobacteriology laboratory is high. The clinico-radiological features were not sufficiently specific to differentiate patients with NTM from patients with MTB. Local studies are needed to explore NTM disease in various developing countries and identify the NTM species causing infections in non-immunosuppressed patients in each locality.

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Mycobacteria other than *Mycobacterium tuberculosis* (MTB), commonly called nontuberculous mycobacteria (NTM), have been implicated in a variety of pulmonary and extrapulmonary diseases such as lymphadenitis, superficial and soft tissue infections and severely disseminated diseases.[1,2] Unlike MTB, which is an obligate human pathogen with no environmental

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reservoir, NTM are commonly isolated from environmental sources such as soil, water and food.[3-6] The NTM are also commonly isolated from asymptomatic individuals. A previous study in Saudi Arabia has demonstrated that up to 30% of asymptomatic individuals with normal chest radiograph have positive mouthwash specimens for NTM.[7] Progress has been made in improving the age-old traditional techniques such as microscopy (Auramine-Rhodamine fluorescent staining), culture and sensitivity techniques (solid, liquid radiometric and non-radiometric systems). Those approaches take time because of the slow growth of mycobacteria. Therefore, rapid methods such as high performance liquid chromatography, thin layer chromatography, RNA sequence and polymerase chain reaction, nucleic acid sequence based amplification assay, and so forth, have paved the way for its rapid detection and treatment.[8-11] However, in most developing countries including Saudi Arabia, many clinicians rely on chest radiograph and smear positivity to diagnose patients with pulmonary tuberculosis due to the lack of sophisticated laboratory facilities. Even when the facility for mycobacterial culture is available, the tests needed to identify the type of mycobacteria (MTB versus NTM) are not readily available. In turn, patients with clinical specimens growing NTM may be mislabeled as MTB and hence receive wrong and unnecessary treatment for long periods of time.

Al-Jarad et al,[12] tried to identify the demographic characteristics of patients with positive respiratory specimens for NTM and compare that to patients with pulmonary tuberculosis.[12] However, some of their patients were HIV positive. Alternately, the literature on imaging appearance of NTM pulmonary infections has tried to identify some features that would differentiate NTM from pulmonary tuberculosis infections.[13] Generally, no clear clinical predictors could be identified. Furthermore, pulmonary NTM is a problem with differing rates in various parts of the world. Studies have shown marked geographic variability in prevalence of pulmonary NTM[14] and it appears that the prevalence of NTM infection in Saudi Arabia is high.[7] In view of the above, we sought to review our experience with NTM isolates and try to identify the clinico-radiological features (using chest radiographs) of patients with positive specimen culture for NTM and compare that to a sample of patients with documented tuberculosis and attempt to identify clinical predictors for NTM infection.

Methods. The study was conducted at King Khalid University Hospital (KKUH), Riyadh, Kingdom of Saudi Arabia, which is an 850 beds tertiary care hospital that accepts referrals from different regions of the Kingdom. The study was conducted in the period between October 1999-April 2002 (retrospectively until 2000 and prospectively from 2000-2002). A laboratory database was used to retrieve all specimens submitted to the hospital mycobacteriology laboratory for mycobacterial smears and cultures. Using this database, the original records of the mycobacteriology laboratory and a review of the patient's health records, a standard proforma[15] was completed that included demographic, clinical, radiological and laboratory information on patients included in this study. The study was approved by the ethics committee at KKUH.

The patients were divided into 2 groups. The first group included all patients who had positive specimen culture (sputum, induced sputum, broncho-alveolar lavage (BAL), pleural effusion, or other sources) for NTM during the study period. The second group consisted of patients who had positive specimen culture for MTB during the study period. In the second group, we created a list of all patients with positive cultures, and subsequently, we selected in order every fourth patient on that list to be included in the study. The decision to send specimens for mycobacterial smear and culture was taken by the treating physician. The presence or absence of symptoms and the presence or absence of chest radiographic abnormalities was documented. Patients were considered symptomatic if they had one or more of the following symptoms; fever, night sweats, cough (productive or dry), hemoptysis, chest pain or weight loss, and asymptomatic if they had none of the above. The presence or absence of previously reported [16] specific risk factors for NTM, MTB, or both were also documented. Risk factors assessed included underlying chronic lung disease, HIV infection, pneumoconiosis, previous history of pulmonary TB, bronchiectasis, diabetes, chronic alcoholics, cancer, chronic renal failure, chemo or radiotherapy, corticosteroids, gastro-intestinal surgery, history of cancer or malnutrition. Chest radiographs were divided into 6 zones: the right and left upper zones, the right and left middle zones, and the right and left lower zones. The pattern and location of the abnormality (apical scarring, parenchymal infiltrate, cavitary disease, bronchiectatic changes, evidence of pleural abnormalities including pleural effusion, or lymph node enlargement) was documented according to the interpretation of the radiologists or the respirologist who were blinded to the patient's microbiology results. The chest radiograph closest to the date of specimen collection was used.

All specimens were digested and decontaminated according to the standards of the Center for Disease Control and Prevention (CDC) Methods. [17] A 5-10ml sample of sputum or other specimens were treated with an equal volume of a solution of sodium

hydroxide and N-Acetyl-L-Cysteine (NaOH/NALC) with the concentration of 2% NaOH and 0.25% NALC for 15 minutes. The mixture was increased to 50 ml by adding sterile distilled water or phosphate buffer and centrifuged at 3500 x g for 15-20 minutes in a refrigerated centrifuge. The sediment was suspended in 1-2 ml of sterile normal saline solution and vortexed for 15-30 seconds. Smears were made from the sediment of each specimen, stained with Ziehl Neelsen (ZN) and Auramine stains. The ZN staining was carried out by the hot method. The Auramine stained slides were examined with a fluorescent microscope. The quantification of the acid-fast bacillus (AFB) was carried out according to the CDC methods.[17]

For culture, solid and liquid media were used. 1. Solid Media: 2 slopes of Lowenstein-Jensen (LJ) medium, one with pyruvate, and the other with glycerol were inoculated with 0.2 ml of the above prepared sediment solution. These slopes were incubated at 37ºC until a positive growth was seen or up to a period of 8 weeks. 2. Liquid Media: 0.5 ml of the above sediment solution was cultured in the automated BACTEC 960 Mycobacterial Growth Indicator Tube (MGIT) system, (Beckton Dickinson, USA) following the manufacturer's instructions. The tubes were incubated at 37ºC until they signal positive or to a maximum of 6 weeks. Smears were made from the growth on the LJ media or from the positive MGIT tubes stained by Gram and ZN stains. A purity plate of blood agar was also made. The slow growing mycobacterial isolates were then differentiated into *Mycobacterium tuberculosis* Complex (MTB complex) and Nontuberculous Mycobacteria (NTM) by culturing on 2 LJ glycerol media, one of these with para-nitro benzoic acid (PNB).[18,19] The MTB complex is inhibited by PNB, whereas this agent does not inhibit NTM. The MTB complex and the NTM were further identified by more conventional tests. The conventional tests used other than rate of growth on LT were nitrate reduction, niacin production, tween hydrolysis, arylsulfatase test and iron uptake. The aim was to show if the isolate belongs to the MTB complex. The few mycobacterial isolates, which showed presumptive ZN and growth morphology of MTB and were not inhibited by PNB were further tested for nitrate reduction and niacin production to confirm that they are not MTB.

Data was expressed as mean ± SD in the text and tables. For comparing continuous variables, the student's t-test was used. For proportions, Chi-square or Fisher's exact test were used. Results were considered statistically significant at the $p<0.05$ levels. Sigma Stat, Version 3 (SPSS, inc, Chicago, Illinois, USA) statistical software was used for analysis.

Results. During the study period, 286 patients had positive mycobacterial cultures from different specimens. Seventy patients (24.5%) grew NTM and 216 (75.5%) grew MTB. All patients with positive culture for NTM were included in the analysis. For patients with MTB, 54 patients were included as per the selection protocol of the study. The distribution of NTM specimens was as follows; spontaneous sputa (92.3%), other respiratory sources (2.9%), other sources (4.8%). For the MTB

Table 1 - Symptoms profile in both groups.

Symptoms profile	NTM N=70* n (%)	MTB N=54* n (%)	*p* value
Presence of symptoms	54 (77)	47 (87)	NS
Productive cough	30 (43)	25 (46)	NS
Non productive cough	6 (8.6)	4 (7.4)	NS
Fever	18 (26)	24 (44)	0.04
Fatigue	1 (1)	2 (4)	NS
Hemoptysis	8 (11)	2 (4)	NS
Chest pain	6 (9)	6 (11)	NS
Night sweats	2 (3)	6 (11)	NS
Short of breath	26 (37)	11 (20)	NS
Weight loss	3 (4)	14 (26)	0.001

*some patients had more than one symptom,
NTM - Nontuberculous Mycobacteria,
MTB - *Mycobacterium tuberculosis*, NS - non-significant.

Table 2 - Radiological findings in both groups as obtained from chest radiographs.

Radiological findings	NTM N=70* n (%)	MTB N=54* n (%)	*p* value
Cavity	3 (4)	5 (9)	NS
Hilar adenopathy	3 (4)	9 (17)	0.02
Bronchiectasis	18 (26)	8 (11)	0.03
Interstitial infiltrates	8 (11)	4 (7)	NS
Alveolar infiltrates	1 (1)	5 (9)	NS
Mixed infiltrates	2 (3)	1 (2)	NS
Pleural effusion	4 (5.7)	2 (3.7)	NS
Pleural thickening	6 (8.6)	8 (14.8)	NS

*some patients had more than one abnormality,
NTM - Nontuberculous Mycobacteria,
MTB - *Mycobacterium tuberculosis*, NS - non-significant.

group, 80% of the positive specimens were of respiratory origin. Smear positivity was significantly more in the MTB group (41%) compared to the NTM group (7%) ($p<0.001$). There was no difference between the 2 groups in all measured demographic variables (age, male to female ratio, past history of tuberculosis, recent history of contact with patients with active pulmonary tuberculosis and the presence for risk factors for tuberculosis). In the NTM group, 3 patients were on steroids, one has HIV, 2 with chronic renal failure and 10 with diabetes mellitus. In the MTB group, one patient was on steroids, no HIV or chronic renal failure patients, and 13 have diabetes.

Table 1 shows the symptoms in each group. Seventy-seven percent of the NTM group were symptomatic compared to 87% in the MTB group. The presence of weight loss and fever were significantly more in the MTB group. All the other symptoms revealed no difference between the 2 groups.

Table 2 demonstrates the radiological findings in both groups. The presence of hilar adenopathy was significantly more among patients with MTB than those with NTM (17% versus 4%, $p=0.02$). However, bronchiectatic changes were seen significantly more among NTM patients compared to patients with MTB (26% versus 11%, $p=0.03$).

Discussion. Even in the 21st century, mycobacterial diseases continue to cause serious public health problems. Although the number of cases of tuberculosis is decreasing in developed countries, NTM are being recovered with increasing frequency.[20] There is evidence that the prevalence of NTM infections is increasing, particularly but not exclusively in non-immunosuppressed patients.[20] The clinical significance of these isolates has to be determined in each case as NTM are prevalent in the environment and colonization is common.[21] In response to this increase in isolation of NTM, the American Thoracic Society (ATS) and British Thoracic Society (BTS) have issued guidelines on the diagnosis and management of NTM infections.[1,2] To establish the diagnosis of NTM disease, both guidelines necessitate the presence of 3 critical elements, namely; compatible clinical presentation, consistent radiographic picture and recovery of NTM from clinical specimens in sufficient numbers or in pulmonary tissue. Therefore, the isolation of NTM from clinical specimens does not inevitably imply NTM disease.

The present study revealed a high prevalence of NTM isolates in a mycobacteriology laboratory in a tertiary care hospital in non-HIV patients. Around one-fourth of the positive mycobacterial cultures grew NTM. Without the proper techniques to identify those isolates, such patients will be mislabeled as MTB; hence, those patients may receive unnecessary treatment for long periods. Even among patient with NTM disease, if the isolates cannot be identified precisely, the patient is likely to receive the wrong combination of antibiotics for an inappropriately short time, which in turn may increase the rate of disease recurrence or mortality.[22]

There was no difference between the 2 groups in demographics. A previous study in the United Kingdom comparing demographic characteristics of patients with pulmonary NTM and pulmonary MTB reported the occurrence of NTM in elderly subjects.[12] Moreover, we could not identify clear clinical or radiological predictors for patients with MTB to help distinguishing them from patients with NTM. History of weight loss and fever as well as radiographic hilar adenopathy was significantly more in MTB patients. Alternatively, radiological evidence of bronchiectasis was more in the NTM group. Bronchiectasis may predispose patients to NTM infection, especially Mycobacterium avium complex. Such observation has been shown clearly in patients with cystic fibrosis.[23] Except for the above, there was no difference between both groups in clinical and radiological profiles. Unfortunately, symptoms and chest radiographic findings in patients with positive NTM specimens are not sufficiently specific to distinguish them from patients with MTB, underlying disease progression or other processes. This fact calls for the importance of having proper laboratory setup and techniques to identify different isolates. The most urgent questions to be addressed by the treating clinician and the mycobacteriology laboratory includes: First, are MTB or NTM involved?, second, when NTM is isolated, does the isolated NTM have any clinical significance (based on clinical, radiological, site of disease and source of specimen); third, if a clinically significant NTM is involved, are efforts made to ensure rapid identification of the type of NTM?; and finally, are susceptibility tests performed for certain species of NTM to ensure starting the proper treatment. As this proposed approach is not feasible in many hospitals in Saudi Arabia and many developing countries, we strongly urge the involved authorities to have central laboratories in different regions to receive and rapidly process such specimens to allow the treating clinician to take the appropriate timely treatment decisions. Otherwise, many patients with NTM infection will receive unnecessary treatment and patients with NTM disease will always receive the wrong combination of treatment. Clinicians should also remember that NTM isolation is common in our patients, especially those with preexisting bronchiectasis, therefore, if the clinical suspicion for MTB is low, anti-tuberculous treatment should not be started based on single

positive AFB smear or culture for mycobacteria, and identification of the isolated species should be sought rapidly. We also stress the importance of processing repeat specimens from such patients, especially if unusual opportunist mycobacteria are identified at sites that do not appear to fit the clinical picture.[2]

In summary, the present study showed that the isolation rate of NTM in a mycobacteriology laboratory is high. As clinico-radiological features are not sufficiently specific to differentiate patients with NTM from patients with MTB, the availability of proper laboratory techniques to identify such isolates becomes essential. In view of the marked geographic variability in the prevalence of NTM disease, local studies are needed to explore NTM disease in various developing countries and identify the NTM species causing infections in non-immunosuppressed patients in each locality.

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