Clinical Significance of the Non-Tuberculous Mycobacteria and the Optimum Methods for Their Isolation

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Introduction

Tuberculosis (TB) continues to cause significant morbidity and mortality worldwide. In 2013, the World Health Organization (WHO) estimated that there were 9 million new cases of TB and 1.5 million deaths attributed to TB. However, there has been a 45% decline in mortality due to TB from 1990 to 2013. The WHO also estimates that there have been 37 million lives saved during this same time frame due to improvements in diagnosis and effective treatments[1].

At the same time we are experiencing a decrease in cases of tuberculosis, we are seeing an increase in disease caused by the non-tuberculous mycobacteria (NTM). Initially, these mycobacteria were seen to cause infections in patients with underlying lung disease and in cases of lymphadenitis in children and in wound infections. We saw the emergence of *Mycobacterium avium-intracellulare* (MAI), now referred to as *M. avium* complex (MAC) as a cause of disease in the early 1980s due to the AIDS epidemic. We also saw the rise of other organisms such as *M. haemophilum*, *M. genavense* and *M. celatum* causing disease in immunocompromised patients.

In the 1985 publication *Public Health Microbiology, A Guide to the Level III Laboratory*, there were 24 NTM organisms listed plus a category called 'other rapid growers'[2]. Now there are approximately 160 different NTM species described. The increase in species numbers is due to the improvement in culture techniques as well as the use of molecular techniques to identify the NTM. In some cases there has been the discovery of entirely new species while in other cases there has been the recognition of certain sub-species as separate entities.

In recent years, there have been an increasing number of NTM infections that have been associated with the non-AIDS population[3]. It is difficult to report on exact numbers as infections with the NTM are generally not reportable. The non-tuberculous mycobacteria are an underestimated problem, especially in countries where

TB is endemic. Gopinath and Sing reported that NTM were found in 17.6% of pulmonary infections that were thought to be caused by multidrug-resistant (MDR) TB and in 12.4% of cases of extra-pulmonary disease originally thought to be due to *M. tuberculosis*[4].

Infections with NTM are not transmitted person to person or animal to human, rather they are transmitted via soil and water. The NTMs cause a variety of infections such as cutaneous infections, pulmonary infections and disseminated disease in susceptible populations. They can be confused with cases of tuberculosis, so culture and definitive identification is required. The NTM can also be found as colonizers or contaminants in clinical specimens. When isolated from a sterile body site, such as a biopsy, there is little doubt as to the pathogenic nature of the isolate. However if isolated from a normally contaminated body site, such as sputum, a single isolate of NTM may not be diagnostic of an infection. Both the

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American Thoracic Society (ATS) and the British Thoracic Society (BTS) recommend three cultures be obtained from non-sterile sites and multiple cultures contain the same organism[3,5]. Both societies also include other clinical criteria that should be met to attribute the isolation of an NTM to clinical disease.

Laboratory Methods

Specimen collection and specimen processing to isolate the NTM are the same as for the isolation of *M. tuberculosis* with some exceptions. Decontamination and digestion should be performed using NaOH at a final concentration of 1.0% along with N-acetyl-L-cysteine. Processing with oxalic acid can be used if the specimen is obtained from a patient with cystic fibrosis[6]. The time for decontamination is critical as contact with NaOH for greater than 15 minutes can result in the destruction of mycobacteria. Respiratory specimens should be centrifuged at a minimum of 3000xg and stained with a fluorochrome stain for maximum sensitivity.

Media Inoculation

Both liquid and solid media should be used to culture mycobacteria. An egg-based medium or Middlebrook 7H11 agar should be included. For specimens obtained from skin lesions, Middlebrook 7H11 can be supplemented with an X factor disk or strip to serve as a source of hemin to isolate *M. haemophilum*. In addition, this media should be incubated at 30°C to isolate this organism as well as *M. marinum* and *M. ulcerans*. Organisms such as *M. xenopi* and *M. thermoresistibile* will grow better at 42°C but can be recovered when grown at 35°C[7].

Liquid Media

There are two major continuously monitored systems for the culture of mycobacteria, the Thermo Scientific™ VersaTREK™ Automated Microbial Detection System and BD BACTEC™ MGIT™ System. Both systems utilize 7H9 broth that is supplemented with growth factors. The VersaTREK System monitors growth by detecting changes in gas pressure in the headspace of the Myco bottle. The MGIT System uses a fluorescent indicator imbedded in silicone on the bottom of the tube. Both systems detect the depletion of oxygen in the medium due to mycobacterial metabolism. The VersaTREK System has no limitations as to the source of specimen whereas the MGIT System has a limitation on blood and urine specimens. There is a paucity of data comparing the two systems, however.

Performance of the VersaTREK System versus the BD MGIT

Since there is no data on direct comparisons between the two systems, a look at historical data can be useful. In the two instances described below, specimens were processed in the same manner at both institutions.

Looking at data from 2006 from a then 500-bed hospital in the Greenwich Village section of Manhattan (Hospital A) from almost 4,000 specimens the laboratory isolated 293 mycobacteria. These included 49 isolates of NTM involving 11 different species and three isolates of *Rhodococcus equi* (Table 1). These organisms were isolated in a laboratory using the VersaTREK System.

Table 1: Mycobacteria isolated from Hospital A in 2006 Using the VersaTREK System

Species	No. Isolates	Species	No. Isolates
M. avium complex	219	*M. xenopi*	3
M. tuberculosis	21	*M. celatum*	3
M. bovis	1	*M. fortuitum*	3
M. gordonae	15	*M. mucogenicum*	1
M. chelonae	9	*M. terrae*	1
M. interjectum	5	*M. scrofulaceum*	1
M. kansasii	4	*R. equi*	3
M. abscessus	4		

Table 2 lists the mycobacteria isolated from an 1100+ bed hospital on the Upper East Side of Manhattan (Hospital B) in 2008 in a laboratory using the MGIT System. There was a total of 313 isolates of mycobacteria from just over 5,000 specimens. Of these, 20 isolates were NTM from 4 different species. However, the 7 *M. fortuitum* isolates were all from the bacteriology section and were not isolated from MGIT tubes.

Table 2: Mycobacteria isolated from Hospital B in 2008 using the MGIT System

Species	No. Isolates
M. avium complex	221
M. tuberculosis complex	72
M. gordonae	7
M. fortuitum (all from bacteriology)	7
M. chelonae	3
M. scrofulaceum	3

Table 3 shows the results of a validation study conducted at Hospital B. Specimens were processed using standard laboratory techniques and inoculated into both a VersaTREK Myco bottle and into a MGIT tube and onto solid media. The order of inoculation of the VersaTREK Myco bottle and the MGIT tube would alternate weekly to avoid any sampling bias. This validation included all specimens except blood and urine specimens received over a 2 month time period.

As depicted in Table 3, both systems were able to detect the 7 specimens containing *M. tuberculosis*. The VersaTREK System detected 9/9 specimens containing MAC whereas the MGIT system failed to detect MAC in one specimen. VersaTREK detected 9/9 specimens containing NTM whereas the MGIT was only able to detect the growth of two isolates of *M. abscessus*.

Table 3: Validation Study VersaTREK vs. MGIT at Hospital B

Species	No. Isolates-VersaTREK	No. Isolates-MGIT
M. tuberculosis complex	7	7
M. avium complex	9	8
M. abscessus	2	2
M. scrofulaceum	2	0
M. kansasii	1	0
M. chelonae	1	0
M. fortuitum	1	0
M. peregrinum	1	0
M. gordonae	1	0

Table 4 shows a comparison of the species of NTM isolated at Hospital C, a 500+ bed community teaching hospital. The data shown from 2013 list all of the NTM isolated from January through June of that year using the MGIT System. The data from 2014 lists the NTM isolated from January through June of that year using the VersaTREK System. Specimens were processed in the same manner and did not include any blood or urine specimens. Isolates obtained only from the liquid media are shown. Isolation rates of *M. tuberculosis* and MAC during these two time frames were comparable as were the number of specimens cultured. In 2013, there were 18 isolates of NTM obtained from the MGIT from five different species. From the VersaTREK Myco bottle there were a total of 32 isolates of NTM from 10 different species and 2 isolates of *Gordonia* spp.

Table 4: Historical Comparison of the Isolation of NTM from Hospital C MGIT vs. VersaTREK Myco

MGIT Jan. to June 2013		VersaTREK Jan. to June 2014	
Species	**No. Isolates**	**Species**	**No. Isolates**
M. abscessus	6	*M. gordonae*	8
M. gordonae	5	*M. abscessus*	7
M. xenopi	3	*M. kansasii*	3
M. fortuitum	3	*M. fortuitum*	3
M. nonchromogenicum	1	*M. mantenii*	3
		M. nebraskense	2
		M. nonchromogenicum	2
		M. flavescens	2
		M. mucogenicum	1
		M. lentiflavum	1
		Gordonia sp.	2

Discussion

While tuberculosis is still the cause of significant morbidity and mortality worldwide, progress has been made in controlling the disease. The WHO's Stop TB Strategy[8] called for a 50% reduction in TB-attributable deaths from 1990 to 2015. As of 2013, there has been a 45% reduction in TB deaths. It also has the goal of eliminating TB as a public health problem by 2050. However with the decline in cases of tuberculosis we are seeing an increase in disease caused by species of the non-tuberculosis mycobacteria. Besides medical reasons for this, we have seen an improvement in technology which allows for the isolation and identification of the increasing number of NTM species.

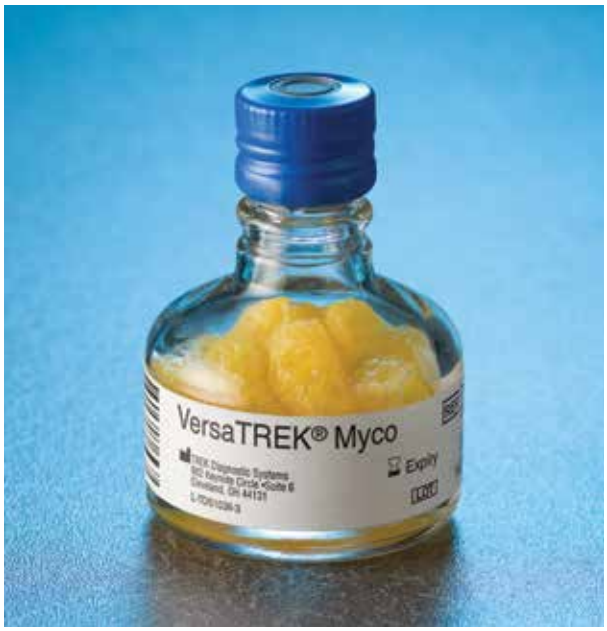
With the increasing importance of the NTMs as a cause of infection it is critical that laboratories have the capability of isolating these organisms from clinical specimens. It has been the recommendation that cultures for mycobacteria consist of both liquid and solid media[3]. It has been the experience of many mycobacteriologists that the use of liquid cultures increases the yield of both *M. tuberculosis* and the NTMs. It is therefore of upmost importance that we obtain a system capable of isolating the NTMs.

There have been no definitive studies comparing the BD MGIT System with the Thermo Scientific VersaTREK Automated Microbial Detection System. There was one report describing an increase in the isolation of NTMs using the VersaTREK as compared to the MGIT[9]. In this current report, both historical data and a validation study point to an increase of isolation rates of NTM using the VersaTREK System as compared to the MGIT. In the first investigation, historical data compared the isolation rates of a teaching hospital from the Lower West Side of Manhattan with a large teaching hospital with a transplant service on the Upper East Side of Manhattan. Specimens were processed in the same manner at both hospitals.

With the patient population of the Upper East Side hospital, it would be anticipated that more isolates of NTM would be obtained at this large teaching hospital. The validation study conducted at this teaching hospital did show discrepancies in the isolation of NTMs from the same specimen. In this study, several NTMs were not detected in the MGIT System. Although this was a limited study there were no NTMs detected in the MGIT that were not detected in the VersaTREK System.

Lastly, a comparison was performed at another institution looking at the isolation rates of NTM from a six month period with the MGIT and the same six month period with the VersaTREK the following year. The methods employed to process specimens were exactly the same during these two time periods. Twice as many species and almost twice the number of NTM isolates were recovered from the VersaTREK as compared to the MGIT.
There were no differences in the isolation rates of *M. tuberculosis* or MAC between these two systems.

All of these studies indicate that the VersaTREK System detects more species of NTM and in greater numbers that does the MGIT System. Both direct comparison and historical studies yielded the same results. Based on this data the VersaTREK System is more reliable than the MGIT System for the growth and detection of NTMs from clinical specimens.



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