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Original Article

Non-tuberculous mycobacteria profiles and their anti-mycobacterial resistance at a major medical center in Lebanon

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Abstract

Introduction: Infection with non-tuberculosis mycobacteria (NTM) has been on the rise globally causing a wide spectrum of respiratory and extrapulmonary infections in humans. Studies on these pathogens from the Middle-East including Lebanon are scarce.

Methodology: This retrospective study addresses the approach used for investigation, speciation and antimicrobial resistance (AMR) profiles of recovered NTM isolates from respiratory sources at a major tertiary care center in Lebanon during two periods (2003-2007 and 2013-2017). Processing of specimens, culture and differentiation of recovered NTM isolates from *Mycobacterium tuberculosis* were done in-house according to standard procedures. Upon request, speciation and AMR testing were performed using molecular and broth dilution methods, respectively, at Mayo Medical Laboratories (Rochester, Minnesota, USA).

Results: Among 108 NTM analyzed isolates, 8 species were revealed during the two periods: *M. simiae* (51% *vs* 61%), *M. avium* complex (MAC) (6 % *vs* 12%) *M. fortuitum* (12% *vs* 5%), *M. gordonae* (6% *vs* 5%), *M. abscessus* (6% *vs* 7%), *M. immunogenum* (12% *vs* 0%), *M. szulgai* (4% *vs* 0%) and *M. peregrinum* (0% *vs* 2%). *M. simiae* isolates showed high susceptibility (93%-96%) to amikacin and clarithromycin, but high resistance to rifampin, ethambutol, ciprofloxacin, rifabutin, linezolid, trimethoprim/sulfamethoxazole and moxifloxacin. MAC isolates were only susceptible to clarithromycin (86%). *M. abscessus* isolates were uniformly susceptible to amikacin (100%).

Conclusion: The revelaed different NTM species, with predominance of *M.simiae* and various AMR profiles provide a current epidemiologic database and help guiding the selection of appropriate empirical therapy once the clinical relevance is established.

Key words: Nontuberculous mycobacteria; *Mycobacterium simiae*; antimicrobial resistance; atypical mycobacteria; Lebanon.

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Introduction

Non-tuberculosis mycobacteria (NTM), also known as mycobacteria other than tuberculosis (MOTT), are free living, non-motile, acid fast bacilli bacteria. They are found ubiquitously in the environment including soil, water, dust, and animal sources [1]. In 1959, Runyon categorized NTM into 4 major groups: photochromogens, scotochromogens, non-chromogens and rapid growers [2]. To date, more than 170 species of NTM have been identified globally, using biochemical testing and gene sequencing [3]. (http://www.bacterio.net/mycobacterium.html). NTM can cause disease in both immunosuppressed and immunocompetent hosts as well as those suffering from chronic diseases. Though pulmonary disease is the most common manifestation, NTM can also cause disseminated, lymphadenitis and cutaneous diseases [4,5].

Studies on the prevalence and incidence of NTM are rare, since diseases caused by these pathogens are considered non-communicable. However, since 2002, increasing global reports about NTM have indicated that *M. avium* complex (MAC) is the predominant species in North America and East Asia, while *M. kansasii, M. xenopi*, and *M. malmoense* are more common in Europe [6].

In the Middle East, a few epidemiological studies on the prevalence of NTM species were reported. For example, *M. xenopi* and *M. simiae* are the most common in Israel [7], *M. fortuitum* and *M. simiae* are the most common in Iran [8], while MAC and *M. abscessus* / *chelonae* predominate in Turkey [8].

In Lebanon, a couple of published articles addressed NTM, mostly as case reports [9–14] but none has comprehensively revealed the present species nor their antimicrobial resistance (AMR) profiles. Besides, the recovery of NTMs has been increasing, with rates reaching 55 to 67% among isolated mycobacteria, as noted for instance in our medical center over the last five years. Thus, this study warranted addressing the approach used in the clinical laboratory testing for the investigation of NTM isolates, as well as revealing the

predominant species and reporting the AMR profiles of NTM isolates recovered from respiratory sources at the Clinical Microbiology Laboratory (CML) of a major Lebanese tertiary care center.

Methodology

Patients and NTM data

This is a retrospective study where the data was generated from investigating Lebanese patients' respiratory specimens (sputum, bronchoalveolar lavage, and/or deep tracheal aspirate) submitted for mycobacterial investigation to the CML of the Department of Pathology and Laboratory Medicine (PLM) at the American University of Beirut Medical Center (AUBMC) during the study periods: 2003-2007 and 2013-2017. The PLM laboratory has been accredited by the College of American Pathologists (CAP) since 2004. The two study periods, a decade apart, were analyzed and compared to determine possible changes in NTM prevalence, and species predominance with time**.**

Culture and identification of mycobacterial isolates

Processing of specimens for mycobacterial culture was done according to their source: sterile specimens were directly processed, whereas non-sterile ones underwent a digestion-decontamination procedure. The processed specimens were inoculated into both a Middlebrook 7H9 broth (Mycobacterial Growth Indicator Tube- MGIT) and a solid based medium (Lowenstein-Jensen), as reported previously [13]. The recovered isolates were identified using MGIT TBc identification test (Becton Dickinson, Sparks, USA) which differentiates *Mycobacterium tuberculosis* (MTB) from NTM. The quality control of testing was ensured using the reference *M. tuberculosis* strain (H37Rv, ATCC 27294).

Speciation of NTM

Speciation of NTM was performed upon the physician's request. If no susceptibility was requested, the NTM isolates were speciated by an in-house developed 16S rDNA sequencing; otherwise, the isolates were referred to Mayo Medical Laboratories (Mayo Clinic, Rochester Minnesota, USA) for both speciation and susceptibility testing. In this case, speciation was done using nucleic acid probes, MALDI-TOF Mass Spectrometry and/ or 16S rDNA sequencing.

Susceptibility testing of non-tuberculosis mycobacteria (NTM)

Susceptibility testing of NTM was also done upon the physician's request. The isolates were tested at Mayo Medical Laboratories using the microtiter broth dilution method (Sensititre, Thermo Scientific,TREK Diagnostic Systems Inc, Oakwood Village, OH, USA), and the results were interpreted according to the antimicrobial panel used for the determined species as presented in Table 1 (according to CLSI guidelines).

Table 1. Panel of antimicrobials and their breakpoints susceptibility used for testing against NTM at Mayo Medical Laboratories.

Antibiotics	Rapid growers	Slow growers		
		MAC	***M. kansasii***	***M. marinum***
	Breakpoints MIC S/ I/ R			
Amikacin	≤16 / 32 / ≥ 64	NI	≤ 32 / - / >32	≤ 32 / - / > 32
Cefoxitin	≤ 16 / 32-64 / ≥ 128			
Ciprofloxacin	≤ 1 / 2 / ≥ 4		≤ 2 / - / > 2	≤ 2 / - / > 2
Clarithromycin	≤ 2/ 4 / ≥ 8	≤ 8 / 16 / ≥ 32	≤ 16 / - / > 16	≤ 16 / - / > 16
Doxycycline	≤ 1 / 2-8 / ≥ 16			≤ 4 / - / > 4
Ethambutol		NI	≤ 4 / - / > 4	≤ 4 / - / > 4
Imipenem	≤ 4 / 8 / ≥ 16			
Linezolid	≤ 8 / 16 / ≥ 32	≤ 8 / 16 / ≥ 32	≤ 16 / - / > 16	
Moxifloxacin	≤ 1 / 2 / ≥ 4	≤ 1 / 2 / ≥ 4	≤ 2 / - / > 2	≤ 2 / - / > 2
Rifabutin		NI	≤ 2 / - / > 2	≤ 2 / - / > 2
Rifampin		NI	≤ 1 / - / > 1	≤ 1 / - / > 1
Streptomycin		NI	NI	
Tobramycin	≤ 2/ 4 / ≥ 8			
TMP/SMX	≤ 2/38 / - / ≥ 4/76		≤ 2/38 / - / > 2/38	≤ 2/38 / - / > 2/38

MAC: *M. avium* complex, MIC: minimum inhibitory concentration, S: susceptible, I: Intermediate, R: Resistant, TMP/SMX: Trimethoprim-Sulfamethoxazole; The testing is carried out on species depending on being rapid or slow growers of NTM. Though few species of slow growers are cited, the susceptibility for other slow growers are tested against the *M. kansasii* antimicrobial panel.

Table 2. Distribution of different MOTT species among isolates requested for speciation at AUBMC: 2003-2007 and 2013-2017.

MOTT species		No (%) of species detected at the different study periods	
		2003-2007 (n = 51)	2013-2017 (n = 57)
Slow growers	*M. simiae*	26 (51)	39 (68)
	MAC	3 (6)	7 (12)
	M. gordonae	3 (6)	3 (5)
	Others	8 (16) *	1 (2) **
Rapid growers	*M abscessus*/*chelonae*	3 (6)	4 (7)
	M. fortuitum	8 (16)	3 (5)

* *M. immunogenum, M. szulgai*; ** *M. peregrinum*, MAC: *M. avium* complex.

Results

Rates of NTM vs TB recovery

The rate of NTM recovery from respiratory tract samples during the two study periods, 2003-2007 and 2013-2017, were 3.5% (3387 total tested) and 4.7% (6543 total tested), respectively. The proportion of recovered MTB against NTM differed between the two study periods. During 2003-2007, the recovery of MTB was higher than that of NTM (average: 62.5% *vs* 37.5%), while in 2013-2017 the trend shifted in favor of NTM (average: 46% *vs* 54%).

Different species of recovered NTM isolates

Overall, 108 NTM isolates were requested for speciation, revealing 8 species, as shown in Table 2. *M. simiae* was the predominant NTM species recovered during both study periods, with increasing rates noted in the second period: 51% in 2003-2007, and 68% in 2013-2017. MAC ranked the second in predominance and showed doubling rates in the second period: 6 % in 2003-2007, and 12% in 2013-2017. *M. gordonae* and *M. abscessus* recovery rates remained very close (5 - 7%) among the two periods. *M. fortuitum* recovery decreased from 12% in 2003-2007 to 5% in 2013-2017. Other species were recovered during only one of the two study periods: *M. immunogenum* (12%), *M. szulgai* (4%) and *M. peregrinum* (2%).

Age and Gender

The age of the patients from which the NTM isolates were recovered ranged between 20 and 87 yrs. Over half of them (57%) fell within the age range of 50 to 80 years. The overall gender distribution shows more NTM recovery from men than women (ratio 1.4:1). This gender difference was noted in the recovery of *M. simiae* (55% *vs* 44%), MAC (80% *vs* 20%), *M. gordonae* (100% *vs* 0%), M. *immunogenum* (67% *vs* 33%), and M. *fortuitum* (55% *vs* 45%), but the trend was reversed for *M. abscessus*/ *chelonae* (29% *vs* 71%).

Antimicrobial susceptibility

The Mayo Clinic panel of antimicrobial agents used against rapid and slow growing NTM isolates, together with the breakpoint interpretation are presented in Table 1. Though the isolate numbers are humble, the susceptibility test findings of different antimicrobials against *M. simiae*, MAC and *M. abscessus* requested for testing in the study period of 2013-2017 are presented in Table 3.

Table 3. Antimicrobials in vitro susceptibility of tested MOTT isolates at AUBMC during the period of 2013-2017.

Antimicrobials	Percentage of susceptible strains among		
	M. *simiae* (n = 24)	*MAC* (n = 7)	M. *abscessus* (n = 5)
Amikacin	93	NA	100
Clarithromycin	96	86	67
Moxifloxacin	30	0	0
Trimethoprim-Sulfamethoxazole	7	NA	0
Linezolid	7	0	0
Rifabutin	7	NA	NA
Ciprofloxacin	0	NA	0
Ethambutol	0	NA	NA
Rifampin	0	NA	NA
Cefoxitin	NA	NA	0
Doxycycline	NA	NA	0
Imipenem	NA	NA	0

NA: Not available.

M. simiae isolates were uniformly resistant to rifampin, ethambutol, and ciprofloxacin. Most isolates were also highly resistant (93%) to rifabutin, linezolid, trimethoprim/sulfamethoxazole, with lower resistance for moxifloxacin (70%). Amikacin and clarithromycin were highly active (93%-96%) against *M. simiae* isolates. MAC isolates were only susceptible to clarithromycin (86%). Among the 5 tested isolates of *M. abscessus*, all were susceptible to amikacin (100%), while being uniformly resistant to the other tested drugs (Table 3).

Discussion

Generally, data on the prevalence and incidence of NTM species are rare. This is probably related to NTM being non-communicable pathogens, in addition to the lack of diagnostic tools and specialized labs to isolate and speciate these organisms.

In our study, the recovery rates of NTM compared to MTB showed a substantial increase with time during the two study periods: from an average of 37.5% in 2003-2007 to an average of 54% in 2013-2017. This goes along similar trends of increasing NTM rates noted in different parts of the world e.g. the United States, Europe, the Middle East, Australia and Asia [6].

Globally, the prevalence of NTM species differs among continents, regions and even different areas within the same country [15]. In Lebanon, the most commonly recovered respiratory NTM species, as revealed in this study, were *M. simiae* (58%), followed by *M. avium* complex (10%), *M. abscessus* (8%) and *M. fortuitum* (8%). These species will be the subject of our discussion in relation to what is reported by other studies, especially from our region.

M. simiae is a slowly growing photochromogen that was first isolated in 1965 from rhesus monkeys. Later on, it has been mostly recovered from water sources. It can colonize the respiratory tract and cause infections in both the immunocompromised and immunocompetent. In humans, this species was first reported from San Antonio, Cuba and Israel. [16–20]. Though *M. simiae* recovery among NTM species from pulmonary infections has been reported worldwide with varying frequency, rates reported from Israel (24%) [7], Iran (24%) [8] and India (22%) [21] ranked among the highest. Similarly, our study revealed increasing recovery rates of *M. simiae*, with time: 51% in 2003-2007 to 68% in 2013-2017. This species has been associated with humid weather and water niches, as reported for Cuba, Arizona, Texas, Iran, Gaza and Israel [13]. The humid weather in Lebanon, a Mediterranean costal country, may also explain the high prevalence of *M. simiae* in this area of the world [13,14]. Few other countries in the Middle East and the Gulf region reported on the recovery of this species, albeit with lower rates such as Oman (8%) and Turkey (2.1%) [8].

The members of the *M. avium* complex (MAC) also belong to the slow growing NTM. They are ubiquitous, and can be readily recovered from soil water, animals, and foodstuffs [4]. Globally, MAC has been reported to be the most common cause of pulmonary disease due to NTM. The contribution of MAC among reported NTM pulmonary isolates varies from 31% in South America to 37 % in Europe, 51-54% in South Africa, North America, and Asia reaching up to 71 % in Australia [15]. These rates are higher than what was revealed in our study though the recovery rate of MAC isolation has doubled from 6% in 2003-2007 to 12% in 2013-2017, thus ranking second after *M. simiae* among NTM isolates recovered from Lebanon. Comparing the latter MAC recovery data from Lebanon to those reported from other Middle Eastern and Gulf countries reveals higher rates than those from Israel (5.5%) [7], and Iran (6%), similar rates to those from Saudi Arabia (13%), and Kuwait (14%) and lower ones than those reported from Turkey (19%) and Oman (69%) [8].

The recovery of rapid growing NTM, mainly *M. fortuitum* and *M. abscessus*, from respiratory specimens also differs among several regions of the world. For example, in the present study, the 7% prevalence of *M. abscessus*/ *chelonae* was close to that reported from Europe (6%-9%) [22], Israel (9.7%) [7], USA and Canada (3%-11%) [6,22] but lower than the rates reported from the Arabian Gulf and Turkey (19%-27%) [8], as well as those from Asia and Brazil (18%-30%) [8,22,23]. *M. abscessus* is the most pathogenic of the rapid growing mycobacteria mostly associated with pulmonary infections, especially in patients with underlying lung disease. It can also cause hematogenously disseminated diseases, surgical wound infections and keratitis [5]. *M. fortuitum* is relatively frequently recovered from different parts of the world. For example, in the Gulf countries (Saudi Arabia, Kuwait, and Iran), they ranged between 31% and 74% while in Turkey and Pakistan their range was 17%-20 % [8]. In Europe, a high prevalence was noted in Greece (63%) while lower rates (2-14%) were reported from other European countries [22]. *M. fortuitum* causes human infections primarily by direct inoculation, including primary skin and soft tissue infections, surgical wound infections, and catheter-related sepsis [5]. Pulmonary disease caused by *M. fortuitum* is rare and most *M. fortuitum* respiratory isolates correspond to patients with underlying pulmonary diseases and

represent colonization or transient infections that don't warrant treatment.

Other NTM species as those recovered in low numbers from our patients i.e. *M. szulgai*, *M. immunogenum* and *M. perigrinum* or those that have not been recovered in our study but were reported elsewhere such as *M. kansasi* and *M. xenopi,* reflect again the regional diversity of NTM species inhabitance [15].

The treatment of NTMs infection is challenging, costly and lengthy. This is mostly attributed to the variety of species involved and their intrinsic resistance to various drugs [5]. Therefore, in addition to knowing the causative NTM species of infection, the appropriate treatment approach necessitates relying on available susceptibility testing for such pathogens. However, the anti-mycobacterial susceptibility testing against NTM isolates remains to suffer from the absence of consensus on standard methodology and guidelines. This, together with the lack of clinical evidence to correlate the *in vitro* testing results with clinical response, constitutes major challenges to physicians in caring of patients infected with NTM [5]. Globally, few reference laboratories have offered *in vitro* anti-mycobacterial susceptibility testing services against NTM isolates and for our laboratory, Mayo Clinic has been the reference lab.

During the two study periods, there were 108 NTM isolates requested for speciation. Susceptibility records were only available from 2013-2017 and included the testing of 39 speciated isolates. In order to reflect relevance, the NTM antimicrobial susceptibility data generated among our tested *M. simiae*, *M. abscessus* and MAC isolates from Lebanon will be discussed and compared to what was reported from other countries.

In our study and among *M. simiae*, only two of the 11 tested antimicrobial agents were associated with high susceptibility: clarithromycin (96%) and amikacin (93%) while the remaining tested agents revealed high resistance (70%-100%). Compared to studies from other countries, the 96% susceptibility to clarithromycin in our study was close to that reported from USA (91%) [24] and Réunion Island (100%) [25], while other countries reported very low susceptibility i.e. Iran (0%) [26], UK (19%) [27], and Netherlands (25%) [28]. For Amikacin, the 93% susceptibility rate found in our isolates was comparable to that from Iran (86%) [26], UK (89%) [27] and Réunion Island (87%) [25] but higher than that reported from USA (14%) [24] and Netherlands (0%) [28]. A relatively high susceptibility to other antimicrobial agents was reported for rifabutin (88%) from Iran [26] and for ciprofloxacin from UK [27] and Réunion Island [25] (64% and 87%, respectively).

In relation to MAC, the 86% susceptibility rate to clarithromycin among the tested isolates in this current study from Lebanon was close to that reported from different parts of the world: USA (99%) [29], England (80%) [27] , Sweden (97%) [30], China (91%) [31], Japan (75%) [32], Taiwan (93%) [33], as well as Pakistan [34] and India (100%, each) [35]. Variation, however, was observed among the susceptibility rates of moxifloxacin and linezolid. For example, the uniform resistance to moxifloxacin among the current isolates from Lebanon mirrored the susceptibility rate reported in Taiwan (4%) [33]. Very low susceptibility rates were also reported from Sweden (15%) [30] and Pakistan (17%) [34]. However, relatively susceptible isolates were reported from Greece (36%) [36], USA (<50%) [37], Netherlands (78%) [28] and China (86%) [31]. Such a wide range of variation in susceptibility was also noted for linezolid: current study from Lebanon (0%), Greece (4%) [36] Taiwan (13%) [33], Sweden (16%) [30], USA (<50%) [37], Pakistan (67%) [34] and China (93%) [31].

The *M. abscessus* susceptibility rates in our study showed a wide range of variation among the 11 tested antimicrobials (Table 3), with noted susceptibility only to amikacin and clarithromycin. The 67% clarithromycin susceptibility rate among the isolates from Lebanon was higher than the 15% and 20% reported from Korea [38] and Iran [26], respectively, close to that reported from Netherlands (62%) [28], but lower than the susceptibility rates reported from Turkey (86%) [39], UK (94%) [40], China (95%) [31], Japan (80%) [41], Pakistan (100%) [34] and Taiwan (93%) [42]. On the other hand, the 100% susceptibility to amikacin in the current study was comparable to that reported from the aforementioned countries (86%-100%) except for that reported from Netherlands (5%) [28] and Korea (68%) [38].

Though this is the first comprehensive study addressing the species profiles and susceptibility testing of NTM isolates recovered from respiratory sources in Lebanon, its limitation is entailed in the fact that the tested isolates were those only based on physicians' requests at one center. Thus, carrying out a multicenter nationwide surveillance on NTM, without restrictions, is warranted to reflect the overall status of NTM in Lebanon, especially in correlation with epidemiologic and clinical significance.

It is worth mentioning that, although the increase in NTM infections concerns mainly the immunocompromised populations, their anticipated

deleterious effect on healthy individuals can also constitute a threat. This is due to the increasing popular trend of water pipe "nergilleh" smoking, especially among young adults, where the recovery of NTM isolates would constitute an alarming threat [43].

Conclusion

In our study, the predominance of *M. simiae*, and the low prevalence of other NTM species reveals a different picture from the prevalence rates and profiles of NTM species reported from the region and other countries. Similarly, differences in AMR profiles are also noted for the NTM species in this study compared to those reported from other countries. Such findings would be currently helpful to physicians, in addition to being an informative epidemiologic database to compare future studies with, and guide the selection of appropriate empirical therapy once the clinical significance of these infections is established.

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