Epidemiology and Clinical Significance of Non-Tuberculous Mycobacterial Isolates in a university hospital in a ten year period

F. Kontos, S. Pournaras

Laboratory of Clinical Microbiology, Attikon Hospital, University of Athens, Greece

P1610

Contact information: Fanourios Kontos
Tel, +30 210 5831900; Email: fankon@gmail.com

OBJECTIVES

Non-tuberculous mycobacteria (NTM) are considered emerging pathogens implicated in lung, lymph node, skin/soft tissue or disseminated infection. This retrospective study assessed the microbiological characteristics and clinical relevance of NTM isolates recovered from patients in Attikon University Hospital, Athens, Greece over the decade 12/2006 – 11/2016.

MATERIALS AND METHODS

Clinical specimens were processed by standard methodology and inoculated into L–J slants, in MGIT960 tubes (Becton Dickinson) or Myco/ F-Lytic (Becton Dickinson) vials (blood, bone marrow). NTM were identified with Genotype Mycobacterium CM and AS (Hain-Lifescience), while 16S rRNA and *hsp65* gene sequencing were applied when necessary [1,2]**. For diagnosis, the criteria of the American Thoracic Society/Infectious Diseases Society of America (ATS/IDSA) 2007 were applied to determine the clinical relevance of recovered isolates** [3]**.**

RESULTS

Number	NTM species	Respiratory specimens	Extrapulmonary specimens	Total (%)	Percentage (%) in 10 European Union countries (according to ref 4)
	SGM (n=144)				
1	*M. lentiflavum*	45	1	46 (24.2)	1.8
2	*M. avium*	28	9	37 (19.5)	23.9
3	*M. gordonae*	14	2	16 (8.4)	18.2
4	*M. kansasii*	5	1	6 (3.2)	3.7
5	*M. intracellulare*	16		16 (8.4)	9.4
6	*M. chimaera*	4		4 (2.1)	0.6
7	*M. yongonense*	3		3 (1.6)	-
8	*M. paraintracellulare*	1		1	-
9	*M. marseillense*	1		1	-
10	*M. marseillense* + *M. fortuitum*	1		1	-
11	*M. celatum*	2		2	<1
12	*M. marinum*		2	2	1.3
13	*M. arupense*	2		2	0.6
14	*M. xenopi*	2		2	8.7
15	*M. simiae*	1		1	0.9
16	*M.simiae* + *M. intracellulare*	1		1	
17	*M. mantenii*		1	1	-
18	*M. malmoense*	1		1	2.1
19	*M. shimoidei* + *M. fortuitum*	1		1	<0.1
20	RGM (n=46)				
21	*M. fortuitum*	20	1	21 (11%)	7.6
22	*M. chelonae*	6	4	10 (5.3%)	3.3
23	*M. abscessus*	1	1	2	3.2
24	*M. abscessus* + *M.intracellulare*	1		1	
25	*M. conseptionense*	2		2	-
26	*M. monacense*	2		2	<0.1
27	*M. elephantis*	1	1	2	<0.1
28	*M. bolletii*		1	1	-
29	*M. massiliense*	1		1	-
30	*M. phlei*	1		1	<0.1
31	*M. peregrinum*	1		1	<0.1
32	*M. canariasense*	1		1	-
33	*Mycobacterium spp.*	1		1	-
Total		165	25	190	

Table 1. Distribution of different NTM species from pulmonary and extrapulmonary specimens

- ***M. avium, M. intracellulare, M. chimaera, M. yongonense, M. marseillense and M.paraintracellulare* are members of *M. avium complex (MAC).***
- ***M. abscessus, M.bolletii and M.massilliense* are members of *M. abscessus complex***
- **SGM, slow-growing mycobacteria; RGM, rapid-growing mycobacteria**

RESULTS

- ✓ During the study period, 314 NTM isolates from 190 different patients were recovered (190 strains)
- ✓ From 186 (97.9%) patients, a single NTM species was recovered (Table 1)
- ✓ For 4 (2.1%) patients, two different NTM species were recovered (Table 1)
- ✓ 189 strains belonged to 27 known *Mycobacterium* species (Table 1)
- ✓ One strain did not belong to any of the officially recognized mycobacterial species (Unidentifiable Mycobacteria - UNM)
- ✓ Between NTM, 144 were slowly growing (SGM) and 46 were rapid growing mycobacteria (RGM) (Table 1)
- ✓ The most frequent NTM were *M. lentiflavum* , *M. avium* , *M. fortuitum*, *M. intracellulare* , *M. gordonae*, *M. chelonae* and *M. kansasii*, which represented 81% of all NTM strains (Fig 1)
- ✓ MAC strains represented 34.2% of all strains

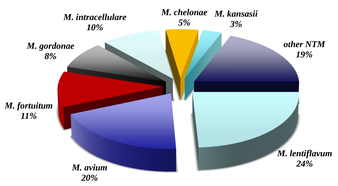


Fig 1. Distribution of the most frequently isolated NTM species

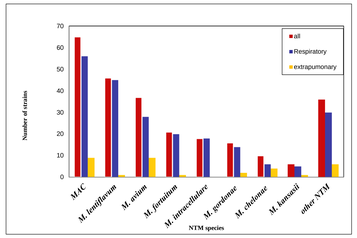
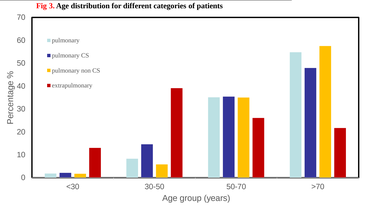


Fig 2. Distribution of most frequent isolated NTM species in pulmonary and extra-pulmonary specimens. Moreover the number of strains belong to MAC were also included.

- ✓ For 166 (87.3%) patients, NTM were recovered from respiratory and for 24 (12.7%) from extrapulmonary specimens (Table 2).
- ✓ NTM recovered from respiratory specimens belonged to 25 species and those from extrapulmonary specimens to 11 species
- ✓ There were 122 men and 68 women; median age was 66.2 years [17-92].
- ✓ Patients with pulmonary NTM were significantly older than those with extrapulmonary isolation (64 vs 51.7 years, p<0.05)
- ✓ 16 patients were HIV-positive

Fig 3. Age distribution for different categories of patients



Characteristics	Pulmonary (n=166)	Pulmonary clinically significant (n=48)	Pulmonary non clinically significant (n=118)	Extrapulmonary (n=44)
Median age, years (range)	68.5 (17-92)	64 (20-90)	70 (17-92)	51.7 (23-76)
Sex male/female	106/60	31/17	75/43	16/8
HIV infected	8	7	1	8

Table 2. Characteristics of 190 patients included in the study

- ✓ For 48 of 166 patients (29.1%), the NTM isolates (13 species) recovered from respiratory specimens were considered as clinically significant according to ATS criteria (Table 3)
- ✓ For 27 (56.3%) of them, smears were positive for acid-fast bacilli.
- ✓ The most frequent clinically significant NTM from respiratory specimens were *M.avium*, *M.intracellulare*, and *M.kansasii*
- ✓ *MAC* represent the 70.8 % of clinically significant strains
- ✓ Also the 64.2% of MAC strains recovered from respiratory specimens were clinically significant (Fig 4)
- ✓ All *M. kansasii* and *M. celatum* strains and the recently described species (2013) *M. yongonense* considered as clinically significant (Fig 4)

No	SPECIES	Total number of strains recovered from respiratory specimens (RS)	Number of strains considered as clinically significant (% of RS strains)	Number of patients with at least one Z-N (+) specimens	Number of HIV(+) patients
1	*M. avium*	28	18 (64.3)	9	6
2	*M. intracellulare*	16	10 (62.5)	5	
3	*M. kansasii*	5	5 (100)	4	1
4	*M. yongonense*	3	3 (100)	2	
5	*M. celatum*	2	2 (100)	1	
6	*M. chimaera*	4	2 (50)	1	
7	*M. fortuitum*	20	2 (10)	0	
8	*M. lentiflavum*	45	1 (2.2)	1	
9	*M. intracellulare* + *M.simiae*	1	1	1	
10	*M. abscessus* +*M.intracellulare*	1	1	1	
11	*M. malmoense*	1	1 (100)	1	
12	*M. marseillense*	1	1 (100)	1	
13	*M. chelonae*	4	1 (25)	0	
	Total	131	48	27	7

Table 3. Characteristics of the 48 clinically significant NTM strains recovered from respiratory specimens

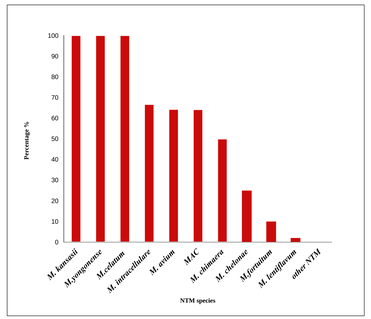


Fig4. Clinical relevance of pulmonary NTM isolates, per species. Percentage (%): Number of strains considered as clinically significant/ total number of pulmonary NTM isolates. Moreover the number of all strains belong to MAC were also included.

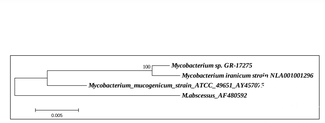


Fig 5. Phylogenetic relationships of the *Mycobacterium* spp. recovered during this study with other *Mycobacterium* type strains based on 16S rDNA gene. Tree was constructed using the neighbor-joining method by MEGA 6.0 software. Bootstrap values were calculated for 1000 replicates. *M. abscessus* was used as an out group. Bar indicates numbers of substitutions per nucleotide position.

Table 4. Details of the Mycobacterium spp. strain recovered during this study. This strain had unique hsp65 and 16S rDNA sequences.

Strain no	Specimen	Characterization	Results of GenBank comparison (differences,%)	
			16S rDNA	*hsp65*
GR-17275	Sputum	Rapidly growing scotochromogenic	*M. iranicum* 1364/1371 (99.5)	*M. iranicum* 316/335 (94.3)

- ✓ **Mycobacterium strain GR-17275 is closely related to the recently described species (2013) *M. iranicum***

- ➢ For 19 of 24 patients (76%), the NTM isolates (8 species) recovered from extra-pulmonary specimens were considered as clinically significant
- ➢ For 6 of them, smears were positive for acid-fast bacilli
- ➢ The most frequent species was *M. avium* (47.4%)
- ➢ Six patients suffered from skin and soft tissue infection
- ➢ 15 patients were immunocompromised (8 with AIDS)
- ➢ 11 patients (8 with AIDS) suffered from disseminated disease

No	Type of specimen	Number of patients	Smear positives	Species	Number of patients with immunosuppression/ HIV(+) patients
1	Blood	6	-	*M. avium*	6/6
2		2	-	*M. chelonae*	3/0
3		1		*M. abscessus*	1/0
4		1		*M. gordonae*	1/0
5		1	-	*M. bolletii*	0
6	Ascetic fluid	1	-	*M. avium*	1/1
7	Synovial fluid	1	1	*M. kansasii*	0
8		1	1	*M. avium*	0
9	pus	2	2	*M. marinum*	1/0
10		1	1	*M. chelonae*	0
11		1	1	*M. avium*	1/1
12		1	0	*M. mantenii*	1/0

Table 5. Characteristics of the 19 clinically significant NTM strains recovered from extrapulmonary specimens

No	Type of specimen	Species (number)
1	Blood	*M.lentiflavum* (1)
2	Urine	*M.chelonae* (1)
3	Urine	*M.gordonae* (1)
4	Urine	*M. elephantis* (1)
5	Urine	*M. fortuitum* (1)
	Total	5

Table 6. Characteristics of the other 5 NTM strains recovered from extrapulmonary specimens

For 118 of 166 patients NTM isolates (21 species) recovered from respiratory specimens were not considered as clinically significant
The most frequent NTM were *M.lentiflavum* , *M.fortuitum* and *M.gordonae*

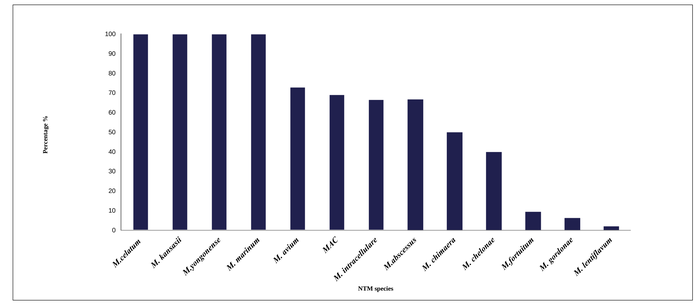


Fig 6. Clinical relevance of NTM isolates, per species. Included the most frequent species. Percentage (%): Number of strains considered as clinically significant/ total number of NTM isolates. Moreover the number of all strains belong to MAC were also included.

CONCLUSIONS

Conclusions: Only 35.3% of patients yielded NTM isolates (belonging to 18/28 of the species) that were linked to human disease. The most common clinically significant isolates were *M. avium* and *M. intracellulare*, which were responsible for 58% of NTM disease. Only 29% of patients with pulmonary NTM isolates met the ATS criteria, mainly because of inadequate sampling of a large number of individuals.

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