

Public Health Ontario | Santé publique Ontario

Not What They're Not but What They Are:

Nontuberculous Mycobacteria and NTM Infections:
The Laboratory

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TIBDN Education Day, Toronto, November 21st, 2019

Outline and objectives – Laboratory Perspective

1. Overview of TB and Mycobacteriology laboratory, Public Health Ontario and mycobacteria epidemiology in Ontario
2. The evolution and ecology of nontuberculous mycobacteria (NTM)
3. Laboratory diagnostic testing and identification of NTM
4. NTM drug susceptibility testing (DST)

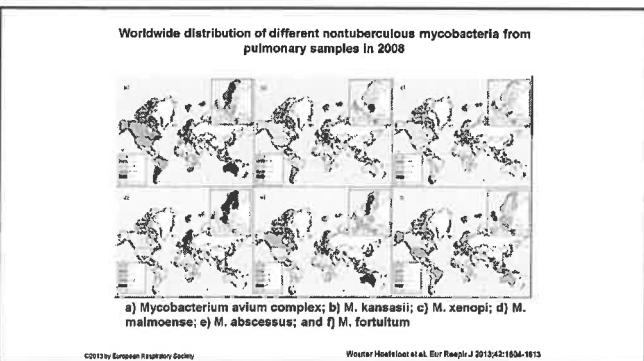
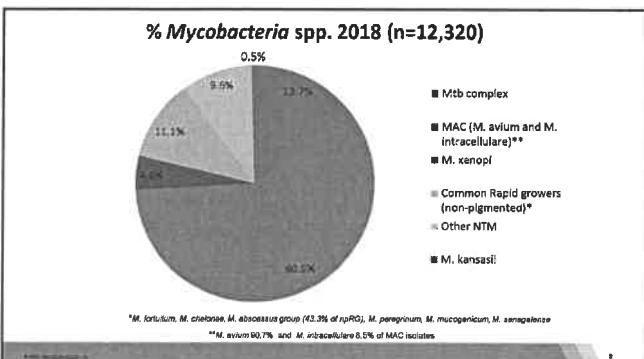
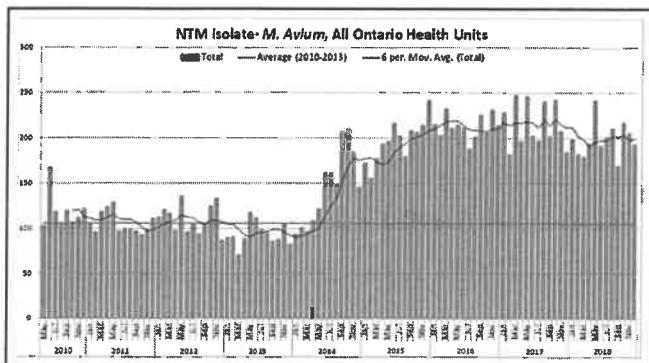
TB and Mycobacteriology Labs in Ontario

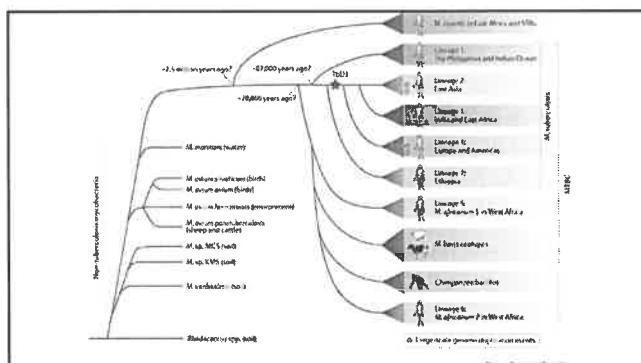
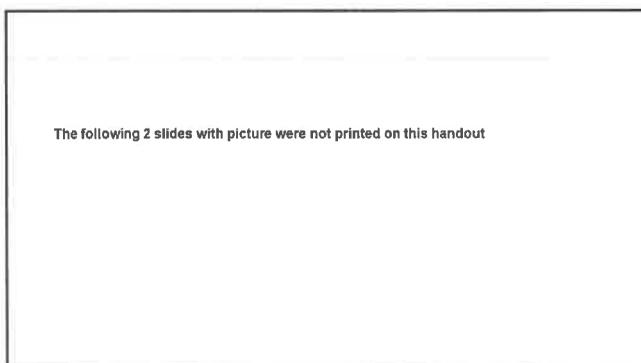
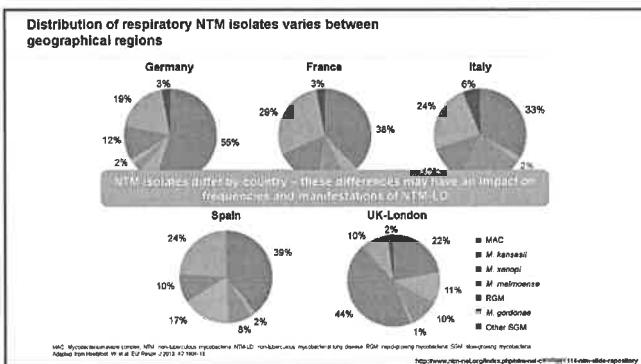
★ Public Health Ontario Lab Network
PHL-Toronto and 3rd Regional PHLs

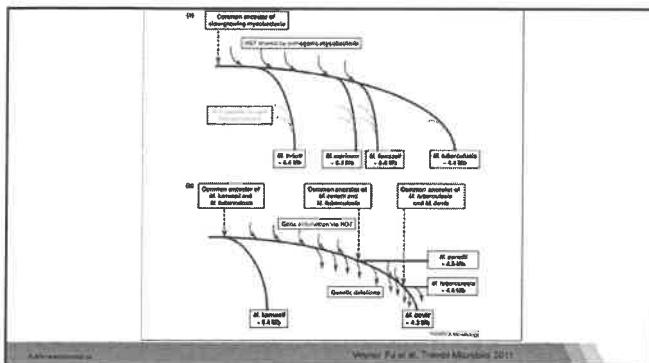
- ◆ Ottawa Hospital
- ◆ Dynacare (community lab)

*as of June 2019, Timmins lab no longer processing specimens









What are NTM?

NOT assigned to either¹:

- Mycobacterium tuberculosis complex
- Mycobacterium kansasii

Also known as:

- Environmentally mycobacteria
- Atypical mycobacteria
- Medical mycobacteria
- Mycobacteria other than tuberculosis

Non-tuberculous
Opportunistic

Non-pathogenic
Pathogenic

¹ Mycobacterium avium, MOTT, mycobacteria other than tuberculosis, NTM, non-tuberculous mycobacteria
 1 Neprina PJ, Dalsgaard J. Clin Infect Dis 2011; 52: 1511-22. 2 Schenkel R, et al. Pneumonia [Epub 2013; 2013: 889014].
 3 Orms RA, Ordway RS. Infect Control Hosp Epidemiol 2004; 25: 89-92. 4 http://www.ntm-net.org/sites/ntm-net-catalog/files/114_ntm-slide-repository_14

NTM vs. *Mycobacterium tuberculosis*: Key distinctions

NTM	<i>Mycobacterium tuberculosis</i>
Not obligate pathogens – normally live free in the environment ¹	Obligate pathogen: require host ²
Low virulence, not usually pathogenic in the absence of predisposing conditions ^{3,4}	Pathogenic ^{2,4}
Human-to-human transmission extremely rare, but some evidence of this in the cystic fibrosis community ⁵	Human-to-human transmission ²
Infection rates increasing, especially in developed countries ⁶	Infection rates decreasing, especially in developed countries ⁶
Large heterogeneous group of species ⁷	Mycobacterium tuberculosis complex contains small group of closely related subspecies ⁷

NTM and *Mycobacterium tuberculosis* differ in terms of pathogenicity, infection rates, and transmission routes⁸

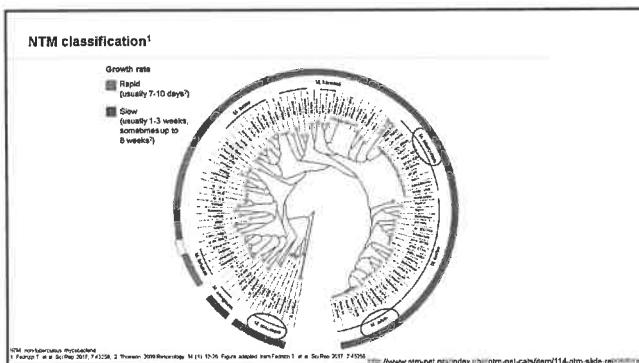
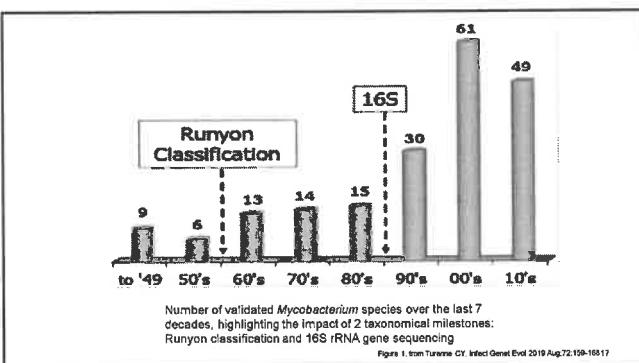
NTM: non-tuberculous mycobacteria
 1 Perea JP, et al. Clin Infect Dis 2004; 37: 89-98. 2 Porta E. Clin Microbiol Rev 2009; 22: 406-10. 3 Trich E, PEWD. Inflamm Allergy Identif 2008; 48: 159-78. 4 Michael P, J. Eur Clin 2015; 143: 1511-27. 5 Broz SK, et al. Int J Tuber Lung Dis 2014; 18: 1574-5. 6 Van Soolingen D, J. Clin Microbiol 2001; 39: 1-2. 7 See NTM-Net.org: http://www.ntm-net.org/sites/ntm-net-catalog/files/114_ntm-slide-repository_14

Classification of Mycobacteria

Four groups of human pathogens:

- M. tuberculosis* complex
- M. leprae*
- Slow-growing NTM
- Rapidly growing NTM

Runyon Group Number	Group Name	Growth Rate	Description
I	Photochromogens	Slow-grower	Pigmentation develops upon exposure to light Growth takes more than 7 days to appear on solid media <i>E.g. M. leprae, M. marinum</i>
II	Scotochromogens	Slow-grower	Pigmentation always present Growth takes more than 7 days to appear on solid media <i>E.g. M. scrofulaceum, M. szulgai, M. gordonae, M. xenopi</i>
III	Nonphotochromogens	Slow-grower	No, weak or late pigmentation, regardless of light exposure Growth takes more than 7 days to appear on solid media <i>E.g. M. avium, M. intracellulare, M. mucogenicum, M. kansasii</i>
IV	Rapid-growers	Rapid-grower	Colonies of NTM that appear on solid media in less than 7 days <i>E.g. M. chelonae, M. abscessus, M. fortuitum, M. smegmatis</i>



Species-level identification of NTM is clinically important

Identification

- Commercially available nucleic acid hybridization probes, e.g. HAIN®
- Based on cell wall protein analysis using mass spectrometry
- Based on analysis of rRNA 16S, 23S rRNA and other target sequences

Species identification is vital to determining the course of treatment and, in the case of recurrent infection, whether it is new or due to a relapse.

It is important to identify NTM-LD caused by *M. abscessus*.

The NTM species should be identified by molecular methods

CF: cystic fibrosis; HAIN: Mycobacterium complex; NTM: non-tuberculous mycobacteria; NTM-LD: non-tuberculous mycobacterial lung disease; PPA: pulmonary granulomatous disease; sputum: sputum, sputum inoculated and stained; T: Gribble DE, et al. Am J Respir Crit Care Med 2007; 175:362-63; 2 Verma AK, et al. Clin Infect Dis 2015; 61:294-3. 3 Jaiswal A, et al. Clin Infect Dis 2015; 61:207-12. 4 Modified from: <http://www.ntm-net.org/sites/ntm-net-catalog/items/114-ntm-slide-repository>

NTM are found throughout the environment

NTM habitats are intimately shared with those of humans

CF: non-tuberculous mycobacteria
Falkinham JC. J Clin Microbiol 2009; 47:356-57. Includes figure from <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2650033/>.
<http://www.ntm-net.org/index.php/ntm-net-catalog/items/114-ntm-slide-repository>

Transmission of NTM

Likely routes of infection

- Inhalation of NTM-laden aerosols or dust
- Ingestion of NTM in water
- Contamination of hospital/clinic supplies and medical equipment
- Person-to-person transmission (evidence weak, but some evidence of transmission in the community)

Gastric mucosal reflux disease has been implicated as a mediator of NTM-LD

Breathing of NTM followed by gastric reflux leading to aspiration into the lung

CF: cystic fibrosis; NTM: non-tuberculous mycobacteria; NTM-LD: non-tuberculous mycobacterial lung disease
1 Falkinham JC. J Clin Microbiol 2009; 47:356-57; 2 Johnson KE, Saito A. J Thorac Dis 2014; 6:210-20; 3 Verma AK, Olson J. Clin Infect Dis 2015; 61:294-3. 4 Modified from: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2650033/>.
<http://www.ntm-net.org/index.php/ntm-net-catalog/items/114-ntm-slide-repository>

The hydrophobic outer membrane supports NTM survival and distribution

- Promotes surface attachment and biofilm formation
- Phagocyte avoidance
- Concentrates bacteria at air/water interface
- Aids aerosol distribution and transmission from vector distribution systems by inhalation

Biofilm formation and hydrophobic characteristics allow colonization of unfavorable habitats and easy spread

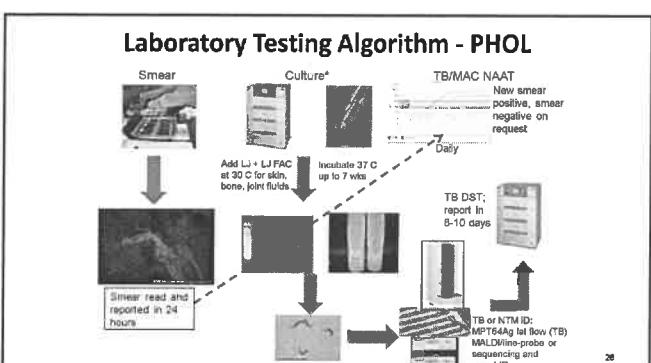
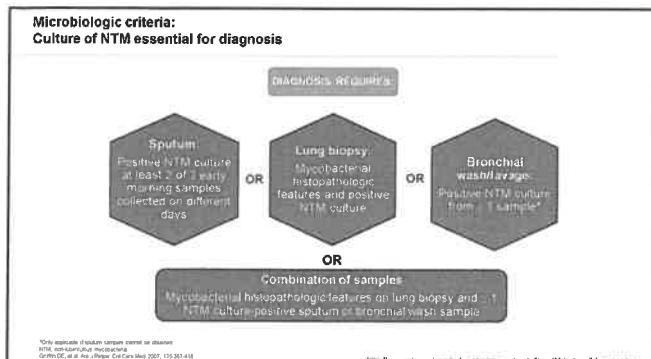
NTM non-tuberculous mycobacteria
Pulm Clin 2008; 30: 356-67
<http://www.ntm-net.org/index.php/ntm-net-cats/item/114-ntm-slide-repository>



Potential for contamination of samples

- Environmental contamination must be avoided during sample collection
- Bronchial washes are less likely to be contaminated than expectorated sputum samples
- If culture reveals NTM that are environmental contaminants or infrequently encountered, expert guidance regarding clinical significance is necessary

NTM non-tuberculous mycobacteria
Gordin DL, et al. Am J Respir Crit Care Med 2007; 175: 851-61
<http://www.ntm-net.org/index.php/ntm-net-cats/item/114-ntm-slide-repository>



M. avium Complex detection from specimens by real-time PCR

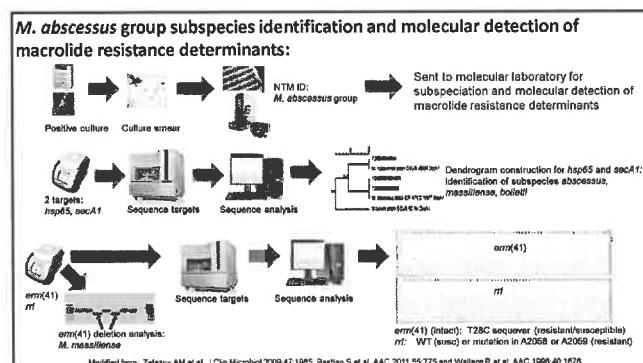
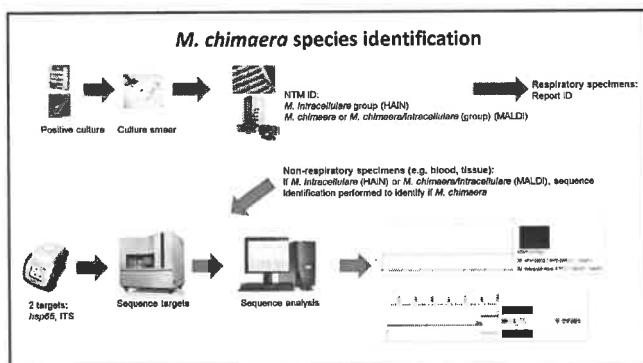
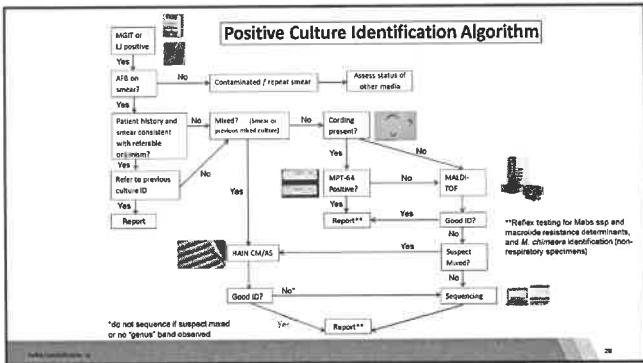
- Duplex assay for MAC and MtbC
- Original assay developed by Wadsworth Laboratory, NY
- Implemented June 6th, 2016

	AFB Positive (%) N = 1201	AFB Negative (%) N = 244	AFB Pos and Neg (%) N = 1445	
	MAC Spec	MAC Neg	MtbC Spec	MtbC Neg
MAC Sensitivity	78.3	77.9	0	0
Specificity	97.3	98.0	100.0	100
PPV	93.2	97.5	0	0
NPV	83.2	80.1	97.4	95.9
Indeterminate*	N = 143	N = 141	N = 13	N = 12
			N = 156 (10.8%)	N = 153

*Includes inhibited specimens.

Performance data Jan 1st, 2018 – Dec 31st, 2018

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NTM DST: 2018 CLSI guidelines M24 3rd edition and M62

- Recommended standard test procedure is broth microdilution
 - Interpretive criteria have been established for MAC, *M. kansasii*, *M. marinum* and RG, as sufficient data exist to support the recommendations
 - MAC: Addition of amikacin breakpoints and interpretive categories (intravenous and liposomal inhaled), confirmed breakpoints for moxifloxacin and linezolid
 - *M. kansasii*: Addition of breakpoints and interpretive categories for minocycline and removal of ethambutol, isoniazid and streptomycin
 - New table for antimycobacterial agents and breakpoints for SG other than MAC and *M. kansasii* (e.g. *M. marinum*)
 - RG: Revision and reformulating of table comments; imipenem breakpoints confirmed, tigecycline listed but only MICs can be reported

NTM DST: 2018 CLSI guidelines M24 3rd edition and M62 cont'd

- Amikacin resistance in MAC and *M. abscessus* may be determined by analysis of 16S rRNA (*rrs*) gene for mutations
 - Induced macrolide resistance in some RG may be detected by prolonging incubation up to 14 days for the final clarithromycin reading
 - Macrolide resistance in *M. abscessus* may be determined by analysis of *erm(41)* (induced) and 23S rRNA (*rrf*) genes (acquired)

Macrolide resistance in *M. abscessus* group: Example Molecular Detection Reports

Acquired Resistance	Mycobacterium avium complex	2010-10-24
Note:	Identified by gene analysis. This is an emerging PCP and XDR resistance-causing bacterium that has been identified as the Public Health Criteria Category for clinical use. It has not been cleared or approved by Health Canada.	
Functional gene at 100%	Functional	2010-10-24
mt gene mutation by sequencing	Not Identified	2010-10-24
Inhibition	Resistant	2010-10-24
		2010-10-24
Note:	The presence of a functional <i>mt</i> gene at 100% mutation is associated with resistance to aztreonam in <i>Acinetobacter baumannii</i> , accompanied with the antibiotic for treatment failure.	
M. avium complex	Mycobacterium avium complex sub. avium	2010-10-24
Note:	Identified by gene sequencing. This is an emerging PCP and XDR resistance-causing bacterium that has been identified as the Public Health Criteria Category for clinical use. It has not been cleared or approved by Health Canada.	
Functional gene at 100%	Functional	2010-10-24
mt gene mutation by sequencing	Not Identified	2010-10-24
Inhibition	Resistant	2010-10-24
		2010-10-24
Note:	The presence of a functional <i>mt</i> gene at 100% mutation is associated with resistance to aztreonam in <i>Acinetobacter baumannii</i> , accompanied with the antibiotic for treatment failure.	

NTM DST: National Microbiology Laboratory, PHAC

- Use commercial Sensititre panels SLOMYCO (slow growers) and RAPMYCO (rapid growers); NTM DST must be requested by treating clinician
 - Additional custom panel added for rapid growers where clofazimine testing requested; if clofazimine requested for slow growers, only custom plate to be used (ETH not in panel):

Thank you!