

Public Health Ontario | Santé publique Ontario

## Not What They're Not but What They Are:

Nontuberculous Mycobacteria and NTM Infections:  
The Laboratory

Frances Jamieson, MD FRCPC  
Medical Lead, TB and Mycobacteriology Laboratory, Public Health Ontario  
TIBDN Education Day, Toronto, November 21<sup>st</sup>, 2019

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### 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)

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### TB and Mycobacteriology Labs in Ontario

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

◆ Ottawa Hospital  
+ Dynacare (community lab)

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

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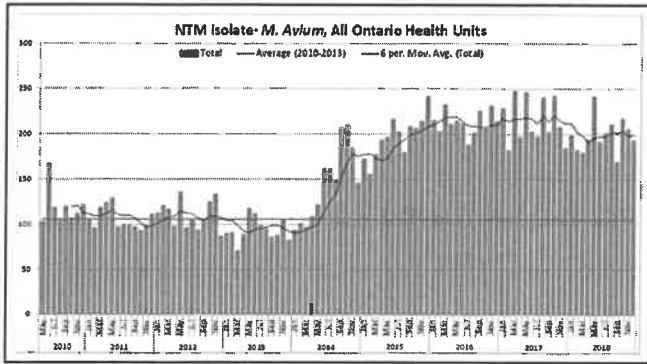
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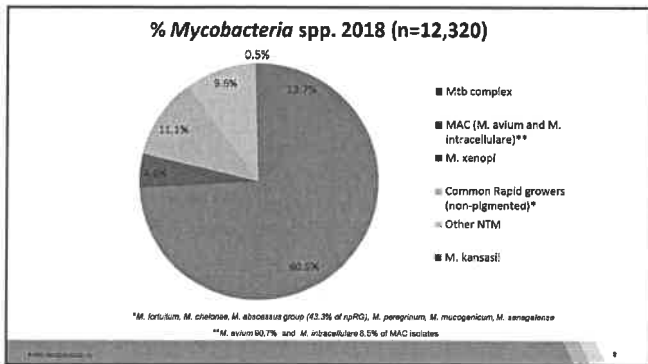
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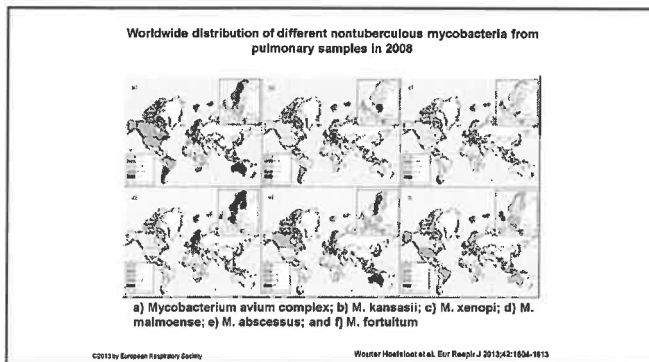
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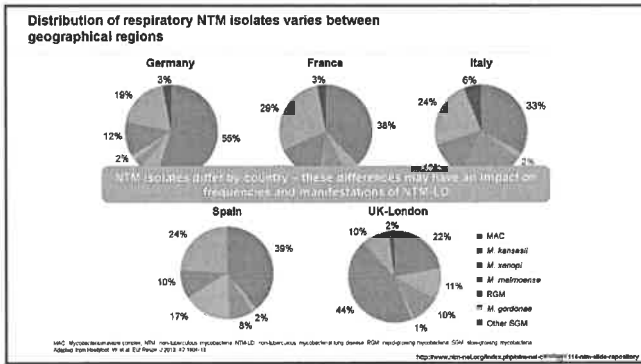
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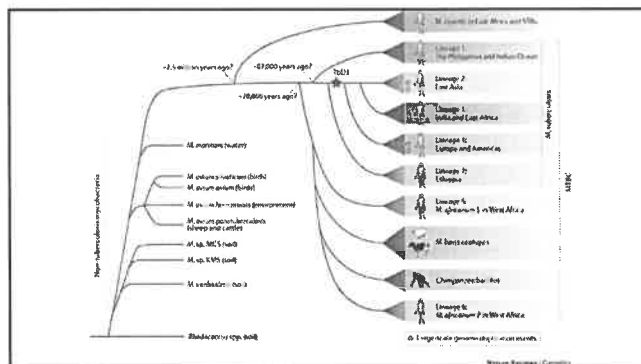
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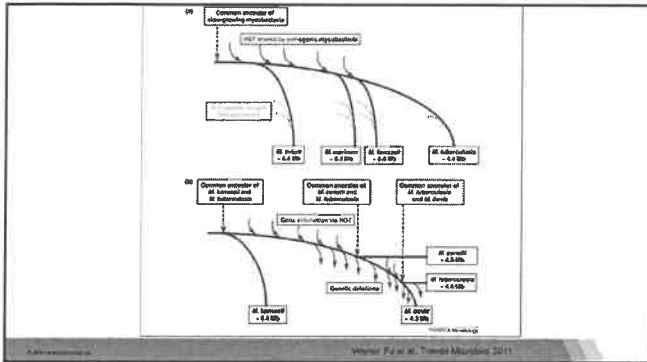
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**What are NTM?**

**NOT assigned to either!**

- Mycobacterium tuberculosis* complex
- Mycobacterium leprae*

**Also known as!**

- Environmental mycobacteria
- Opportunistic mycobacteria
- Atypical mycobacteria
- Mycobacteria other than *tuberculosis* (MOT)

NTM

**Non-pathogenic**

They live in soil, water, and air. They are not usually considered as pathogens. They are a common part of the environment. They are not usually considered as pathogens.

**Opportunistic**

They cause disease in immunocompromised individuals. They are not usually considered as pathogens.

1. NY, United States. Mycobacterium. NTM. <http://www.cdc.gov/dpdx/ntm/index.php?ntm-net-cats&nav=114-456-repository>  
 2. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1111111/>  
 3. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1111111/>  
 4. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1111111/>

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**NTM vs. *Mycobacterium tuberculosis*: Key distinctions**

NTM	<i>Mycobacterium tuberculosis</i>
Not obligate pathogens – normally live free in the environment <sup>1</sup>	Obligate pathogens: require host <sup>2</sup>
Low virulence: not usually pathogenic in the absence of predisposing conditions <sup>3</sup>	Pathogenic <sup>4</sup>
Human-to-human transmission extremely rare, but some evidence of this in the cystic fibrosis community <sup>5</sup>	Human-to-human transmission <sup>6</sup>
Infection rates increasing, especially in developed countries <sup>7</sup>	Infection rates decreasing, especially in developed countries <sup>8</sup>
Large heterogeneous group of species <sup>9</sup>	<i>Mycobacterium tuberculosis</i> complex contains small group of closely related subspecies <sup>10</sup>

1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 are based on differences in pathogenesis, infection rates, and transmission routes.

1. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1111111/>  
 2. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1111111/>  
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 10. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1111111/>

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**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 E.g. <i>M. leoprii</i> , <i>M. neoaurum</i>
II	Scotochromogens	Slow-grower	Pigmentation always present Growth takes more than 7 days to appear on solid media E.g. <i>M. scrofulaceum</i> , <i>M. szulgai</i> , <i>M. goodii</i> , <i>M. neoaurum</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 E.g. <i>M. avium</i> , <i>M. intracellulare</i> , <i>M. fortuitum</i> , <i>M. goodii</i>
IV	Rapid-growers	Rapid-grower	Colonies of NTM that appear on solid media in less than 7 days E.g. <i>M. chelonae</i> , <i>M. abscessus</i> , <i>M. fortuitum</i> , <i>M. neoaurum</i>

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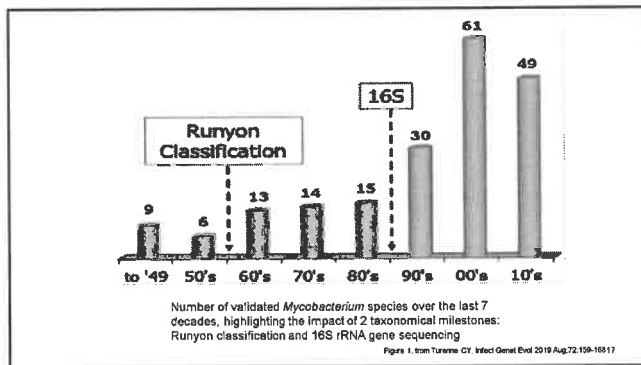
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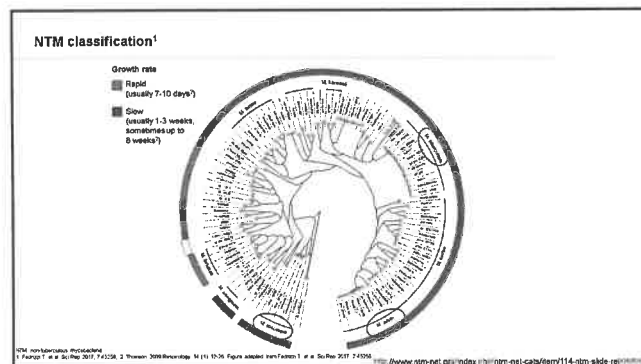
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**The hydrophobic outer membrane supports NTM survival and distribution**

**Cell wall**

- Lipopolysaccharide
- Mycolic acid
- Peptidoglycan/Arabinan layer
- Plasma membrane
- Protein

**Mycobacterium**

- Promotes surface attachment and biofilm formation
- Physique wash-out
- Concentrates bacteria at air/water interface
- Add aerosol distribution and transmission from water distribution systems by inhalation
- Optim formation and hydrophobic characteristics allow colonization of unfavorable habitats and easy spread

© 2014, The University of Texas at Dallas, Center for Experimental Research on Tuberculosis  
February 20, 2014, 1:00 PM - 1:15 PM

http://www.rnm-net.org/index.php/rnm-net/cats/item/114-rnm-slide-repository

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**"Good quality lab work cannot compensate for a poor quality specimen"**

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**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

© 2014, The University of Texas at Dallas, Center for Experimental Research on Tuberculosis  
February 20, 2014, 1:00 PM - 1:15 PM

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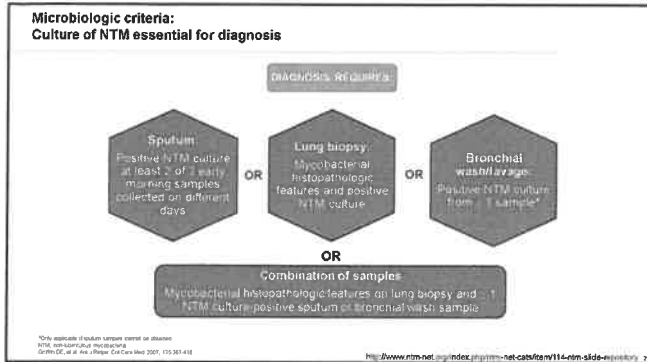
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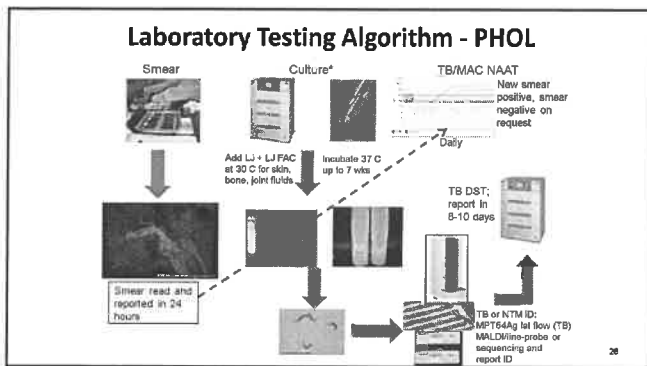
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**M. avium Complex detection from specimens by real-time PCR**

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

	AFB Positive (%) N = 1201		AFB Negative (%) N = 244		AFB Pos and Neg (%) N = 1345	
	MAC	Mtbc	MAC	Mtbc	MAC	Mtbc
MAC Sensitivity	78.3	77.9	0	0	77.6	77.1
Specificity	97.3	98.0	100.0	100	72.8	98.5
PPV	93.2	97.5	0	0	99.2	97.5
NPV	83.2	80.1	97.4	96.9	86.9	84.9
Indeterminate*	N = 143	N = 141	N = 13	N = 12	N = 156 (10.8%)	N = 153

\*includes inhibited specimens

Performance data Jan 1<sup>st</sup>, 2018 – Dec 31<sup>st</sup>, 2018

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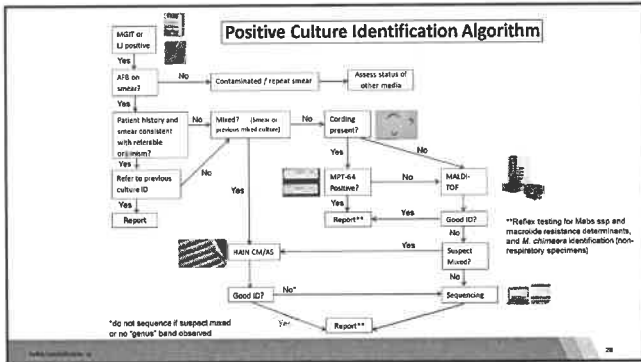
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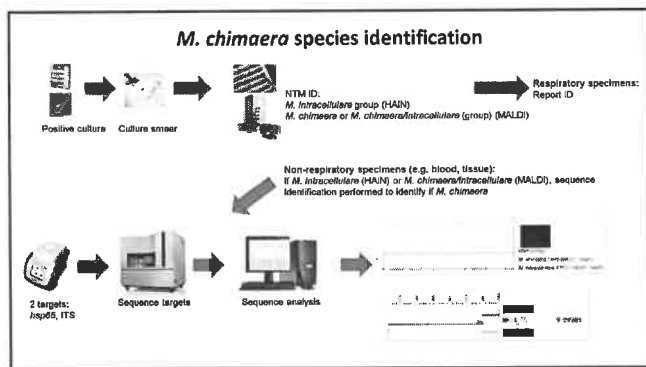
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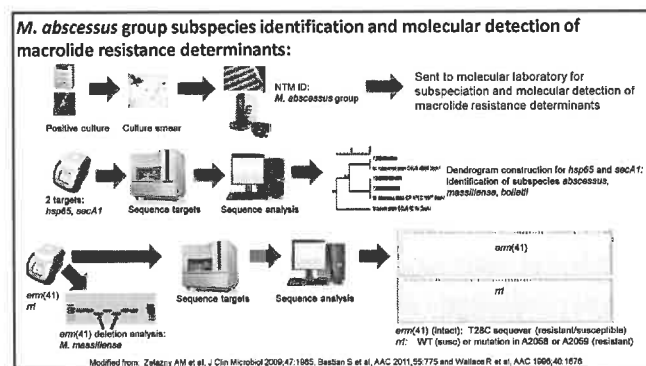
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**NTM DST: 2018 CLSI guidelines M24 3<sup>rd</sup> edition and M62**

- 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 reformatting of table comments; imipenem breakpoints confirmed, tigecycline listed but only MICs can be reported

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**NTM DST: 2018 CLSI guidelines M24 3<sup>rd</sup> 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 (*rrl*) genes (acquired)

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

*M. abscessus* subsp. *abscessus* Mycobacterium abscessus subsp. abscessus 2019-10-24

Note: Identified by gene analysis. This is an endpoint PCR and longer sequencing-based assays that are being validated at the Public Health Ontario Laboratory for clinical use. It has not been assessed or approved by Health Canada.

Test	Result	Interpretation	Date
Functional <i>erm(41)</i> gene	Non-functional		2019-10-24
<i>erm(41)</i> gene mutation by sequencing	Not detected		2019-10-24
16S gene mutation by sequencing	Detected		2019-10-24
Interpretation	Resistant		2019-10-24

Note: The presence of a functional *erm(41)* gene among *M. abscessus* spp. is associated with macrolide resistance (e.g. clarithromycin, azithromycin) with the potential for resistance failure.

*M. abscessus* subsp. *abscessus* Mycobacterium abscessus subsp. abscessus 2019-10-28

Note: Identified by gene analysis. This is an endpoint PCR and longer sequencing-based assays that are being validated at the Public Health Ontario Laboratory for clinical use. It has not been assessed or approved by Health Canada.

Test	Result	Interpretation	Date
Functional <i>erm(41)</i> gene	Functional		2019-10-28
<i>erm(41)</i> gene mutation by sequencing	Not detected		2019-10-28
16S gene mutation by sequencing	Not Detected		2019-10-28
Interpretation	Resistant		2019-10-28

Note: The presence of a functional *erm(41)* gene among *M. abscessus* spp. is associated with macrolide resistance (e.g. clarithromycin, azithromycin) with the potential for treatment failure.

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