Clinical and Laboratory Aspects of Nontuberculous Mycobacteria

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• No financial interests to declare
Objectives

1. Describe clinical manifestations of nontuberculous mycobacteria

2. Discuss the epidemiology of nontuberculous mycobacteria

3. Explain the clinical algorithm for the laboratory diagnosis of nontuberculous mycobacteria
Topics

- Review of NTM diseases
- Epidemiology of nontuberculous mycobacteria
- Processing of clinical specimens, especially specimens from Cystic Fibrosis patients
- Identification of NTM - how far should we go?
- Antimicrobial susceptibility testing of NTM
- Hospital-acquired infections with NTM-outbreaks and pseudo-outbreaks
PNTM Disease?

- **Clinical** – Cough, fatigue, weight loss
- **Radiograph** - X-ray (nodular or cavitary opacities) or HRCT (multifocal bronchiectasis w/multiple small nodules)
- **Bacteriology:**
  - Pos culture from at least 2 separate expectorated sputum samples
  - Pos culture from at least 1 bronchial wash or lavage
  - Transbronchial biopsy or lung biopsy with mycobacterial histopathologic features and pos culture for NTM or biopsy showing mycobacterial histopathologic features and 1 or more sputum or bronch washings that are culture pos for NTM

ATS/IDSA AJRCCM 2007. 175:367
Pulmonary:
MAC, M. kansasii, M. xenopi, M. malmoense, M. abscessus

Disseminated:
MAC, M. kansasii, M. haemophilum, M. marinum, M. genavense, M. chelonae, M. abscessus, M. fortuitum

Skin/Soft Tissue/Catheter
MAC, M. marinum, M. haemophilum, M. abscessus, M. chelonae, M. fortuitum, M. mucogenicum
M. scrofulaceum replaced by M. avium

Shift took place between 1975 and 1985

Municipal water treated now with chloramine instead of chlorine; Legionella pneumophilia disappeared, NTM increased…
Most frequently isolated:

- M. avium complex (e.g., M. intracellulare, M. avium, M. chimaera)  
  N. America
- M. abscessus and its subspecies  
  Europe
- M. simiae  
  Israel

Less frequently isolates:

- M. kansasii
- M. fortuitum
NTM Infections in Cardiac Surgery Patients Linked to Heater-Coolers

Patients undergoing heart transplantation are more susceptible to NTM infection, as a result of their transplant-associated immunosuppression.

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Reported Tuberculosis (TB) Cases
United States, 1982–2015*

*As of June 9, 2016.
TB Case Rates, by Age Group and Sex, United States, 2015*

* As of June 9, 2016.
PNTM Medicare Beneficiaries

- **White**
  - Males: 100
  - Females: 150

- **Black**
  - Males: 75
  - Females: 125

- **Asian/Pacific Islander**
  - Males: 300
  - Females: 200

- **Hispanic**
  - Males: 100
  - Females: 100

**PNTM Cases per 100,000 Persons**

AJRCCM 2012 Adjemian et al
**Measurements and Main Results:** In 2010, we estimated 86,244 national cases, totaling to $815 million, of which 87% were inpatient related ($709 million) and 13% were outpatient related ($106 million). Annual state estimates varied from 48 to 12,544 cases ($503,000–$111 million), with a median of 1,208 cases ($11.5 million). Oceanic coastline states and Gulf States comprised 70% of nontuberculous mycobacterial disease cases but 60% of the U.S. population. Medical encounters among individuals aged 65 years and older ($562 million) were twofold higher than those younger than 65 years of age ($253 million). Of all costs incurred, medications comprised 76% of nontuberculous mycobacterial disease expenditures. **Projected 2014 estimates resulted in 181,037 national annual cases ($1.7 billion).**
Four integrated health care delivery systems*, 1991-2007

<table>
<thead>
<tr>
<th>NTM Species</th>
<th>Pulm. Cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>*M. avium complex</td>
<td>1,495</td>
<td>(80.1%)</td>
</tr>
<tr>
<td>*M. chelonae/abscessus</td>
<td>225</td>
<td>(12.1%)</td>
</tr>
<tr>
<td>*M. fortuitum</td>
<td>106</td>
<td>(5.6%)</td>
</tr>
<tr>
<td>*M. kansasii</td>
<td>102</td>
<td>(5.5%)</td>
</tr>
<tr>
<td>*M. simiae</td>
<td>53</td>
<td>(2.8%)</td>
</tr>
<tr>
<td>*M. xenopi</td>
<td>33</td>
<td>(1.7%)</td>
</tr>
</tbody>
</table>

*KP Southern California, KP Southern Colorado, Group Health, Geisinger

AJRCCM 2010 Prevots et al.
> 8,800 isolates were analyzed using *rpoB* gene sequencing

Seven *Mycobacterium* species and subspecies accounted for ~80% of all isolates tested

- **24.4%** *M. abscessus*, incl. all 3 subspecies
- **19.9%** *M. avium*
- **16.4%** *M. intracellulare* **42.3%**
- **6.0%** *M. chimaera*
- **5.1%** *M. fortuitum*
- **3.8%** *M. gordonae*
- **3.7%** *M. chelonae*

National Jewish Health – unpublished data
NTM in CF – Age Related

- MABSC-positive patients
- MAC-positive patients

Review of NTM diseases

Epidemiology of nontuberculous mycobacteria

Processing of clinical specimens, especially specimens from Cystic Fibrosis patients

Identification of NTM - how far should we go?

Antimicrobial susceptibility testing of NTM

Hospital-acquired infections with NTM-outbreaks and pseudo-outbreaks
• NTM are an increasing problem in patients with cystic fibrosis (CF), in large part because recovery of these organisms is hampered by the presence of *Pseudomonas aeruginosa* in the respiratory tract of these patients, which rapidly overgrows mycobacteria in culture.

• **Specific decontamination methods are used**
  - oxalic acid method
  - two-step NALC-NaOH-oxalic method. However, this method may affect the mycobacteria viability, and its effect on the recovery of *M. abscessus* in pediatric CF patients is unknown.

• The ability of a **chlorhexidine decontamination method** vs. the NALC-NAOH-oxalic acid method to recover NTM from patients with cystic fibrosis has been compared. - The chlorhexidine method recovered twice as many NTM, despite a higher contamination rate. (Ferroni et al. J Clin Microbiol. 2006;44:2237-2239).
Review of NTM diseases
Epidemiology of nontuberculous mycobacteria
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Hospital-acquired infections with NTM-outbreaks and pseudo-outbreaks
The genus *Mycobacterium* is the only genus in the family *Mycobacteriaceae* and is related to other mycolic acid-containing genera.

High G+C contents of the DNA of *Mycobacterium* species (61 to 71 mol%, except in *M. leprae* [55%]) is within the range of other mycolic acid-containing genera:

- *Gordonia* (63 to 69 mol%)
- *Tsukamurella* (68 to 74 mol%)
- *Nocardia* (64 to 72 mol%)
- *Rhodococcus* (63 to 73 mol%)
- *Segniliparus* (68 to 72 mol%)
Mycobacterium sp.

Source: http://www.bacterio.net/mycobacterium.html

Number of species cited in this file: 177
Number of subspecies cited in this file: 13
- **M. abscessus subs. abscessus; M. abscessus subsp. bolletii; M. abscessus subsp. massiliense**

- **M. sarraceniae; M. helvum** – pitcher plant
- **M. lutetiense; M. montmartrense; M. arcueilense** – water
- **M. paraintracellulare** – Korea, pulmonary
- **M. icosiumassiliensis** – water
- **M. virginiense** – US, tenosynovitis, osteomyelitis
- **M. alsense** – Denmark & Italy, pulmonary
- **M. malmesburyense** - water, soil, cattle, African buffalo
- **M. oryzae** - paddy cultivated soils, Western Ghats of India
M. avium complex includes:

four **M. avium** subspecies

*M. avium* subsp. *avium*, *M. avium* subsp. *hominissuis*,
*M. avium* subsp. *silvaticum* and *M. avium* subsp. *paratuberculosis*

seven other species

*M. intracellulare*, *M. marseillense*, *M. timonense*,
*M. bouchedurhonense*, *M. colombiense*, *M. vulneris*
and *M. chimaera*
Molecular TB Assays

1987  Roberts MC – DNA Probes for identification
1991  Cave et al – IS6110 for fingerprinting
1991  Eisenach et al – PCR from sputum
1992  Boettger et al – *Mycobacterium genavense*
1993  Telenti et al – rpoB sequencing
2006  Somoskovi et al – MDR screen in AFB+ sputum
2010  Helb et al – Fully integrated sample processing
2010  Lotz et al – MALDI-TOF MS for identification
• **Nucleic acid probe kits**
• **High Performance Liquid Chromatography (HPLC)** – cell wall
• **PCR Restriction Analysis (PRA)**
• **Line Probes**
• **DNA sequencing**
• **MALDI-TOF MS (Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry)** – protein
DNA probe hybridization results
All the *M. chimaera* strains presented identical reactivity with the DNA probes tested. In the AccuProbe system, they hybridized with probes for MAC and *M. intracellulare* but not with the probe for *M. avium*.

False-Positive Results for *M. celatum* with the AccuProbe *M. tuberculosis* Complex Assay

*M. celatum* type 1 was found to cross-react in the AccuProbe *M. tuberculosis* complex assay. Subsequently, we found a statistically significant increase in the relative light units with lower temperatures, suggesting that it is necessary to perform this AccuProbe assay at between 60 and 61°C.
Evaluation of the GenoType Mycobacterium Assay for Identification of Mycobacterial Species from Cultures

The DNA strip assay was evaluated for the ability to differentiate mycobacterial species. The test is based on a PCR technique targeting a 23S rRNA gene region, followed by reverse hybridization and line probe technology. Concordant results were obtained for 137 (92.6%) of 148 mycobacterial strains with the CM assay and 133 (89.9%) of 148 mycobacterial strains with the AS assay.

GenoType NTM-DR for Identifying *Mycobacterium abscessus* Subspecies and Determining Molecular Resistance

Performance of a new line probe assay for identifying the subspecies and determining the macrolide and aminoglycoside resistance levels of 50 *Mycobacterium abscessus* isolates. Agreement of GenoType NTM-DR results with sequencing and phenotypic resistance results was 92% for subspecies identification and 98% for determining molecular and phenotypic resistance.

Laboratory Aspects of "Mycobacterium genavense," a Proposed Species Isolated from AIDS Patients

The mycolic acid pattern of patients' isolates closely resembled that of the type strain of *M. simiae* when analyzed by one- and two-dimensional thin-layer chromatography and by high-performance liquid chromatography. Whole-cell fatty acid analyses by gas-liquid chromatography distinguished the isolates from *M. simiae* but misidentified them as *M. fortuitum*. Sequence determinations of the hypervariable regions of the 16S rRNA gene indicate that these organisms belong to the recently proposed new species "*M. genavense."

Currently we use \textit{rpoB} and 16S sequencing technology for molecular identification

\begin{itemize}
  \item \textbf{Pros}
    \begin{itemize}
      \item Sensitive
      \item Accurate
      \item Specific
      \item \textbf{Gold Standard}
    \end{itemize}
  \item \textbf{Cons}
    \begin{itemize}
      \item Costly
      \item Labor intensive, takes a few days for ID results
    \end{itemize}
\end{itemize}
**MALDI-TOF MS can reliably and rapidly identify**

- approximately 88% of *Mycobacterium* species, 90% of *Nocardia* species, and 51% of other aerobic actinomycetes encountered in routine clinical practice at a tertiary medical center/reference laboratory.
  - Using a custom, enhanced library and a streamlined extraction procedure
- Described the ability of the manufacturer’s library to identify these groups of organisms and described the effects of lowering the accepted cutoff score from 2.0 to 1.7
  - As the manufacturer continues to expand its database, many laboratories will have the ability to identify many of the isolates they routinely encounter using MALDI-TOF MS.
  - An expanded custom library may ultimately be the most useful tool for identification of the uncommon species encountered most often in a reference laboratory setting.

M. abscessus [rough + smooth] & M. avium [translucent]
All NTM isolates from individuals with CF should undergo molecular identification to identify the *species*... *M. abscessus* should be identified to the *subspecies* level.
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Mycobacterium tuberculosis complex
Mycobacterium avium complex
Mycobacterium kansasii
Mycobacterium marinum
Miscellaneous slowly growing NTM
Rapidly growing mycobacteria (RGM)

CLSI M24-A2 (2011) Susceptibility testing of mycobacteria, nocardia, and other aerobic actinomycetes – Approved standard, second edition (in revision)
Broth microdilution method (for research use only) with TREK Sensititre 96-well plate for Rapid Growing Mycobacteria (RGM) (RAPMYCO; Thermo Scientific, Cleveland, OH)

These include *Mycobacterium fortuitum* group (*M. fortuitum*, *M. peregrinum*, and the species included in the former *M. fortuitum* third biovariant complex), *M. chelonae*, *M. abscessus*, *M. massiliense*, *M. bolletii*, *M. mucogenicum*, and *M. smegmatis* group (*M. smegmatis*, *M. goodii*, *M. wolinskyi*).
If growth in the growth control well is insufficient after 72 h, re-incubate and read at day 4. If growth is still insufficient in the growth control well, incubate one more day to day 5. If no or very poor growth is observed after 5 days, test should be repeated.

For *M. abscessus* group isolates, plates should be incubated and read again for clarithromycin at day 14 to account for possible inducible resistance to this drug, unless resistance is recognized earlier or determined by a molecular assay, *erm*(41).
One of the most serious drawbacks of designing a potent therapeutic regimen for slowly growing NTM in the clinical practice is that current drug susceptibility testing of slowly growing NTM is lacking correlation with clinical outcome, with the exception of clarithromycin.
The intended use of the Trek Diagnostics Sensititre SLOMYCO broth microdilution (research use only) is for the determination of MIC of antimicrobial agents against slowly growing NTM, e.g., *Mycobacterium avium* complex, *M. arupense*, *M. malmoense*, and *M. nebraskense*. 
The following therapeutic compounds are included: clarithromycin, rifabutin, ethambutol, isoniazid, moxifloxacin, rifampin, trimethoprim/sulfamethoxazole, amikacin, linezolid, ciprofloxacin, streptomycin, doxycycline, and ethionamide.

Some laboratories may use a custom-made microtiter plate to determine combination MICs for ethambutol and rifampin.
<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N=2,318</strong></td>
<td>%</td>
<td>EFFECT</td>
</tr>
<tr>
<td>1,642</td>
<td>71</td>
<td>SYNERGISTIC</td>
</tr>
<tr>
<td>584</td>
<td>25</td>
<td>ADDITIVE</td>
</tr>
<tr>
<td>91</td>
<td>4</td>
<td>NO EFFECT</td>
</tr>
</tbody>
</table>
Erythromycin Methylase Gene (41)

- Macrolide antibiotics activate the \textit{erm}41 gene
- Results in inducible (delayed) resistance to clarithromycin and/or azithromycin
- \textbf{Mutations} or deletions \textit{inactivate} this gene resulting in \textit{macrolide susceptibility}
- Presence of wildtype or a mutated sequence differs within the 3 subspecies
### TABLE 3. TREATMENT RESPONSES FOR PATIENTS WITH MYCOBACTERIUM ABSCESSUS AND MYCOBACTERIUM MASSILIENSE LUNG DISEASE

<table>
<thead>
<tr>
<th></th>
<th>M. abscessus (n = 24)</th>
<th>M. massiliense (n = 33)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptomatic response</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improved</td>
<td>18 (75%)</td>
<td>32 (97%)</td>
<td>0.040</td>
</tr>
<tr>
<td>Unchanged</td>
<td>4 (17%)</td>
<td>1 (3%)</td>
<td></td>
</tr>
<tr>
<td>Worsened</td>
<td>2 (8%)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Radiographic response on HRCT</td>
<td></td>
<td></td>
<td>0.003</td>
</tr>
<tr>
<td>Improved</td>
<td>10 (42%)</td>
<td>27 (82%)</td>
<td></td>
</tr>
<tr>
<td>Unchanged</td>
<td>7 (29%)</td>
<td>5 (15%)</td>
<td></td>
</tr>
<tr>
<td>Worsened</td>
<td>7 (29%)</td>
<td>1 (3%)</td>
<td></td>
</tr>
<tr>
<td>Microbiologic response</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Initial sputum conversion and maintenance of conversion</td>
<td>6 (25%)</td>
<td>29 (88%)</td>
<td></td>
</tr>
<tr>
<td>Initial sputum conversion, with sputum relapse</td>
<td>4 (17%)</td>
<td>3 (9%)</td>
<td></td>
</tr>
<tr>
<td>Failure to sputum conversion</td>
<td>14 (58%)</td>
<td>1 (3%)</td>
<td></td>
</tr>
</tbody>
</table>

*Definition of abbreviation: HRCT = high-resolution computed tomography.*

Koh. AJRCCM 2011.
<table>
<thead>
<tr>
<th>Species</th>
<th>Number Identified</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. abscessus ssp. abscessus</em></td>
<td>1,470</td>
<td>71.7%</td>
</tr>
<tr>
<td><em>M. abscessus ssp. massiliense</em></td>
<td>420</td>
<td>20.5%</td>
</tr>
<tr>
<td><em>M. abscessus ssp. bolletii</em></td>
<td>110</td>
<td>5.4%</td>
</tr>
<tr>
<td>Other</td>
<td>58</td>
<td>2.4%</td>
</tr>
</tbody>
</table>


National Jewish Health: Unpublished data
**M. massiliense** is positive for the *erm(41)* gene but contains a 273-bp deletion within the gene rendering the gene nonfunctional.
We recommend that the CLSI should evaluate the current clarithromycin susceptibility breakpoints for *M. abscessus* subsp. *abscessus*, and we further propose that isolates with a clarithromycin MIC of 8 µg/mL (currently considered resistant) should have repeat MIC testing and/or *erm* gene sequencing performed. Sequencing of the *erm*(41) gene is preferred, as sequencing can produce an answer more quickly than repeating the MIC tests.
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- Hospital-acquired infections with NTM - outbreaks and pseudo-outbreaks
Intrinsic Contamination of Heater-Cooler Devices Used in Cardiac Surgery with *Mycobacterium chimaera* – United States

- Environmental samples vs. clinical samples
- Single nucleotide polymorphisms (SNPs) were called and used to infer a maximum likelihood tree
- **Results** from pairwise comparisons among all sequences across a core genome of approximately 4Mb revealed a maximum of **3 SNPs** between any two isolates related to the outbreak investigation, versus approximately **80-300 SNPs** between the outbreak isolates and the epidemiologically unlinked isolates

Notes from the Field: *Mycobacterium chimaera* Contamination of Heater-Cooler Devices Used in Cardiac Surgery — United States  Morbidity Mortality Weekly Report. October 14, 2016. 65(40);1117–18
M. mucogenicum


<table>
<thead>
<tr>
<th>Location</th>
<th>Source of Water</th>
<th>Mycobacterium</th>
</tr>
</thead>
<tbody>
<tr>
<td>UK</td>
<td>Hospital water</td>
<td><em>M. xenopi</em></td>
</tr>
<tr>
<td>USA</td>
<td>Municipal water</td>
<td><em>M. avium</em></td>
</tr>
<tr>
<td>USA</td>
<td>Hospital, house and reservoir water</td>
<td><em>M. avium</em></td>
</tr>
<tr>
<td>Canada</td>
<td>Hot-tub water</td>
<td><em>M. avium</em></td>
</tr>
<tr>
<td>USA</td>
<td>Various vegetables</td>
<td><em>M. avium</em></td>
</tr>
<tr>
<td>USA</td>
<td>Nail salon (whirlpool bath)</td>
<td><em>M. mageritense</em></td>
</tr>
<tr>
<td>USA</td>
<td>Hospital hot water</td>
<td><em>M. avium</em></td>
</tr>
<tr>
<td>Australia</td>
<td>Spa water</td>
<td><em>M. avium</em></td>
</tr>
<tr>
<td>Korea</td>
<td>Hospital tap water</td>
<td><em>M. mucogenicum</em></td>
</tr>
<tr>
<td>USA</td>
<td>Potting soil</td>
<td><em>M. intracellulare</em></td>
</tr>
<tr>
<td>Japan</td>
<td>Residential water</td>
<td><em>M. avium</em></td>
</tr>
<tr>
<td>USA</td>
<td>Hospital, municipal water</td>
<td><em>M. phocaicum</em></td>
</tr>
<tr>
<td>Australia</td>
<td>Residential, municipal water</td>
<td><em>M. kansasii</em></td>
</tr>
<tr>
<td>Australia</td>
<td>Municipal, residential, swimming pool w.</td>
<td><em>M. abscess</em></td>
</tr>
</tbody>
</table>

Halstrom et al Int J Mycobacterioli 2015. 4:81-91
Advancing our understanding of NTM in the CF airway

Molecular Core
Director Michael Strong, PhD
whole genome sequencing
> 300 WGS

NTM Culture, Biorepository, and Coordinating Core
Director: Charles Daley, MD
Co-Director Max Salfinger, MD
- culture
- basic molecular identification
- antimicrobial susceptibility
- isolate banking
- data coordination
> 500 isolates

CFF Care Centers

Clinical Research Core
Director, Stacy Martiniano, MD
Co-Director Jerry Nick, MD
Clinical data & Supports trials
PREDICT PATIENCE

https://www.nationaljewish.org/Colorado-CF-Research-and-Development-Program/Home
On the Horizon...
• Identification
• Drug resistance
• Genotyping
• Undiscovered biomarkers
As we approach the “affordable” bacterial genome, the challenge remains with the analysis.

But, let’s not simplify the Data Generation – mycobacteria are by nature very difficult to work with. Many are slow growing, difficult to culture, difficult to lyse, some may have nucleases. A superb molecular biologist is key.
• Bi-monthly newsletter
• Please feel free to sign up – it is free 😊


• Or [Google](http://www.google.com)
Thank-you!

salfingerm@njhealth.org