An update on the diagnosis and management of non-tuberculous mycobacterial infections – Perspectives from the Singapore Laboratory

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Declaration

• Funded by Bayer (OA2017-008)
OBJECTIVE

• Scope – excludes *M. leprae*
• NTM issues – globally and locally
• Identification
• Susceptibility testing
• Role of WGS
Positive Cultures Identified at CTBL, 1998-2018
TB vs NTM

UK 6.1/100k, USA 1-6.6/100k
Canada: 29.3 to 41.3/100k

MGIT testing
Emerging novel NTM

>40 newly described in 5yrs

Forbes, JCM 2018
NTM Epidemiology

- Ubiquitous in environment
- Not transmitted from person-to-person

BTS: Minimise transmission of *M. abscessus* for CF patients (Grade B)

- Chronicity, difficult to treat. Identification matters to the patient chronically infected
Laboratory diagnosis

- Standard processes for mycobacterial cultures/smears
  BTS: Minimum of 2 sputum samples on separate days
- No Oropharyngeal swabs, serology testing
Indications for identification

• Serious comorbidities
• Affects prognosis
• Pathogenic, resistant species – *M. abscessus*
• Public health implications
• Specimen type, AFB smears, # of positive cultures (Lab: ATS criteria)

Recommendations:

1. Clinically significant NTM isolates should be routinely identified to the species level. An important exception is MAC because the differentiation between *M. avium* and *M. intracellulare* is not yet clinically significant. Although not routinely recommended, this differentiation may be important epidemiologically and, in the future, therapeutically (C, III).

2. The RGM (especially *M. chelonae, M. abscessus, and M. fortuitum*) should be identified to species level using a recognized acceptable methodology, such as PRA or biochemical testing, not HPLC alone (A, II).

3. Susceptibility of RGM for eight agents, including amikacin, cefoxitin, clarithromycin, ciprofloxacin, doxycycline, linezolid, sulfamethoxazole, and tobramycin, can also be used to facilitate identification of *M. abscessus, M. chelonae, and M. fortuitum* (C, III).

4. Communication between the clinician and laboratory is essential for determining the importance and extent of the identification analysis for a clinical NTM isolate (C, III).

Box 1 Clinical and microbiological criteria for diagnosing non-tuberculous mycobacterial lung disease (modified with permission from Griffith et al)

**Clinical (both required)**

1. Pulmonary symptoms, nodular or cavitary opacities on chest radiograph, or a high-resolution CT scan that shows multifocal bronchiectasis with multiple small nodules. And
2. Appropriate exclusion of other diagnoses.

**Microbiological**

1. Positive culture results from at least two separate expectorated sputum samples, if the results are non-diagnostic, consider repeat sputum AFB smears and cultures. Or
2. Positive culture results from at least one bronchial wash or lavage. Or
3. Transbronchial or other lung biopsy with mycobacterial histopathological features (granulomatous inflammation or AFB) and positive culture for NTM or biopsy showing mycobacterial histopathological features (granulomatous inflammation or AFB) and one or more sputum or bronchial washings that are culture-positive for NTM.

*All NTM isolates from respiratory samples should be identified to at least species level using validated molecular or mass spectrometry techniques. (Grade B)*

*Isolates of M. abscessus should be subspecies using appropriate molecular techniques. (Grade C)*

*If person-to-person transmission of M. abscessus is suspected, isolates should be typed, preferably using whole genome sequencing (Grade C).*
LAB – IDENTIFICATION OF NTMS

American Thoracic Society Documents

An Official ATS/IDSA Statement: Diagnosis, Treatment, and Prevention of Nontuberculous Mycobacterial Diseases

Non-sterile sputum samples

Clinically significant

PRELIMINARY REPORT ON 22/12/15:

PRESUMPTIVE NONTUBERCULOUS MYCOBACTERIA

METHOD: TBCID

DATE OF REPORTING: 11/02/16

Abnormal.

FINAL REPORT ON 30/12/15:

THIS ISOLATE HAS BEEN IDENTIFIED AS

M. CHELONAE CPLX (GPIII, M.ABS) (M. CHELONAE COMPLEX, INCLUDING M. ABSCESSUS)
NTM: diagnosed by exclusion
Mycobacteria classification

Growth on Solid Media

Slow (>7 days)
- MTBC
  - Nonchromogens
    - M. avium
    - M. intracellulare
    - M. haemophilum
  - Photochromogens
    - M. kansasii
    - M. marinum

Rapid (< 7 days)
- Scotochromogens
  - M. gordonae
- M. abscessus
- M. fortuitum
Identification of NTMs

Universal
- Sequencing: 16S rRNA, Hsp 65, NGS
- PCR-RFLP

Specific
- Line probe assays: LIPA, Hain
- Accuprobes

Generic
- MALDI-TOF
- HPLC

Complex groups
- M. abscessus- M. chelonae
- M. fortuitum species group
- M. kansasii
- M. avium-intracellulare scrofulaceum
- M. terrae

Challenges
- M. abscessus
- M. haemophilum
- M. leprae
- M. chimaera
Molecular identification methods

- GenProbe (Hologic) –
  Very accurate and reliable – MGIT and solid media
  FDA-approved, CAP recommended
  requires a high load, limited to *M. tuberculosis*, *M. avium complex*, *M. kansasii*, *M. gordonae*
An ode to HPLC

- High performance liquid chromatography
  Reference method (US CDC)
  Inexpensive, reliable and robust
  Viewable result
  In-house open library, High load
- Gas liquid chromatography
  Sherlock LCS – Mycobacteria/Mycolic (25/38 species, E-FAME (22, environmental)
  Sherlock DNA – 16SrRNA sequences
  >80, Combined analysis FAME-DNA
• Detection of proteins

• Additional extraction methods – from MGIT, solid media, Myco-FLytic

• Bruker and Vitek-MS (SARAMIS v4.12, IVD 3.0)
### TABLE 1 Identification of Mycobacterium spp. grown on solid medium 7H10 by Vitek MS Saramis v4.12 and IVD v3.0®

<table>
<thead>
<tr>
<th>Organism</th>
<th>RGM</th>
<th>SGM</th>
<th>IVD v3.0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%) of isolates identified by Saramis v4.12 RUO</td>
<td>No. (%) of isolates identified by IVD v3.0</td>
<td>No. (%) of isolates identified by Vitek MS Saramis v4.12 RUO</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (106)</td>
<td>88 (100)</td>
<td>32 (32)</td>
<td>32 (36)</td>
</tr>
<tr>
<td>Species level ID</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complex level ID</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Misidentified</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incorrect</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**MYCO SPECIES**

- *M. abscessus*: 29 (28 [97]), 1
- *M. avium complex*: 11 (9 [62]), 2
- *M. chelonae*: 2, 2
- *M. fortuitum sp sp*: 14 (14)
- *M. gordonae*: 8 (6 [75]), 2
- *M. haemophilum*: 5 (4 [80]), 1
- *M. kansasii*: 14 (12 [86]), 2
- *M. lentiflavum*: 1, 1
- *M. marinum*: 1, 1
- *M. mucogenicum*: 1, 1
- *M. simiae*: 2, 2
- *M. scrofulaceum*: 1, 1
- *M. tuberculosis*: 16, 16
- *M. ulcerans*: 1, 1

**TOTAL** 106, 97 (92), 8, 1

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**CTBL**

Vs GenProbe, LiPA, Hain CMAS

SARAMIS better than IVDv2

Leyer, JCM 2017
MALDI-TOF vs sequencing

- Good as a general test
- Shared resource
- Does not subspeciate for *M. abscessus, M. intracellulare*
- High inocula
- Customizable, systems are improving (Bruker RUO v5, 164)
Case example: workflow for discovering new pathogens

- Bronchiectasis, AFB3+
- No ID on Vitek, LiPA, Hain AS – myco sp

M. basiliense

903442
Merged Bruker spectra
Smith et al

Vitek MS
No identification result

WGS: M. basiliense

Hain CM

M. scrofulaceum / M. paraffinicum / M. parascrofulaceum

M. haemophilum / M. palustre / M. nebraskense / M. malmoense
Genetic speciation

• Some genes are better than others, depending on the species versus 16SrRNA – other bacteria
• hard to split complexes
• Multigene approach better

Adekambi, 2004: 16SrRNA, hsp65, sod, rpoB

Böttger, 1996
# Sequencing

<table>
<thead>
<tr>
<th>Advantage</th>
<th>16SrRNA</th>
<th>Alternate gene</th>
<th>Alternate methods</th>
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<tbody>
<tr>
<td>Universal gene target</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Large reference: publications, NCBI reference</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Commercial kits</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Use full sequence, 100%</td>
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<td>-</td>
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</table>

<table>
<thead>
<tr>
<th>Disadvantage-Interspecies</th>
<th>16SrRNA</th>
<th>Alternate gene</th>
<th>Alternate methods</th>
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<tbody>
<tr>
<td>M. abscessus vs M. chelonae</td>
<td>rpoB, hsp65, sodA</td>
<td>-</td>
<td>MALDI</td>
</tr>
<tr>
<td>M. kansasii vs M. gastri</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M. marinum vs ulcerans</td>
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<td>-</td>
<td>-</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Intraspecies</th>
<th>16SrRNA</th>
<th>Alternate gene</th>
<th>Alternate methods</th>
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<tbody>
<tr>
<td>MTBC</td>
<td>gyrB</td>
<td>Hain MTBC</td>
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<tr>
<td>M. avium</td>
<td>ITS, rpoB, hsp65</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M. intracellulare</td>
<td>ITS, rpoB, hsp65</td>
<td>-</td>
<td>MALDI+/+</td>
</tr>
<tr>
<td>M. abscessus complex</td>
<td>Hsp65, rpoB, ITS, erm41, secA1</td>
<td>-</td>
<td>MALDI+/+</td>
</tr>
<tr>
<td>M. fortuitum gp</td>
<td>rpoB</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

ITS: 23S-16SrRNA region

CLSI MM18 2nd Edn
Line Probe Assays

- PCR based, Fujirebio
- 1st line tests
- From liquid broth, solid media
- Fast TAT
- InnoLiPA: 16-23S rRNA ITS region
• 23SrRNA
Scenario 1: *M. abscessus* complex

<table>
<thead>
<tr>
<th>Name</th>
<th>Complete 16S rRNA Gene Sequence</th>
<th>( \text{rpo} \beta ) Gene Sequence</th>
<th>( \text{erm}(41) ) Gene Sequence</th>
<th>( \text{erm} ) (41) Functional</th>
<th>Whole-Genome Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. abscessus</em> or <em>M. abscessus</em> subsp. abscessus or <em>M. abscessus</em> sensu stricto</td>
<td>Identical to <em>M. bolletii</em> and <em>M. massiliense</em></td>
<td>Unique to <em>M. abscessus</em></td>
<td>Unique to <em>M. abscessus</em></td>
<td>Yes*</td>
<td>Unique to <em>M. abscessus</em></td>
</tr>
<tr>
<td><em>M. bolletii</em> or <em>M. abscessus</em> subsp. bolletii</td>
<td>Identical to <em>M. abscessus</em> and <em>M. massiliense</em></td>
<td>Unique to <em>M. bolletii</em></td>
<td>Unique to <em>M. bolletii</em></td>
<td>Yes</td>
<td>Unique to <em>M. bolletii</em></td>
</tr>
<tr>
<td><em>M. massiliense</em> or <em>M. abscessus</em> subsp. massiliense</td>
<td>Identical to <em>M. abscessus</em> and <em>M. bolletii</em></td>
<td>Unique to <em>M. massiliense</em></td>
<td>Unique to <em>M. massiliense</em></td>
<td>No</td>
<td>Unique to <em>M. massiliense</em></td>
</tr>
</tbody>
</table>

*Definition of abbreviation: M. = Mycobacterium.*

*Exception is approximately 15% of isolates that have a point mutation (28 T to C) that renders the gene nonfunctional (17).*

- **Lab:** As the test calls it
- **Subspeciation is recommended:** (BTS Grade C)
  - Reinfection vs relapse
  - Resistance matters: Clarithromycin, aminoglycosides
  - Academic requirement
- **How:** Sequence (target, WGS), Hain NTMDR
### Case 2: *M. chimaera* (or Mr I can be Agent A)

CTBL: 93% of *M. intracellulare* sec MAC-A were *M. chimaera*

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**Table 1. Comparison of the results of different molecular markers for isolates identified by Lipav1 as MAIS or MYC probe positive**

<table>
<thead>
<tr>
<th>Isolate no.</th>
<th>Lipav1</th>
<th>AccuProbe</th>
<th>Lipav2</th>
<th><em>hop65</em>-PRA</th>
<th><em>hop65</em> sequence</th>
<th>ITS sequence</th>
<th>16S rDNA sequence</th>
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<tbody>
<tr>
<td>1</td>
<td>MAIS</td>
<td>ND</td>
<td>MAIS-MIP</td>
<td><em>M. haemophilum</em></td>
<td><em>M. haemophilum</em> (98%)</td>
<td>MAIP (100%)</td>
<td>ND</td>
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<tr>
<td>2</td>
<td>MAIS</td>
<td>MIN</td>
<td>MAIS-MIN1</td>
<td><em>M. intracellular</em></td>
<td><em>M. intracellular</em> (98%)</td>
<td>MIN (99%)</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>MAIS</td>
<td>MIN</td>
<td>MAIS-MIN2</td>
<td><em>M. intracellular</em></td>
<td><em>M. intracellular</em> (98%)</td>
<td>MIN (99%)</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>MAIS</td>
<td>MIN</td>
<td>MAIS-MIN3</td>
<td><em>M. intracellular</em></td>
<td><em>M. intracellular</em> (98%)</td>
<td>MIN (99%)</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>MAIS</td>
<td>MIN</td>
<td>MAIS-MIN4</td>
<td><em>M. intracellular</em></td>
<td><em>M. intracellular</em> (98%)</td>
<td>MIN (99%)</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>MAIS</td>
<td>MIN</td>
<td>MAIS-MIN5</td>
<td><em>M. intracellular</em></td>
<td><em>M. intracellular</em> (98%)</td>
<td>MIN (99%)</td>
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<td>7</td>
<td>MAIS</td>
<td>MIN</td>
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<td><em>M. intracellular</em></td>
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<td>8</td>
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<td>MIN</td>
<td>MAIS-MIN7</td>
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<td><em>M. intracellular</em> (98%)</td>
<td>MIN (99%)</td>
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<td>9</td>
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<td>10</td>
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<td>MIN</td>
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<td><em>M. avium</em></td>
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<td>Mac (100%)</td>
<td>M. intracellular* (100%)</td>
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<td>Mac (100%)</td>
<td>M. intracellular* (100%)</td>
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<td>Mac (100%)</td>
<td>M. intracellular* (100%)</td>
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<td>Mac (100%)</td>
<td>M. intracellular* (100%)</td>
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<td>Mac (100%)</td>
<td>M. intracellular* (100%)</td>
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<td>Mac (100%)</td>
<td>M. intracellular* (100%)</td>
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<td>Mac (100%)</td>
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<td>Mac (100%)</td>
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<td>M. intracellular* (100%)</td>
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<td><em>M. avium</em></td>
<td><em>M. avium</em> (98%)</td>
<td>Mac (100%)</td>
<td>M. intracellular* (100%)</td>
</tr>
<tr>
<td>34</td>
<td>MAIS</td>
<td>MAC</td>
<td>MAIS</td>
<td><em>M. avium</em></td>
<td><em>M. avium</em> (98%)</td>
<td>Mac (100%)</td>
<td>M. intracellular* (100%)</td>
</tr>
<tr>
<td>35</td>
<td>MAIS</td>
<td>MAC</td>
<td>MAIS</td>
<td><em>M. avium</em></td>
<td><em>M. avium</em> (98%)</td>
<td>Mac (100%)</td>
<td>M. intracellular* (100%)</td>
</tr>
</tbody>
</table>

Lebrun, JCM 2005
Solution: Hain NTMDR

- Subspecies *M. abscessus*, *M. avium* complex (incl. *M. chimaera*)
- Genotypic testing for macrolide and aminoglycoside resistance
- Resistance: absence of mutation ≠ no resistance
<table>
<thead>
<tr>
<th>No.</th>
<th>Species</th>
<th>Hain GenoType NTM-DR</th>
<th>WGS-derived sequence identity match with <em>M. chimaera</em> reference strain % (n)</th>
<th>NCBI BLAST result</th>
</tr>
</thead>
<tbody>
<tr>
<td>INNO-LIPA</td>
<td>Identification</td>
<td>16S-ITS1</td>
<td>23S</td>
<td>WGS</td>
</tr>
<tr>
<td>22</td>
<td><em>M. intracellulare</em> (tec MAC-A)</td>
<td><em>M. chimaera</em></td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td><em>M. intracellulare</em> (tec MIN-A, B, C, D)</td>
<td><em>M. intracellulare</em></td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>3</td>
<td><em>M. avium</em> complex</td>
<td><em>M. intracellulare</em></td>
<td>99 (2)</td>
<td>99</td>
</tr>
<tr>
<td>1</td>
<td><em>M. intracellulare</em> (tec MAC-A)</td>
<td><em>M. chimaera</em> (reference strain)</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

**Reference strains:**

<table>
<thead>
<tr>
<th>No.</th>
<th>Species</th>
<th>Hain GenoType NTM-DR</th>
<th>WGS-derived sequence identity match with <em>M. chimaera</em> reference strain % (n)</th>
<th>NCBI BLAST result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>M. intracellulare</em> (tec MIN-A, B, C, D)</td>
<td><em>M. intracellulare</em></td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>1</td>
<td><em>M. avium, M. paratuberculosis, M. suisaticum</em></td>
<td><em>M. avium</em></td>
<td>98</td>
<td>99</td>
</tr>
<tr>
<td>1</td>
<td><em>M. chelonae complex (egr III, M. abscessus)</em></td>
<td><em>M. abscessus subsp. massiliense</em></td>
<td>95</td>
<td>91</td>
</tr>
<tr>
<td>1</td>
<td><em>M. chelonae complex (egr III, M. abscessus)</em></td>
<td><em>M. abscessus subsp. bolletti</em></td>
<td>95</td>
<td>91</td>
</tr>
<tr>
<td>1</td>
<td><em>M. kansasii</em> group I</td>
<td>Other mycobacteria species</td>
<td>97</td>
<td>96</td>
</tr>
<tr>
<td>1</td>
<td><em>M. fortuitum – M. peregrinum</em> complex</td>
<td>Other mycobacteria species/High GC gram positive bacteria</td>
<td>95</td>
<td>92</td>
</tr>
</tbody>
</table>

Lo, ASM 2019
Investigating heater-cooler water samples

Fig 1: Conventional identification vs identification via Hain GenoType NTM-DR

Lo, ASM 2019
Whole Genome Sequencing

Identification from positive cultures
Adv: Multiple genes

Direct detection from clinical sample?

Tortoli, Clin Micro Rev 2014

adapted from Pankhurst, Lancet, 2015
WGS for NTMs

‘Universal’ procedure
Costs equivalent
Analysis – no commercial system

Wee, ESM 2019
M. chimaera
UK, Swiss, NDL, USA, Australia (250 isolates)
24 fm cardiac surgery-related patients (21), 218 from hospitals HCU's, LivaNova (formerly Sorin) and Maquet (Rastatt, Germany) HCU production sites, and unrelated environmental sources and patients, as well as eight Mycobacterium intracellulare isolates.

M. abscessus
Preferred typing for outbreak investigations (BTS 2018)

Van Ingen, Lancet Inf Dis 2017
Direct detection from specimens

- No US-FDA cleared assays
- NTM by clinical/radiological suspicion and negative TB PCR test
- LDT – PRA, Hain Direct, LiPA

Table 3. Detection and Identification of Mycobacteria in Extrapulmonary Specimens

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Correct GM10 band</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present/Absent</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. tuberculosis POS</td>
<td>13/0</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>M. tuberculosis NEG</td>
<td>0/5</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>M. kansas only POS</td>
<td>1/0</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>M. kansas only NEG</td>
<td>0/20</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>MAC only POS</td>
<td>1/1</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>MAC only NEG</td>
<td>0/19</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>M. kansas with MAC POS</td>
<td>2/0</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>M. kansas with MAC NEG</td>
<td>0/19</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>OVERALL POS/NEG</td>
<td>17/1</td>
<td>94.4%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>96.5%</td>
</tr>
</tbody>
</table>

Hain Direct validation, ESM, Wang SX, 2009

- Majority of NTM-positive smears
  - M. abscessus
  - M. kansasii
  - M. avium complex

- Routine use of non-culture-based detection methods is not recommended at the present time. (Grade D)
- A validated rapid method should be used to detect NTM in respiratory samples. (Grade D)

BTS 2017
Susceptibility testing

• Perform only when disease is suspected and treatment is an option (severity, risk of progression, comorbidity, treatment goals).

Lab: ATS criteria

• More variables than TB – species, limited options

• Where possible, report MICs and critical concentrations

• Test to guide, but not dictate, treatment regimens (BTS)

• Need for monitoring

• Rapid growers, *M. avium* complex, *M. kansasii*
CLSI changes for DST

- **Reference method**: Microbroth dilution, serial two-fold dilution, provide the MIC and interpretation
- **SGM**: Interpretative criteria and new QC organisms (*M. marinum* ATCC 927) provided
- **Rapid growers** – report tigecycline MICs, imipenem interpretation
- **M. abscessus** subspeciation strongly recommended
M. abscessus DST

Interpretation of clarithromycin susceptibility results for M. abscessus

<table>
<thead>
<tr>
<th>Clarithromycin susceptibility day 3-5</th>
<th>Clarithromycin susceptibility day 14</th>
<th>Genetic implication</th>
<th>M. abscessus sub-species</th>
<th>Macrolide susceptibility phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible</td>
<td>Susceptible</td>
<td>Dysfunctional erm(41) gene</td>
<td><em>M. a. massiliense</em></td>
<td>Macrolide susceptible</td>
</tr>
<tr>
<td>Susceptible</td>
<td>Resistant</td>
<td>Functional erm(41) gene</td>
<td><em>M. a. abscessus</em> <em>M. a. bolletti</em></td>
<td>Inducible macrolide resistance</td>
</tr>
<tr>
<td>Resistant</td>
<td>Resistant</td>
<td>23S ribosomal RNA point mutation</td>
<td>Any</td>
<td>High level constitutive macrolide resistance</td>
</tr>
</tbody>
</table>

Treatment: M. abscessus-pulmonary disease

<table>
<thead>
<tr>
<th>M. abscessus-pulmonary disease</th>
<th>Antibiotic regimen:</th>
</tr>
</thead>
</table>
| Clarithromycin susceptible isolates or Inducible macrolide resistant isolates | **Initial phase: ≥ 1 month**  
- iv amikacin 15mg/kg daily or 3x per week  
- iv tigecycline 50mg twice daily  
- and where tolerated: iv imipenem 1g twice daily and where tolerated oral clarithromycin 500mg twice daily or oral azithromycin 250-500mg daily  
- **Continuation phase:**  
  - nebulised amikacin  
  - oral clarithromycin 500mg twice daily or oral azithromycin 250-500mg daily
| | **Continuation phase:**  
- nebulised amikacin  
- oral clarithromycin 500mg twice daily or oral azithromycin 250-500mg daily  
- 1-3 of the following guided by drug susceptibility and patient tolerance: oral clofazimine, linezolid, minocycline, moxifloxacin, cotrimoxazole |

Key:
- Patients with clarithromycin resistant isolates may benefit from longer duration iv antibiotic treatment
- Substitute iv / nebulised amikacin for an alternative antibiotic if M. abscessus is resistant to amikacin (MIC >64mg/L or known to have a 16S rRNA gene mutation)
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- Substitute iv / nebulised amikacin for an alternative antibiotic if M. abscessus is resistant to amikacin (MIC >64mg/L or known to have a 16S rRNA gene mutation)

Low rates of constitutional resistance (2.4%) Chew, J Med Micro 2017
**M. abscessus DST**

**Interpretation of clarithromycin susceptibility results for M. abscessus**

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<tr>
<td>Susceptible</td>
<td>Resistant</td>
<td>Functional erm(41) gene</td>
<td>M. a. abscessus M. a. bolletti</td>
<td>Inducible macrolide resistance</td>
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<tr>
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<td>Resistant</td>
<td>23S ribosomal RNA point mutation</td>
<td>Any</td>
<td>High level constitutive macrolide resistance</td>
</tr>
</tbody>
</table>

Genetic resistance detection by HAIN NTMDR

Low rates of constitutional resistance (2.4%) Chew, J Med Micro 2017

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**M. abscessus** and aminoglycosides

- **Sensititre plate (RAPMYCO):** Amikacin, cefoxitin, ciprofloxacin, clarithromycin, doxycycline, imipenem, linezolid, moxifloxacin, Bactrim, tigecycline, tobramycin
- **Genotypic:** Hain NTMDR test
- If ≥64µg/ml, retest or confirm with Hain NTMDR or *rrs* sequencing. If resistance is confirmed, make a comment that resistance is greater than that expected for the species.

---

**Table 2:** Mutations in the *rrs* gene and the corresponding wild type and mutation bands (3-5)

<table>
<thead>
<tr>
<th>Failing wild type band</th>
<th>Analyzed nucleic acid position</th>
<th>Developing mutation band</th>
<th>Mutation</th>
<th>Phenotypic resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A1400G</td>
<td></td>
<td>Aminoglycosides</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>RGM species and antimicrobial agent (no. of isolates tested)</th>
<th>No. (% of isolates)</th>
<th>MIC (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Susceptible</td>
<td>Intermediate</td>
</tr>
<tr>
<td><strong>Mycobacterium abscessus</strong> Amikacin (313)</td>
<td>282 (90)</td>
<td>28 (9)</td>
</tr>
</tbody>
</table>

Tang, Clin Micro Infect 2015
**M. avium and M. kansasii DST**

- Test on isolate before starting treatment and with treatment failure or MAC is recultured after culture conversion (3-6mths, or earlier)
- Treatment continues for a minimum of 12 months after culture conversion
- $, delays)

<table>
<thead>
<tr>
<th></th>
<th><strong>M. avium</strong></th>
<th></th>
<th><strong>M. kansasii</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treatment</td>
<td>Test</td>
<td>Treatment</td>
</tr>
<tr>
<td>1st line</td>
<td>Rifampicin Ethambutol Azithromycin / Clarithromycin</td>
<td>Clarithromycin</td>
<td>Rifampicin Ethambutol Isoniazid or Azithromycin or Clarithromycin</td>
</tr>
<tr>
<td>2nd line</td>
<td>Severe as above +/- amikacin</td>
<td>Moxifloxacin Linezolid</td>
<td>CLR-resistant Rifampicin Clarithromycin</td>
</tr>
<tr>
<td>Others</td>
<td>X Azithromycin (use CLR) X clofazimine</td>
<td>X Isoniazid (poor correlation)</td>
<td></td>
</tr>
</tbody>
</table>
Assessing the microbiological response to treatment

<table>
<thead>
<tr>
<th>Definitions for Microbiological Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Culture conversion</strong>: three consecutive negative mycobacterial sputum cultures collected over a minimum of three months, with the time of conversion being the date of the first of the three negative mycobacterial cultures. In patients unable to expectorate sputum, a single negative mycobacterial culture of a CT-directed bronchial wash is indicative of culture conversion.</td>
</tr>
<tr>
<td><strong>Recurrence</strong>: two positive mycobacterial cultures following culture conversion. If available, genotyping may help distinguish relapse from reinfection.</td>
</tr>
<tr>
<td><strong>Refractory disease</strong>: failure to culture convert after twelve months of NTM treatment. Poor prognosis!</td>
</tr>
</tbody>
</table>
Conclusion

• Identification, molecular methods and MIC testing - very important

\textit{M. abscessus} - should be subspeciated

• Challenges faced - $\$

• Focus areas: Direct NAAT, Role of pharmacists, combination testing, correlative studies
• Annual Report, SGH Department of Pathology, 1998 – 2018.
• Practice Guidelines for Clinical Microbiology Laboratories: Mycobacteria. Forbes BA, Hall GS, Miller MB, Novak
References

- Product Insert, INNO-LiPA MYCOBACTERIA v2, Fujirebio
- Whole Genome Sequencing (WGS) for the subspeciation of clinical isolates of Mycobacterium abscessus complex in a Clinical Laboratory. 2019. Wee JFJ, Lee BWM, Choo PH, Sng L. 40th Annual Meeting of the European Society Mycobacteriology, Valencia, Spain.