Diagnosis of nontuberculous mycobacterial infections

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Conflicts of interest: Research grants and consultancies for Hain Lifesciences 2015
NTM infections

- **Respiratory infections**
  - Cystic fibrosis?
  - Other respiratory diseases?
  - Previous TB?
  - Smoker, 60-yo woman,

- **Extra-respiratory infections**
  - HIV positive AIDS
  - Immunodeficiency
  - Corticosteroid or immunosuppressing treatment
  - Iatrogenic (surgery, injection, plastic procedures)

ATS/IDSA criteria guidelines 2007 for diagnosis of NTM infections

• The criteria apply to lung diseases only, and to symptomatic patients with radiographic opacities
• These criteria fit best with *Mycobacterium avium* complex (MAC), *M. kansasii*, and *M. abscessus*.
• Needs
  – to define criteria for HAI infections
  – to update criteria according to recent findings on *M. abscessus*

Diagnosis of respiratory infections due to NTM

• Is there a real NTM infection?
  – Clinical symptoms +
  – Radiologic symptoms
  – AND
  – Microbiology positive
Which samples and how many?

• Respiratory infections:
  – Problem of contamination by the environment
  – Problem of colonization different from the infection
  ⇒ several samples > one sample
  ⇒ Repeat the sampling if positive

• Extra-respiratory infections:
  – Sample from the site of the infection + BLOOD (specific blood culture)
  – If non sterile site: several samples > one sample
Procedures in the CM lab for cultures

• As usual for mycobacteria (NALC-NAOH decontamination)
• Liquid and solid media for culture
• Temperature
  – 37°C for all, 30°C for some of them, 42°C if needed for M. xenopi
  – during 2 to 3 months!

Be aware that the presence of NTM in the environment may depend where the patient and you are living.

Rio Grande, USA
(Bland et al. (2005))

Hoefsloot W et al. 2013
NTM are present in water networks

Does this patient have a real NTM infection?

- The name of the NTM species
  - Some are more commonly pathogenic than others

- Number of positive samples with regard to the NTM species isolated:
  - At least 2 separated sputum samples for *M. avium* complex (especially *M. chimaera*)
  - One positive sample may be enough for *M. kansasii*
  - Many samples positive for some species (*M. fortuitum, M. abscessus*)
  - If repeated weeks later, they will still be positive in case of infection

Choosing the NTM isolate

• From a clinical suspicion of NTM infection or asystematic culture for tuberculosis diagnosis?
• Microscopy result: AFB positive – sample?
• = Positive culture with AFB but no MTB…
  – Solid / liquid media
  – Quantity in non protected specimens
Do you know the mycobacterial species involved?

• **NO**: do sampling again and microbiological examination in a lab with NTM expertise

• **YES**: What is its name?
  Some are more commonly pathogenic than others
  – how this species was identified?
  – At the Complex level
  – At the species level
  – At the subspecies or variant level?
Identification of NTM

Molecular identification
- Target (*rrs, rrl, ITS, hsp65, rpoB, gyrB...*)
- Probes
- Reverse hybridization
- PCR sequencing
- Whole genome sequencing

Mass spectrometry MALDI-ToF
- Apparatus (Brucker, Shimadzu, ...)
- Data base (biotyper, vitekMS, andromas..)
- Protein extraction

Rapidly growing mycobacteria

gene or sequence based phylogeny
hsp65, rpoB, rrs, rrl, gyrB, ITS, ...

slowly growing mycobacteria

Roth 1998, Dai J. 2011; Adekambi 2003, Mc Nabe 2004,
Whole genome based phylogeny
Tortoli et al.
Infect Genet Evol 2017
**M. abscessus** bacterial specificities

- Strains distributed into subspecies (*rpoB*, *hsp65*)
- One species (> 70% DNA-DNA)

- **subsp. abscessus**
- **subsp. bolletii**
- **subsp. massiliense**

MLST (argH, cya, glpK, gnd, murC, pta, purH)

Adekambi 2006, Leao IJSM 2010; Cho YJ Plos one 2013, Macheras 2014
**M. abscessus** genome characteristics

- 5,067,172 bps ATCC strain
- Genes transferred from other RGM and non-mycobacterial bacteria
- Confirms the 3 subspecies with exchanges and mosaic genomes

Gene flow between the three subspecies

Identification by MALDI-TOF

**Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF MS)**

1. **Apparatus**
   - Microflex (Bruker Daltonics)
   - Vitek MS (bioMerieux)

2. **Data bases**
   - Mycobacterium Library v1.0 bioTyper (Bruker)
   - Vitek MS
   - Andromas

**Unknown microorganism**

Select a colony

Smear a thin-layer onto a MALDI target plate

Generate MALDI-TOF profile spectrum

BioProfiler
Data interpretation

Identified species

Buchan 2014, Lever 2017, Alcaide 2018

Courtesy of J Gonzalez
Give a precise identification of the NTM isolate

- All clinically relevant isolates of NTM should be identified by molecular methods
- MALDI-Tof can be used on pure cultures
- Identification is needed to the species level and to the subspecies level if needed (CM lab with NTM expertise)

- **Identify follow-up isolates** of patients undergoing treatment
- Isolates of patients who are being treated for NTM pulmonary disease **should be frozen and saved** in order to distinguish reinfection from relapse when recurrence occurs
Warning introduction on antimycobacterial susceptibility testing

• Few is known about susceptibility testing and resistance of non tuberculous mycobacteria

• It is different from what is known for tuberculosis and *Mycobacterium tuberculosis* complex

• Need to follow the recommendations
Has the patient previously been treated by antibiotics?

- For an infection due to the same NTM species?
- Is it a relapse or a new case?
- Having treated for another infection?
  - Chronic broncho-pneumopathy
  - General disease: diabetes, renal failure, transplant....

=> If Yes, I need to perform AST and I will search acquired or secondary resistance to antibiotics
Detection of acquired resistance

<table>
<thead>
<tr>
<th>Mycobacterial species</th>
<th>Mandatory</th>
<th>Supplement</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. avium</em> complex</td>
<td>Clarithromycin</td>
<td>amikacin</td>
</tr>
<tr>
<td><em>M. kansasii, M. szulgai</em></td>
<td>Rifampicin</td>
<td>If rif-R</td>
</tr>
<tr>
<td><em>M. abscessus</em></td>
<td>Clarithromycin</td>
<td>Amikacin</td>
</tr>
<tr>
<td><em>M. bolletii</em></td>
<td>Clarithromycin</td>
<td>amikacin</td>
</tr>
<tr>
<td><em>M. massiliense</em></td>
<td>Clarithromycin</td>
<td>amikacin</td>
</tr>
<tr>
<td><em>M. chelonae</em></td>
<td>Clarithromycin</td>
<td>tobramycin</td>
</tr>
<tr>
<td><em>M. fortuitum</em></td>
<td>Ciprofloxacin</td>
<td></td>
</tr>
</tbody>
</table>
Acquired resistance to macrolides in NTM

Relapse cases of NTM infection due to:
- M. avium
- M. intracellulare
- M. abscessus
- M. chelonae

mutations in the rrl gene encoding 23S RNA at positions A2058 or A2059

Intrinsic resistance to macrolides in mycobacteria

⇒ Inducible 23S RNA methylation at A2058 due to the *erm* gene
# ermA41 sequevars in *M. abscessus*

<table>
<thead>
<tr>
<th>Subspecies</th>
<th>ermA41 sequevar</th>
<th>MIC90 clarithromycin (mg/L) after 14 days</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. massiliense</em></td>
<td>Deletion of 276 pbs</td>
<td>1</td>
</tr>
<tr>
<td><em>M. abscessus</em></td>
<td>ermA41 C28 (R10)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>ermA41 T28 (W10)</td>
<td>&gt;256</td>
</tr>
<tr>
<td><em>M. bolletii</em></td>
<td>ermA41 T28 (W10)</td>
<td>&gt;256</td>
</tr>
</tbody>
</table>

Relation between *erm41* polymorphism and clinical efficacy

<table>
<thead>
<tr>
<th>Table 3. Treatment Responses for Patients with <em>Mycobacterium abscessus</em> and <em>Mycobacterium massiliense</em> Lung Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td><strong>M. abscessus</strong></td>
</tr>
<tr>
<td>(n = 24)</td>
</tr>
<tr>
<td>Symptomatic response</td>
</tr>
<tr>
<td>Improved</td>
</tr>
<tr>
<td>Unchanged</td>
</tr>
<tr>
<td>Worsened</td>
</tr>
<tr>
<td>Radiographic response on HRCT</td>
</tr>
<tr>
<td>Improved</td>
</tr>
<tr>
<td>Unchanged</td>
</tr>
<tr>
<td>Worsened</td>
</tr>
<tr>
<td>Microbiologic response</td>
</tr>
<tr>
<td>Initial sputum conversion and maintenance of conversion</td>
</tr>
<tr>
<td>6 (25%)</td>
</tr>
<tr>
<td>Initial sputum conversion, with sputum relapse</td>
</tr>
<tr>
<td>4 (17%)</td>
</tr>
<tr>
<td>Failure to sputum conversion</td>
</tr>
<tr>
<td>14 (58%)</td>
</tr>
</tbody>
</table>

Molecular detection of resistance in NTM detecting intrinsic and acquired resistance

<table>
<thead>
<tr>
<th>Species</th>
<th>Antibiotic</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. avium complex</em></td>
<td>Clarithromycin / azithromycin</td>
<td><em>rrl</em></td>
</tr>
<tr>
<td></td>
<td>Amikacin</td>
<td><em>rrs</em></td>
</tr>
<tr>
<td><em>M. kansasii, M. szulgai</em></td>
<td>rifampicin</td>
<td><em>rpoB</em></td>
</tr>
<tr>
<td><em>M. chelonae</em></td>
<td>Clarithromycin / azithromycin</td>
<td><em>rrl</em></td>
</tr>
<tr>
<td><em>M. fortuitum</em></td>
<td>fluoroquinolones</td>
<td><em>gyrA</em></td>
</tr>
<tr>
<td><em>M. abscessus</em></td>
<td>Clarithromycin / azithromycin</td>
<td><em>erm41, rrl, rrs</em></td>
</tr>
</tbody>
</table>

Phenotypic antibiotic susceptibility testing for NTM
(CLSI 2011 guidelines, revised 2018, EUCAST in preparation)

• Broth microdilution in 7H9
  – 0.5 Mc Farland inoculum
  – final concentration of $5 \times 10^5$ cfu/ml
  – pH 7.3 for macrolides and others (MH+OADC)
  – Use of control strains
  – Second reading with extended incubation for macrolides
  – No Tween80: increase the permeability and artificially decrease the MIC
AST in liquid medium

MIC = 4 mg/L

Positive growth control
Problem of heterogenous populations ex. *M. abscessus* and clarithromycin / Etest method
Distribution of clarithromycin MIC at early reading time (ERT) and late reading time (LRT) for 165 clinical isolates of *M. abscessus*
Clarithromycin MIC with regard to the subspecies at early reading time (white) and late reading time (black)

**erm41 massiliense**

![Graph showing Clarithromycin MIC (mg/L) for erm41 massiliense]

**erm41 abscessus C28**

![Graph showing Clarithromycin MIC (mg/L) for erm41 abscessus C28]

**erm41 bolletii**

![Graph showing Clarithromycin MIC (mg/L) for erm41 bolletii]

**erm41 abscessus T28**

![Graph showing Clarithromycin MIC (mg/L) for erm41 abscessus T28]

Mougari F. et al. 2016
Molecular detection of resistance ex. Commercial test GenoType NTM-DR

M. avium complex
M. abscessus
M. cheloneae

erm41 M. abscessus
macrolide
Intrinsic resistance

Macrolide and aminoglycoside acquired resistance in NTM
Conclusions

• NTM respiratory infections will be more often diagnosed
• Identification of species and subspecies is mandatory
• \textit{erm41} sequevar and \textit{rrl} genotype to be determined before starting a treatment by clarithromycin or azithromycin
• Needs to know more about
  – Mode of contamination? Source and reservoir?
  – Difference between colonization and infection
  – Relapse and reinfection
  – Antibiotic susceptibility to antibiotics others than macrolides and aminoglycosides
  – New active antibiotics?
Coming soon.......
ESGMYC
ESCMID STUDY GROUP FOR MYCOBACTERIAL INFECTIONS

European Society of Clinical Microbiology and Infectious Diseases

- Created in 2011
- Elections for the board (chair, secretary, treasurer) this year!
- About 140 members: mostly clinical microbiologists and infectious diseases specialists
- Topics: Tuberculosis, leprosy and infections due to nontuberculous mycobacteria
- Chair: Delia Goletti; secretary: Mateja Jankovic; treasurer: Miguel Santin

- Visit our Website / www.escmid.org/esgmyc
  - For more information
  - TO BECOME A MEMBER (Free for ESCMID members)
  - group meeting on Tuesday 13.15 room G106-107
Mycobacteroides (Mycobacterium) abscessus complex, Mycolicibacterium (Mycobacterium) fortuitum, and Mycobacteroides (Mycobacterium) chelonae??
Comparaison génomique entre mycobactéries
Scheme of selection of resistant mutant during treatment is similar to what described in tuberculosis and leprosy.