The enigma of *Mycobacterium abscessus* infection

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Conflicts of interest

Research grants or consultancies for:
- Biomérieux 2011, 2013
- Hain Lifescience 2012-2015
- Becton-Dickinson 2009-2014
Number of publications related to *M. abscessus*
**M. abscessus** is a Nontuberculous mycobacteria (NTM)

<table>
<thead>
<tr>
<th>Strict pathogen</th>
<th>Opportunistic pathogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present in Patients and animal with disease</td>
<td>Soils, water and intermittent commensalism</td>
</tr>
<tr>
<td>Highly transmissible</td>
<td>Rare transmission</td>
</tr>
<tr>
<td><strong>M. tuberculosis</strong> → TUBERCULOSIS</td>
<td>About 150 species of Nontuberculous mycobacteria (atypical mycobacteria)</td>
</tr>
<tr>
<td>M. leprae → LEPROSY</td>
<td></td>
</tr>
<tr>
<td>Biosafety level 3</td>
<td>Biosafety level 2</td>
</tr>
</tbody>
</table>
Rapidly growing mycobacteria

but one ribosomal operon

Like slowly growing mycobacteria

Dai 2011
**M. abscessus** bacterial specificities

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

**Subspecies distribution**

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

**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

NTM infections

- Respiratory infections
  - Cystic fibrosis
  - Other respiratory diseases
  - Previous TB
  - Hot tub Spa
  - Smoker
  - 60-yo woman

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

**M. abscessus** infection specificities

- Frequent pulmonary infections in cystic fibrosis and 60 y.o. woman
- Frequent epidemic infections due to health care contaminations
- Rare isolation from the environment (surface and network waters, soils, devices)
- Resistant to nearly all antibiotics (intrinsic resistance)
- High rate of failure of antimicrobial treatment
- Recurrences even after long time treatment (relapse? reinfection?)

Health care associated infections

- Large outbreak in South America (Viana-Niero 2008, Cardoso-Leao 2009)
  - *M. abscessus* subsp. *massiliense*
- South Corea (Choi 2011)
  - 52 cases 2007-2008
  - Acupuncture (Koh 2010, Tang 2006)
- Surgical tourism (Furuya 2008, MMWR 2014)
- Direct injection (Zhibang Y 2002)
- Cytokine-induced killer cell therapy for body beautification health boosting (Liu R 2013)
### Table 1 Sites of isolation of *M. abscessus* from human samples 2001-2010

<table>
<thead>
<tr>
<th>Site of isolate</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>14 (2.9)</td>
</tr>
<tr>
<td>Bones and joints</td>
<td>5 (1)</td>
</tr>
<tr>
<td>Cutaneous/Soft tissue</td>
<td>108 (22.2)</td>
</tr>
<tr>
<td>Eye</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td>Lymph node + Other</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>3 (0.6)</td>
</tr>
<tr>
<td>Peritoneal</td>
<td>2 (0.4)</td>
</tr>
<tr>
<td>Post surgical/ Medical access device</td>
<td>19 (3.9)</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>331 (68.1)</td>
</tr>
<tr>
<td>Unknown</td>
<td>2 (0.4)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>486 (100)</strong></td>
</tr>
</tbody>
</table>
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*

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

Identification of *M. abscessus*

- Acid fast bacilli
- Rapidly growing mycobacteria
- Biochemical characterization: not done any more in clinical microbiology
- *rpoB, hsp65, 23 SRNA (rrl), ITS* for molecular identification of *M. abscessus* (not 16S RNA!)
- *rpoB, hsp65, MLST, erm41* for molecular identification of subspecies: massiliense, bolletii and abscessus
- *erm41, rrl and rrs* for molecular detection of intrinsic and acquired resistance

*M. abscessus* subspecies detection by MALDI-Tof

**FIG 2** MALDI-TOF spectral profiles and characteristic peaks for each of the three *Mycobacterium abscessus* subspecies. Accurate analysis with ClinProTool software highlighted five discriminating peaks (m/z 2,081, 3,123, 3,378, 3,463, and 3,378).

ECCMID 2016  
Fangous MS et al. JCM 2014
Antibiotic susceptibility testing in *M. abscessus*

• Is this a real mycobacterial infection or colonization
• Does the patient previously been treated by antibiotics?
  – Relapse or new case?
  – Previous receipt of antibiotics for other infections?
  – Cystic fibrosis (azithromycin long term ttt)
• Only for antibiotics known for efficacy and resistance (macrolides and aminoglycosides)
  – Intrinsic resistance = *erm*41 sequevar / macrolide; amikacin and not tobramycin

Phenotypic antibiotic susceptibility testing for *M. abscessus* (CLSI 2011 guidelines 2011)

- **Microdilution**
  - 0.5 Mc Farland inoculum
  - 5 $10^5$ cfu/ml
  - pH 7.3 for macrolides and others (MH+OADC)
  - Control strains
  - Not more than 5 days except macrolides
  - No Tween80: increase the permeability and artificially decrease the MIC
AST in liquid medium: microdilution for rapid growers

MIC = 4 mg/L

Positive growth control
Problem of heterogenous populations ex. *M. abscessus* and clarithromycin / Etest method

ECCMID 2016 E. Cambau
Antibiotic resistance pattern of *M. abscessus*

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt; (mg/l)</th>
<th>Susceptibility*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clarithromycin</td>
<td>0.25 / 256</td>
<td>S / R</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>32</td>
<td>I</td>
</tr>
<tr>
<td>Imipenem</td>
<td>16</td>
<td>I</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>8</td>
<td>R</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>16</td>
<td>R</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>&gt; 8/152</td>
<td>R</td>
</tr>
<tr>
<td>Co-amoxyclov</td>
<td>&gt; 64/32</td>
<td>R</td>
</tr>
<tr>
<td>Amikacin</td>
<td>16</td>
<td>S</td>
</tr>
<tr>
<td>Linezolid</td>
<td>32</td>
<td>R</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>0.25-0.5</td>
<td>S</td>
</tr>
<tr>
<td>Clofazimine</td>
<td>0.25</td>
<td>S</td>
</tr>
</tbody>
</table>

* With regard to breakpoints defined for other bacteria

Acquired resistance to macrolides (Clarithromycin/azithromycin) in *M. abscessus*

⇒ High level resistance (MIC > 256 mg/l) in isolates with *rrl* mutations (a2058, a2059)

Intrinsic resistance to macrolides in *M. abscessus*

⇒ Inducible 23S RNA methylation at A2058 due to the *erm41* gene

Polymorphism of *erm41* within the *M. abscessus* species and subspecies

<table>
<thead>
<tr>
<th>Subspecies</th>
<th><em>erm(41) sequevar</em></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></td>
<td><em>erm (41)</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C28 (R10)</td>
<td>2</td>
</tr>
<tr>
<td><em>M. abscessus</em></td>
<td><em>erm (41)</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T28 (W10)</td>
<td>&gt;256</td>
</tr>
<tr>
<td><em>M. bolletii</em></td>
<td><em>erm (41)</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T28</td>
<td>&gt;256</td>
</tr>
</tbody>
</table>

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 masiliense**

![Graph showing Clarithromycin MIC (mg/L) for M. masiliense with isolates (N) distribution.]

**erm41 abscessus C28**

![Graph showing Clarithromycin MIC (mg/L) for M. abscessus C28 with isolates (N) distribution.]

**erm41 bolletii**

![Graph showing Clarithromycin MIC (mg/L) for M. bolletii with isolates (N) distribution.]

**erm41 abscessus T28**

![Graph showing Clarithromycin MIC (mg/L) for M. abscessus T28 with isolates (N) distribution.]

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

**erm41 M. abscessus**

- **macrolide**
  - natural resistance

- **Macrolide and aminoglycoside**
  - acquired resistance

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Amino Acid</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>rrs MUT1</td>
<td>A1408G</td>
<td>T1496A</td>
</tr>
<tr>
<td>rrs MUT2</td>
<td>A2058C</td>
<td>C1409T</td>
</tr>
<tr>
<td>rrs MUT3</td>
<td>A2058G</td>
<td>A2058T</td>
</tr>
<tr>
<td>rrs MUT4</td>
<td>A2059C</td>
<td>A2059T</td>
</tr>
</tbody>
</table>

Other Species: includes *M. scrofulaceum, M. interjectum, M. kansasii, M. malmoense, M. haemophilum, M. palustria*
Relation between *erm41* polymorphism and clinical efficacy of macrolides

**Table 3. Treatment responses for patients with Mycobacterium abscessus and Mycobacterium massiliense lung disease**

<table>
<thead>
<tr>
<th></th>
<th><em>M. abscessus</em> (n = 24)</th>
<th><em>M. massiliense</em> (n = 33)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Symptomatic response</strong></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><strong>Radiographic response on HRCT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improved</td>
<td>10 (42%)</td>
<td>27 (82%)</td>
<td>0.003</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><strong>Microbiologic response</strong></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>

Aminoglycoside resistance

Intrinsic resistance due to AAC2, APH3’

Acquired resistance to amikacin = modification of 16S subunit
- *rrs* (16 SRNA) mutations
  - most often a1408g
  - T1406a, C1409t, g1491t

In vivo activity of other antibiotics

Figure 2. Lung (A) and spleen (B) colony-forming unit (CFU) counts in nude mice treated with cefoxitin (300 mg/kg, 4 times daily) clarithromycin (100 mg/kg/d), and amikacin (150 mg/kg/d), alone and in combination (trial 3).

Spleen

Lungs

Lerat JD 2013
M. abscessus is rarely found in the environment

- Australia
  - 14 abscessus, 1 bolletii were isolated from water
  - Urban water network, tank water, swimming pool
- South Africa: PCR positive in water
- Taiwan?
- USA?? Pseudo-outbreaks in 1994 et 1997
- In the past, difficult to distinguish from M. chelonae?

M. abscessus genotyping: relapse, reinfection? transmission? Reservoir?
Disease progression with regard to genotypes
Shin SJ 2013

Figure 3. Relationships between VNTR genotype, disease phenotype, and disease progression in *Mycobacterium abscessus* and *Mycobacterium massiliense* lung disease. The genetic distance was calculated as the Manhattan distance of each (A) *M. abscessus* and (B) *M. massiliense* clinical isolate from the reference strains ATCC19977 and CIP108297, respectively. To determine whether the individual characteristics of disease could be discriminated based on the VNTR genotype, a comparison was made between the patients with stable and progressive disease of the nodular bronchiectatic form and the fibrocavitary form. The horizontal bar through each scatter plot indicates the mean value of genetic distance for the group. *P*<.05 and ***P***<.001 were considered significant.
Conclusions on *M. abscessus*

- Will be more and more diagnosed
- Identification of species and subspecies is mandatory
- `erm41` sequevar and `rrl` genotype to determine before treatment by clarithromycin or azithromycin
- Needs to know more about
  - Mode of contamination? Reservoirs?
  - Difference between colonization and infection
  - Relapse and reinfection
  - Antibiotic susceptibility to antibiotics others than macrolides and aminoglycosides
  - New active antibiotics?
Perspectives: New guidelines for the treatment of NTM infections

• Memorandum of understanding between
  – American Thoracic Society
  – European Respiratory Society
  – Infectious Disease Society of America
  – European Society of Clinical microbiology and Infectious Diseases (ESGMYC study group)

• 2013-2016

ESGMYC
European Society of Clinical Microbiology and Infectious Diseases

ECCMID 2016
E. Cambau
ESGMYC
European Society of Clinical Microbiology and Infectious Diseases

• Created in 2011
• Elections for the board (chair, secretary, treasurer) this year!
• About 66 active members: mostly clinical microbiologists and infectious diseases specialists
• Topics: Tuberculosis, leprosy and infections due to nontuberculous mycobacteria

• Visit our Website / www.escmid.org/esgmyc
  – For more information
  – TO BECOME A MEMBER (Free for ESCMID members)
  – group meeting on Monday 13.15 room 101
Did you isolate or see a case of infection due to *Mycobacterium abscessus*: 

A. in your whole life? 

B. last year? 

C. last month?
Is the differenciation between M. abscessus subspecies (abscessus abscessus, bolletii, massiliense) done at your place (hospitals, labs, wards) ?

A. Yes

B. No
Do you test susceptibility to clarithromycin by incubating the plates during 14 days?

A. Yes

B. No
Do you use molecular detection of resistance to any antibiotic for M. abscessus?

A. Yes

B. No