

# How can we deal with Non Tuberculous Mycobacteria

Pr. Jean-Louis Herrmann  
INSERM-Versailles University UMR1173  
Microbiology Department, Raymond Poincaré Hospital  
Versailles and Garches, France  
[jean-louis.herrmann@aphp.fr](mailto:jean-louis.herrmann@aphp.fr)

# Definitions

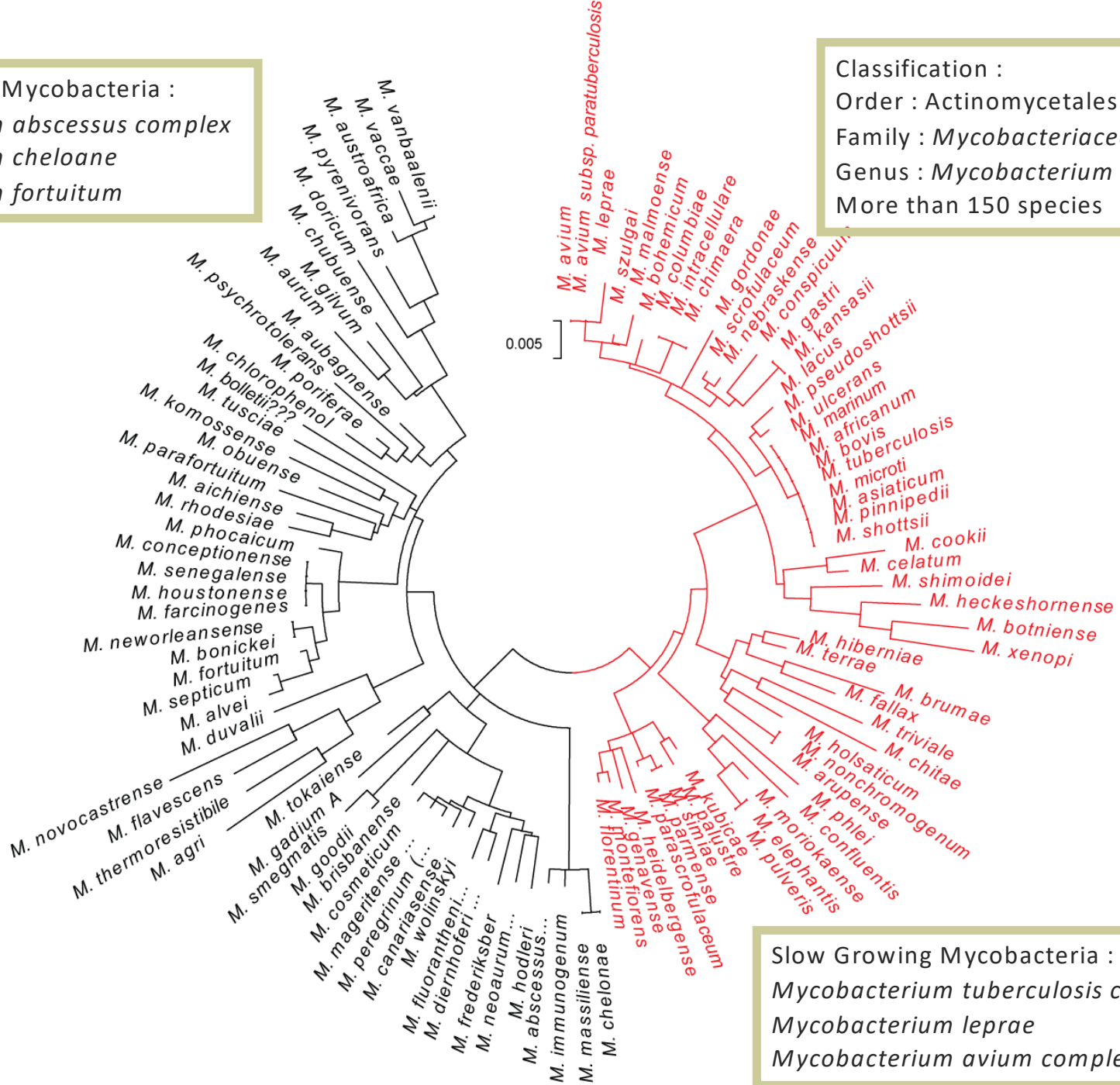
- Nontuberculous mycobacterial (NTM) species are mycobacterial species other than those belonging to the *Mycobacterium tuberculosis* complex (eg, *M. tuberculosis*, *M. bovis*, *M. africanum*, and *M. microti*) and *M. leprae*.
- NTM are generally free-living organisms that are ubiquitous in the environment. There have been more than 200 NTM species identified.

# Recognition of the great distance we have travelled over the last 30 years

- NTM lung diseases
  - Pathophysiology, including mechanisms of acquisition
  - Epidemiology, including estimates of disease prevalence
  - Mycobacteriology (Molecular biology, Maldi-Tof)
  - Even treatment strategies
- The more we know about NTM respiratory pathogens, the less Mtb is a pertinent model for them, in the realm of therapy

Rapid Growing Mycobacteria :  
*Mycobacterium abscessus* complex  
*Mycobacterium chelonae*  
*Mycobacterium fortuitum*

Classification :  
 Order : Actinomycetales  
 Family : *Mycobacteriaceae*  
 Genus : *Mycobacterium*  
 More than 150 species



Slow Growing Mycobacteria :  
*Mycobacterium tuberculosis* complex  
*Mycobacterium leprae*  
*Mycobacterium avium* complex

# Inside the *Mycobacterium* genus

- Only a very small number are strict pathogens for men and animals
  - *M. tuberculosis* complex
  - NTM
    - Strict pathogens
      - *M. marinum*
      - *M. ulcerans*
    - Opportunistic pathogens: *M. avium*, *M. intracellulare*, *M. kansasii*, *M. xenopi*...
    - Rapid growing mycobacteria
      - Opportunistic pathogens: *M. abscessus*, *M. chelonae*, *M. fortuitum*
      - Saprophytic bacteria: *M. smegmatis*, *M. goodii*...
- The key difference between pathogenic and non pathogenic:
  - to replicate and persist within antigen presenting cells (macrophages, dendritic cells)

# Species identification

- 30 years ago: the Runyon classification
  - Only 40 to 50 species
- HPLC, DNA probes, gene sequencing techniques (16S, hsp60, rpoB, 16-23S...)
  - More than 200 species
- Multi-gene sequencing, WGS
- MALDI-Tof

# Epidemiology

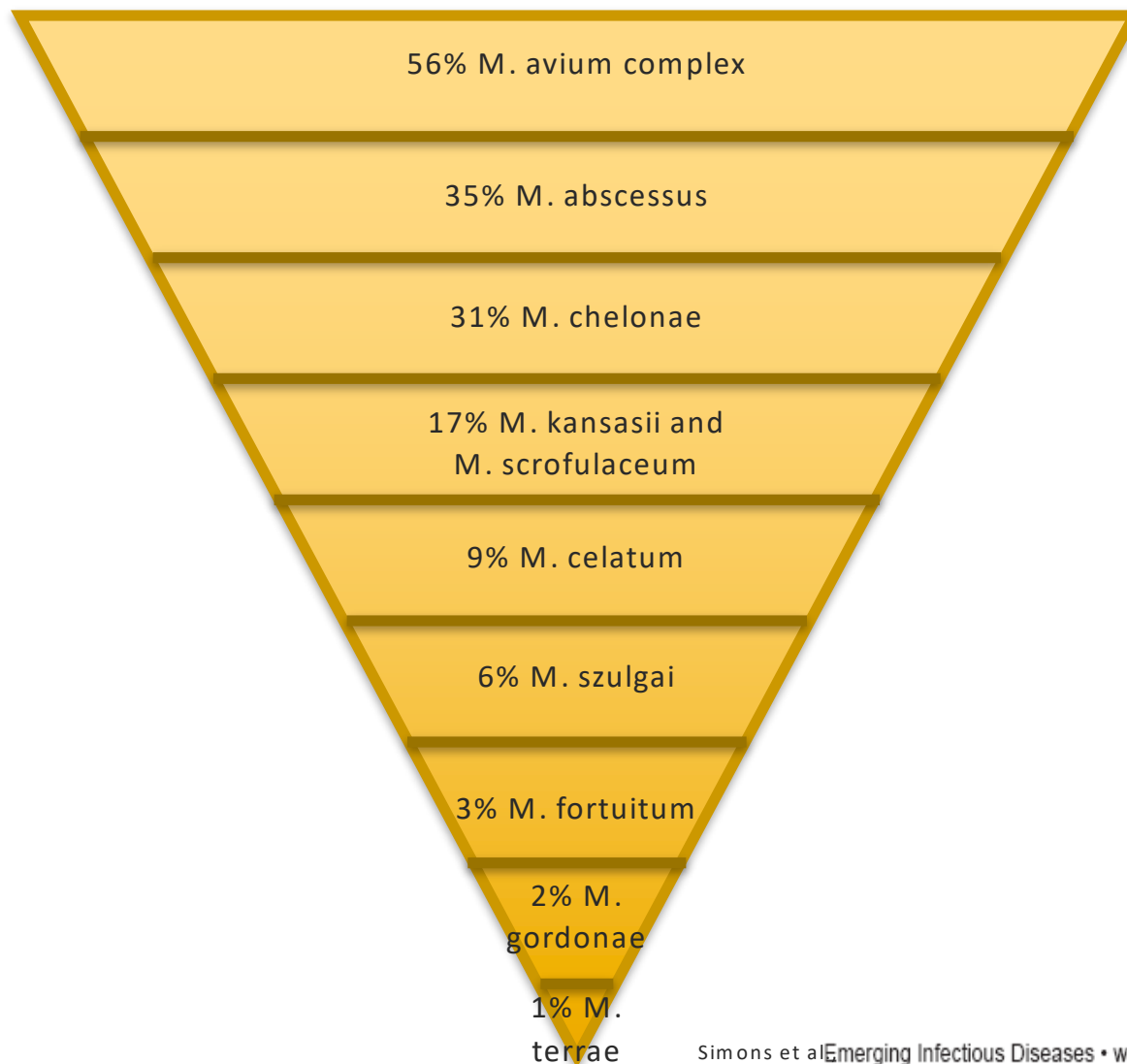
- With Or Without HIV ?
- Impact of the CD4 T-cells count
- Mendelian deficit (IL-12 – IFN $\gamma$  axis)
- TNF blockers
- chronic lung infections account for up to 90 percent of patient encounters due to NTM

# Epidemiology

- Non-tuberculous mycobacterial infections have been increasing worldwide over the last 2 decades (Griffith et al., 2007)
- incidence and prevalence of NTM lung disease: problematic
  - disease reporting is not mandatory
  - there must be an assessment of the clinical significance of individual NTM isolates, as opposed to *M. tuberculosis*, where each isolate is assumed to be associated with true disease.
- In Europe: NTM-Network European Trials Group (NET) framework
  - [www.ntm-net.org](http://www.ntm-net.org) (Eur Respir J. 2013 Dec;42(6):1604-13):
  - 20 182 patients, from 62 laboratories in 30 countries across six continents.
  - 91 different NTM species were isolated (MAC, *M. goodii*, and *M. xenopi*)
  - species distribution among NTM isolates from pulmonary specimens in the year 2008 differed by continent and differed by country within these continents



Clinical relevance of pulmonary non tuberculous mycobacterium (NTM) isolates, Asia, 1971–2007. Relevance per species was defined as percentage of patients with pulmonary NTM isolates meeting the American Thoracic Society criteria.



# Epidemiology

- In Oregon (Cassidy *et al.* 2009): 2005-2006
  - 5.6 cases/100 000 persons, including 6.4 cases/100 000 women and 4.7 cases/100 000 men.
  - The prevalence was highest in patients over 50 years of age with 15.5 cases/100 000 persons.
  - MAC was the most common species identified in pulmonary cases (4.7cases/100 000 persons).
- In the USA (Prevots *et al.* 2010; Winthrop *et al.* 2010):
  - 1.7 cases/100,000 persons in Southern Colorado to 6,7/100,000 in California.
  - MAC was the most common species identified in pulmonary cases (4.7cases/100,000 population)
  - If matching NTM isolation with clinical history: 8,6/100,000 population (2005-2006)
  - The prevalence was highest in patients  $\geq$  50 years of age with 20.4 cases/100 000 population.

# Epidemiology

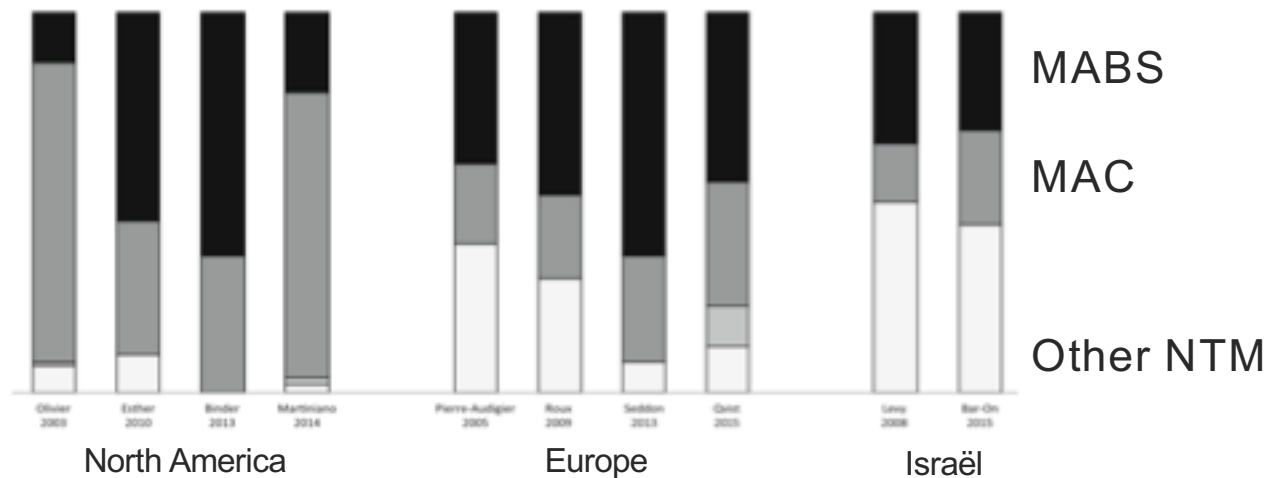
- In Canada (Marras *et al.* 2007)
  - from 9.1/100 000 population in 1997 to 14.1/100 000 population in 2003.
- In France: (Dailloux *et al.*, 2006)
  - 0.74, 0.73 and 0.72 cases per 100,000 inhabitants in 2001, 2002 and 2003, respectively.
  - MAC pulmonary disease: 0.2 per 100,000 inhabitants between 2000-2002

# NTM Epidemiology

- Amongst TB positive patients
  - In Iran: 10.2% of TB positive patients
  
- Amongst the population
  - Without any risk factor
    - In Japan: from 2001 – 2009: 11 and 10.1 / 100,000
    - *M. intracellulare*, and *M. avium*
  - With risk factor:
    - Immunotherapy:
      - 56 269 older adults with RA: 37 cases of TB and 211 cases of NTM disease

# NTM Epidemiology and Cystic Fibrosis

- NTM prevalence range: 3-23% in CF patients (**mean around 9%**)
- Species:  
Majority of NTM are **MAC** or **MABS** and they are the most clinically significant  
**MAC 75% - 25 % MABS** (*M. abscessus* and *massiliense*)
- Geographical variation:



- **MABS accelerate lung function decline**

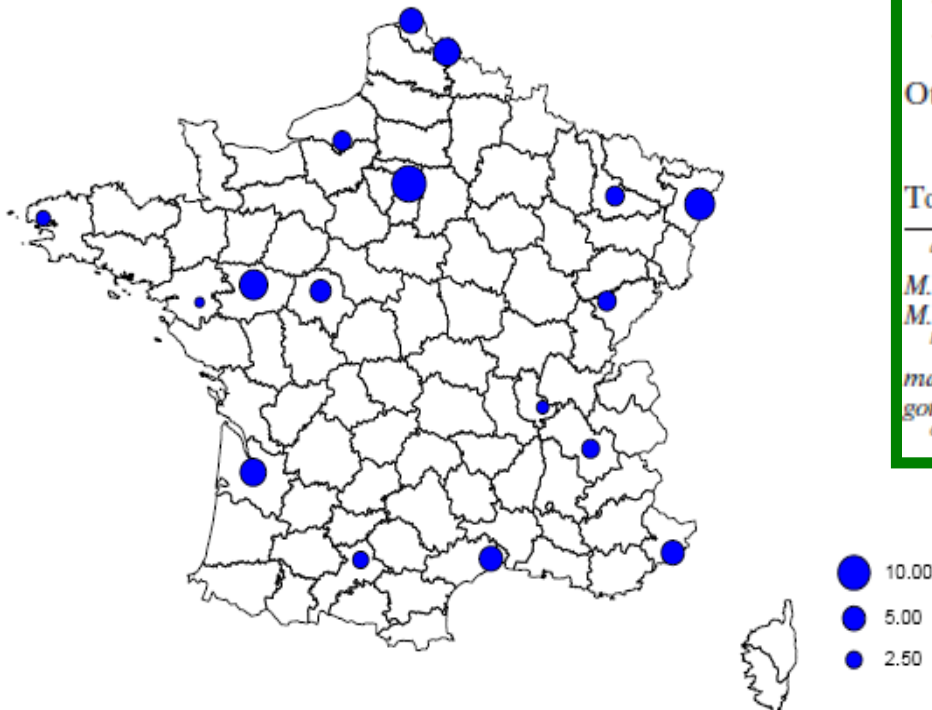
(Lévy, EID, 2008; Esther, JCF, 2010 ; Roux, JCM, 2009; Olivier AJRMCCM, 2007)

# Non Tuberculous Mycobacteria (NTM) and Cystic Fibrosis

- Since the 90's: increasing number of studies have reported the isolation of NTM from the respiratory tract of patients with CF
  - 1,8 à 19,5% of patients infected
- *M. avium* complex (MAC) and MABS account for over 95% of CF cases
  - MAC in North America, MABS in Western Europe and in Israel
- One multicenter prevalence study (USA) Prevalence of 13%.  
Olivier KN et al. (2003). Am J Respir Crit Care Med. 167:828-834.
  - *M. avium* complex: 1<sup>st</sup> isolated NTM, *M. abscessus*: 2<sup>nd</sup> isolated NTM
  - Patients aged > 10 years.
- 3 studies in paediatric hospitals (France) : Prevalence 6,6-9,8%,  
Fauroux et al. (1997) Pediatr Infect Dis J. 16:354-358. Sermet-Gaudelus et al. (2003). Emerging infect. dis. 9(12): 1587-91. Pierre-Audigier C et al. (2005) J Clin Microbiol. 43:3467-3470.
  - *M. abscessus* : 1<sup>st</sup> isolated NTM,
  - All patients included were less than 24 years of age.

# Results from the national observatory of atypical mycobacterial infections in CF patients (France, 2004)

- 1582 patients included (41 out of 49 competence centres for CF)
- Prevalence of 6.6%
  - 3.7 to 9.6%



NTM group	No. (%) of patients with:			
	NTM positivity	Previous NTM positivity <sup>c</sup>	ATS criteria met	Positive AFB smear
MABSC	50	15 (30.0)	40 (80.0)	24 (48.0)
<i>M. abscessus</i>	30	9 (30.0)	23 (76.7)	15 (50.0)
<i>M. bolletii</i>	9	4 (44.4)	8 (88.9)	4 (44.4)
<i>M. massiliense</i>	11	2 (18.2)	9 (81.8)	5 (45.5)
MAC	23	4 (17.4)	17 (73.9)	11 (47.8)
<i>M. avium</i>	15	1 (6.7)	10 (66.7)	6 (40.0)
<i>M. intracellulare</i>	8	3 (37.5)	7 (87.5)	5 (62.5)
Other species <sup>a</sup>	34	0 (0)	0 (0)	0 (0)
Total	104 <sup>b</sup>	19 (18.3)	57 (54.8)	35 (33.7)

<sup>a</sup> *M. gordonae* (16 patients), *M. chelonae* (8 patients), *M. fortuitum* (2 patients), *M. xenopi* (2 patients), *M. peregrinum* (2 patients), *M. immunogenum* (1 patient), *M. lentiflavium* (1 patient), *M. manitobense* (1 patient), and *M. simiae* (1 patient).  
<sup>b</sup> Two different mycobacterial species were isolated from three patients (*M. massiliense* and *M. peregrinum*, *M. abscessus* and *M. simiae*, or *M. chelonae* and *M. gordonae*).  
<sup>c</sup> Known positivity for the same NTM species prior to inclusion.

Recovered NTM species

# Prevalance of NTM infection in Scandinavian CF patients

retrospective observational study; identified all Scandinavian CF patients with a positive NTM culture from airway secretions from 2000 to the end of 2012

overall period prevalence of patients with at least one positive NTM culture of 11% (157/1411)

*Mycobacterium abscessus* complex (MABSC) (45%) and *Mycobacterium avium* Complex (MAC) (32%) were the predominant species

Younger patients were more prone to MABSC ( $p < 0.01$ ).

Despite treatment, less than one-third of MABSC patients with repeated positive cultures cleared their infection and a quarter had a lung transplant or died.



# The role of NTM in Infectious diseases (1)

- The history of Nontuberculous Mycobacteria (NTM) dates back to the late 19th century when "tuberculosis" in chickens was first described in 1868: *M. avium* (1890)
- Calmette, in 1920, considered the existence of Mycobacteria other than *M. tuberculosis* and *M. bovis* as causative agents of infection in humans [*Grosset and Meyer, 1980*].

## The role of NTM in Infectious diseases (2)

- The first cases of lung disease due to *M. avium* complex (MAC) was described in 1943 in a man with underlying silicosis [*Wallace R. History of MAC*]
- Interest in NTM increased when HIV infected patients began to develop infections distributed throughout the body due to various NTM species, particularly *M. avium*. [*Griffith et al., Am J Respir Crit Care Med 2007;175:367-416.*]

# The role of NTM in Infectious diseases (3)

- Pulmonary infections (Wolinski, 1979; Griffith et al., 1997 and 2007)
  - *M. kansasii*, *M. avium* complex, *M. abscessus* complex, *M. xenopi*, *M. malmoense*, *M. szulgai*, *M. chimerae*, and *M. simiae*
- Superficial lymphadenitis, especially cervical lymphadenitis, in children caused mostly by:
  - MAC, *M. scrofulaceum*, and, in northern Europe, *M. malmoense* and *M. haemophilum*.

# The role of NTM in Infectious diseases (4)

- Skin, soft tissue, or bone
  - *M. marinum*, *M. ulcerans*, *M. abscessus* complex, *M. chelonae*; *M. fortuitum*, *M. terrae* complex
  - Notion of nosocomial infections with the RGM, *M. xenopi*
- Disseminated disease in severely immunocompromised patients
  - most commonly caused by MAC
  - less commonly by the rapidly growing mycobacteria [RGM], eg, *M. abscessus*, *M. fortuitum*, and *M. chelonae*
  - Immunocompromised patients; IL-12/23-IFN- $\gamma$  deficiency; TNF $\alpha$  blockers, corticosteroids...
  - Transplantation
- Multiresistance to antiseptics, antibiotics, disinfectants

# *M. ulcerans*



Buruli ulcer  
Nodules  
Ulcerations  
oedema



Aquatic bugs

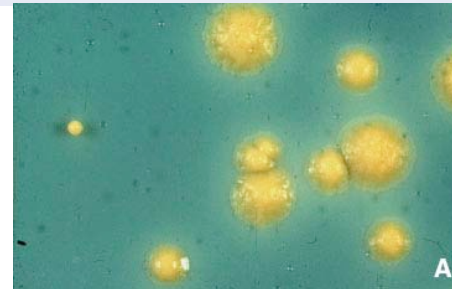
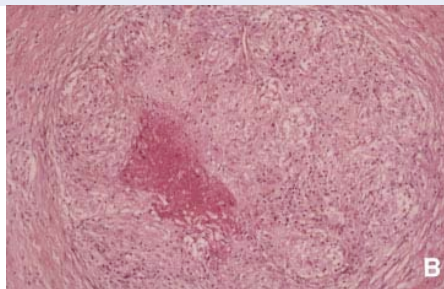
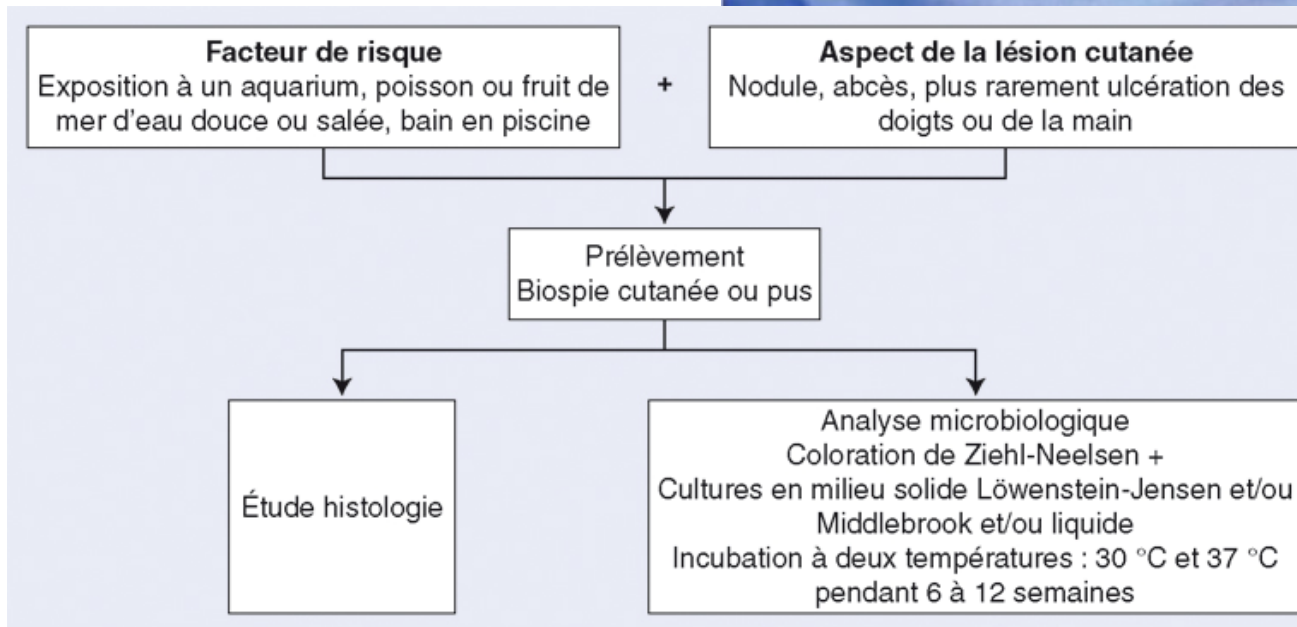


Mycolactone

Toxin belonging to the family of macrolides

Highly cytotoxic, with immunomodulatory activity

# *M. marinum*



## Other NTM involved



- 50 to 60% of nosocomial infections due to *M. abscessus*
- Direct inoculation or usage of contaminated solutions
- Cutaneo- mucous infections (abscesses)
- In Colombia: 350 out 2000 patients contaminated by a lidocaine solution
- Plastic, cardiac or orthopedic surgery: 45% of extra-pulmonary infections due to *M. abscessus*

# With major examples

- The large outbreaks are due to:
  - Injection of adrenal cortex extract
  - Mesotherapy
  - Abdominoplasty
  - Tattooing, piercing
- Two major examples
  - American « lipotourists » who underwent abdominoplasty in the Dominican republic
  - Hundreds of subjects after laparoscopic surgeries and cosmetic procedures in Brazil





# NTM infections of the lungs

- often occur in the context of preexisting lung disease
- COPD, bronchiectasis, pneumoconiosis, cystic fibrosis, and previous tuberculosis.
- As a result, the clinical manifestations of NTM lung disease are often similar to those of the underlying disease.
- cough, fatigue, malaise, fever, weight loss, dyspnea, hemoptysis, and chest discomfort.
- Similar symptoms are also present in patients with NTM lung disease who do not have preexisting pulmonary disease.

# Description of patients with NTM lung infections

- Kim et al., recently reported a characteristic morphotype (body habitus) in 63 patients (95% female, 91% white, 68% lifetime nonsmokers)
  - the BMI was significantly lower
  - the height significantly greater than matched controls
  - higher rates of scoliosis (51%),
  - pectus excavatum (11%),
  - mitral valve prolapse (9%),
  - cystic fibrosis transmembrane conductance regulator gene (CFTR) mutations (36%)
  - there were no recognized immune defects (cell-mediated dysfunction or cytokine-pathway abnormalities) identified in these patients.

# Meta-Analysis of Lung NTM infections in Asia

- Most patients with pulmonary NTM infections in Asia were:
  - male (543/689),
  - 8 studies reported mean ages of 50–70 years.
  - One third of patients had a history of tuberculosis (TB) (252/689).
  - HIV co-infection was less prevalent among patients with localized pulmonary NTM infections (15/280).
- Clinical signs of NTM disease mimicked those typical of TB:
  - most frequently chronic cough (255/268),
  - followed by hemoptysis (82/268),
  - fever (47/268), and weight loss (40/268).
  - Radiographically, 39% (405/1,044) had cavitations and 44% (461/1,044) had bronchiectasis.



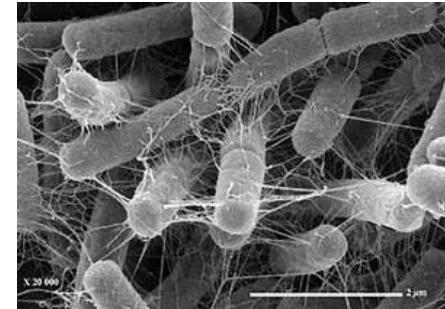
**Table 1. Most frequently identified nontuberculous mycobacteria in medical laboratories and their presence in the environment**

Environmental presence	Potentially pathogenic	Usually saprophytic
<b>Natural environment</b>		
Frequent	MAC <sup>a</sup> <i>M. scrofulaceum</i> <i>M. fortuitum</i>	<i>M. gordonae</i> <i>M. terrae</i> <i>M. nonchromogenicum</i>
Infrequent	<i>M. avium</i> <i>M. intracellulare</i> <i>M. chelonae</i> <i>M. malmoense</i> <i>M. simiae</i> <i>M. asiaticum</i> <i>M. marinum</i>	<i>M. flavescens</i> <i>M. vaccae</i> <i>M. aurum</i> <i>M. gastri</i> <i>M. smegmatis</i> <i>M. thermoresistibile</i>
<b>Environment modified by man</b>		
Frequent	<i>M. kansasii</i> <i>M. xenopi</i> <i>M. avium</i> MAC	<i>M. gordonae</i>
Infrequent		
Not (yet) found in the environment	<i>M. ulcerans</i> <i>M. haemophilum</i> <i>M. genavense</i> <i>M. szulgai</i> <i>M. shimoidei</i> <i>M. celatum</i>	<i>M. triviale</i>

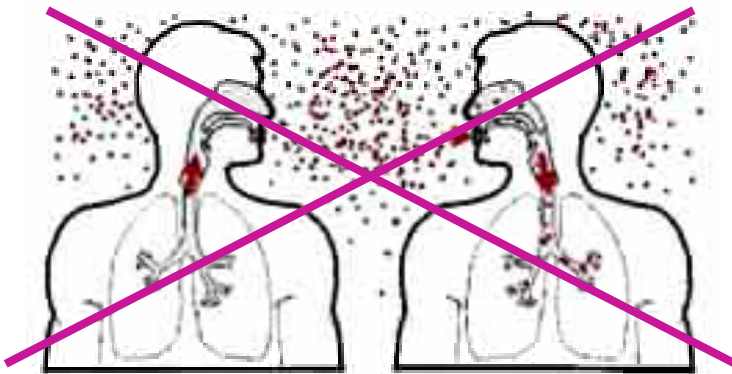
## Impact of the environment

<sup>a</sup>The *Mycobacterium avium* complex (MAC) includes several environmental species that resemble *M. avium* and *M. intracellulare* or that have intermediate characteristics common to these species. Reproduced with permission [10].

# NTM colonisation/transmission



Biofilm + + +

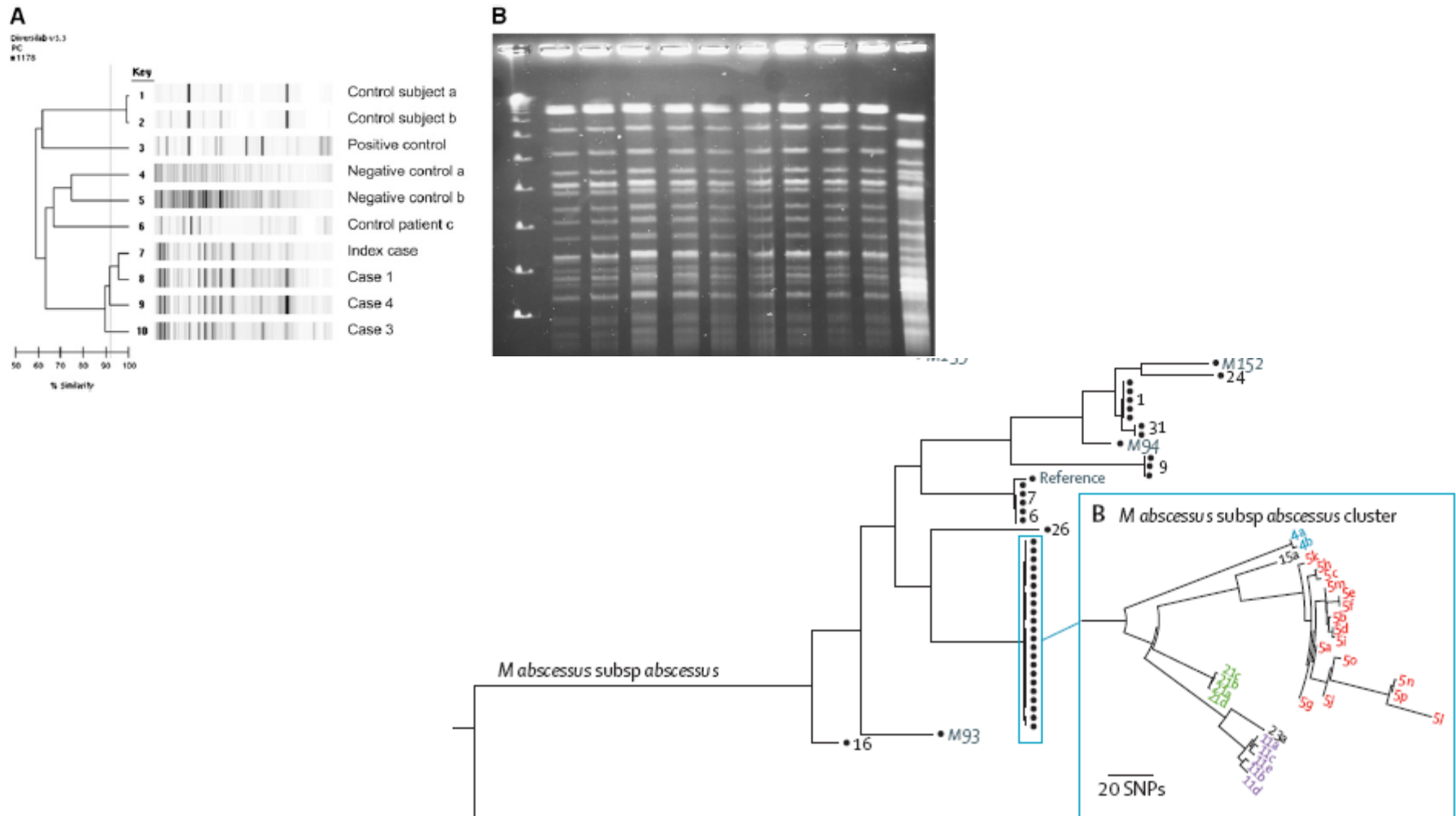


Two recent reports of  
of *M. abscessus* human to human  
transmission

# *Mycobacterium chimaera* infections after cardiac surgery

- In 2011 two patients with a history of cardiac surgery were diagnosed with *Mycobacterium chimaera* infections at the University Hospital Zurich, Switzerland.
- One patient had a prosthetic valve endocarditis
- the other patient presented with disseminated disease with multiple positive cultures including from blood.
- At that time, rare cases of prosthetic valve endocarditis due to fast-growing non-tuberculous mycobacteria (NTM) had been reported.
- The uncommon finding
  - two immunocompetent patients with endovascular infections
  - due to slowly growing NTM
  - prompted an outbreak investigation in the hospital, focusing on water sources.
- **contaminated heater-cooler units**
- In Italy, in 2017, *M. chimaera* infections risk was 0.4-1 patient every 1000 cardiac procedures

# Transmissibility of *M. abscessus* complex strains in CF patients



(Aitken, *AJRCCM*, 2012 ; Bryant, *Lancet*, 2013)



# MAC infections in the context of HIV co-infection

- MAC infection is most commonly seen among AIDS patients with CD4 counts <50 cells/mm<sup>3</sup>.
- Infection probably occurs most commonly through inhalation or ingestion. There is no need for isolation of hospitalized patients with MAC infection since person-to-person transmission does not appear to be common.
- The two principal forms of MAC infection in HIV are disseminated and localized disease. The most common symptoms of disseminated MAC include fever, night sweats, abdominal pain, diarrhea, and weight loss.
- The diagnosis is confirmed by the isolation of MAC from the blood in patients with disseminated disease or by isolation of the organism from a lymph node in localized disease.
- Treatment, is multi-drug therapy which should include a macrolide

# MYCOBACTERIUM AVIUM COMPLEX — *M. avium*, *M. intracellulare* and *M. chimaera*.

- The symptoms and signs of MAC lung disease are variable, not specific, and are influenced by whether the patient has pre-existing symptomatic lung disease. They include cough (productive or dry), fatigue, malaise, weakness, dyspnea, chest discomfort, and occasionally hemoptysis
- Examination of the lungs is often normal but, since these infections frequently coexist with underlying lung disease such as chronic obstructive pulmonary disease (COPD) or bronchiectasis
- Two major clinical presentations
  - Disease in those with known underlying lung disease, primarily white, middle-aged, or elderly men, often alcoholics and/or smokers with underlying chronic obstructive pulmonary disease.
  - Disease in those without known underlying lung disease predominantly in nonsmoking women over age 50 who have interstitial patterns on chest radiography

# *MYCOBACTERIUM KANSASII*

- Unlike other NTM, *M. kansasii* has never been found in soil or natural water supplies, but has been recovered consistently from tap water in cities where *M. kansasii* is endemic. Thus, there may be an association between clinical disease and potable water supplies.
- *M. kansasii* usually presents as lung disease that is nearly identical to tuberculosis,
  - although fever may be less common,
  - chest pain (82%), cough (84%), hemoptysis (38%), fever and night sweats (39%)
  - cavitation occurred in 85 to 95% of cases
- Disseminated infection is a rare complication that occurs in immunocompromised hosts such as those with HIV infection.

# NTM infections and TNF $\alpha$ blockers

- Hundred and five reports met NTM disease criteria.
  - The median age was 62 years,
  - 65% of patients were female
  - the majority of patients had rheumatoid arthritis.
  - NTM infections were associated with all available TNF- $\alpha$  blockers
  - MAC was the NTM species most commonly implicated,
  - Extrapulmonary disease was common (44%),
  - 9% of patients had died at the time their infection was reported.
  - **TNF $\alpha$  blockers are an important predisposing factor for sometimes serious, even fatal, NTM infection and must be used with extreme caution in patients with NTM disease.**

# Rapidly Growing Mycobacteria

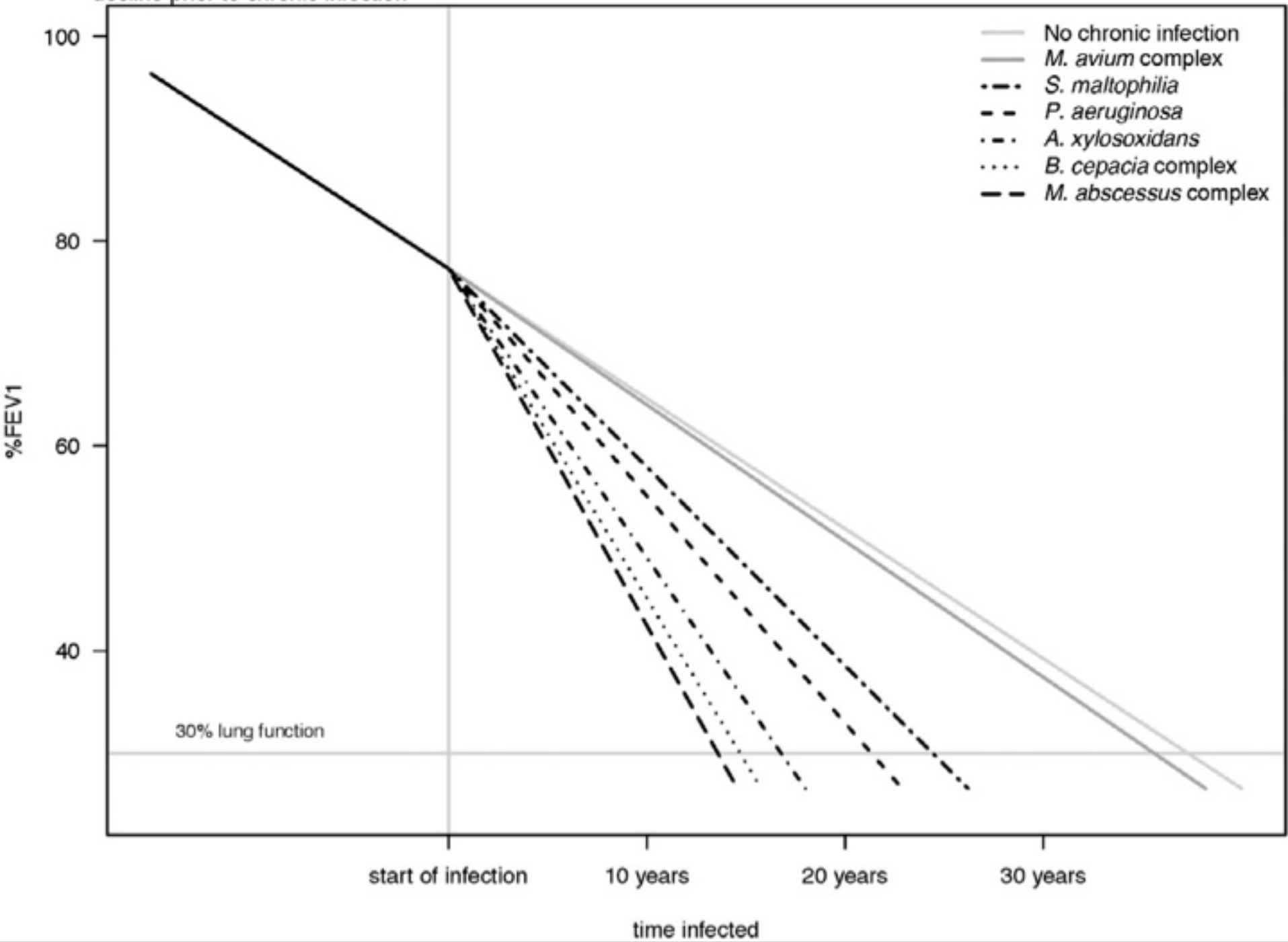
- Rapidly growing mycobacteria (RGM) include three clinically relevant species: *M. abscessus*, *M. chelonae*, *M. fortuitum*
- The RGM are environmental organisms found worldwide that usually grow in culture in less than one week following initial isolation, and may sometimes grow on standard microbiologic media
- Pulmonary disease due to rapidly growing mycobacteria (RGM) is predominantly due to *M. abscessus* (80%) and *M. fortuitum* (15%)

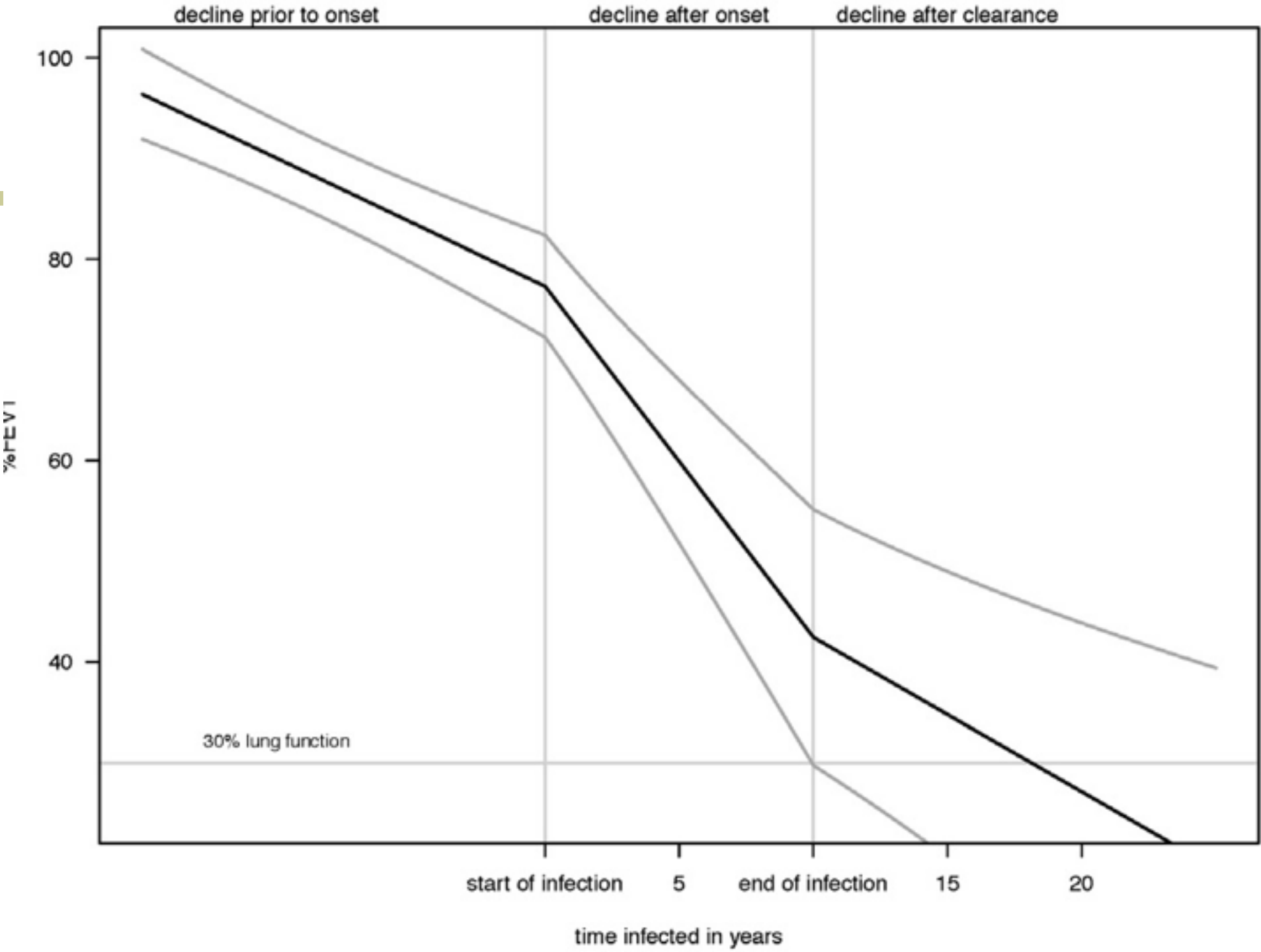
# *Mycobacterium abscessus* and impact on lung function

Longitudinal registry study of 432 patients with cystic fibrosis  
53,771 lung function measures between 1974 and 2014

- Infections with a significant impact on rate of decline in %FEV1 were
  - ***Mycobacterium abscessus*** complex with  $-2.22\%$  points per year (95% CI  $-3.21$  to  $-1.23$ ),
  - *Burkholderia cepacia* complex  $-1.95\%$  (95% CI  $-2.51$  to  $-1.39$ ),
  - *Achromobacter xylosoxidans*  $-1.55\%$  (95% CI  $-2.21$  to  $-0.90$ ),
  - *Pseudomonas aeruginosa*  $-0.95\%$  (95% CI  $-1.24$  to  $-0.66$ ).
- Clearing *M. abscessus* complex was associated with a change to a slower decline, similar in magnitude to the pre-infection slope.
- *M. abscessus* complex was associated with the worst impact on lung function.

decline prior to chronic infection







## ATS 2007 and diagnostic recommendations

- These criteria denote that to diagnose pulmonary NTM disease, clinical, radiological, and microbiological evidence of disease should be gathered.

**Table 1** Summary of the American Thoracic Society diagnostic criteria for pulmonary nontuberculous mycobacterial infection

<b>Clinical</b>
1. Pulmonary symptoms, nodular or cavitary opacities on chest radiograph, or a high-resolution computed tomographic scan that shows multifocal bronchiectasis with multiple small nodules
and
2. Appropriate exclusion of other diagnoses.
<b>Microbiologic</b>
1. Positive culture results from at least two separate expectorated sputum samples (If the results from the initial sputum samples are nondiagnostic, consider repeat sputum acid-fast bacillus (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
4. Expert consultation should be obtained when NTM are recovered that are either infrequently encountered or that usually represent environmental contamination
5. Patients who are suspected of having NTM lung disease but who do not meet the diagnostic criteria should be followed until the diagnosis is firmly established or excluded
6. Making the diagnosis of NTM lung disease does not, per se, necessitate the institution of therapy, which is a decision based on potential risks and benefits of therapy for individual patients

Source: Adapted from Griffith DE, Aksamit T, Brown-Elliott BA, et al; ATS Mycobacterial Diseases Subcommittee; American Thoracic Society; Infectious Disease Society of America. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med* 2007;175(4):367–416.

# What respiratory tract samples should be used to evaluate individuals for suspected NTM pulmonary disease?

- **culture and smears for acid fast bacilli from sputum, induced sputum, bronchial washings or broncho-alveolar lavage samples can be used to evaluate individuals suspected to have NTM pulmonary disease.**
- **Recommend against the routine use of transbronchial biopsies to detect NTM in individuals with CF suspected to have NTM pulmonary disease.**
- **Recommend against the use of oro-pharyngeal swabs to perform diagnostic smears and cultures in individuals with CF suspected to have NTM pulmonary disease.**

# How should respiratory tract samples from individuals with CF be cultured for NTM?

- **an NTM culture should be processed within 24 hours of collection to optimise the detection of NTM in respiratory samples. If a delay in processing is anticipated, refrigeration of samples is advised.**
- Recommend that respiratory tract samples should be cultured using both solid and liquid media.
  - Temperature range: 25° to 42°
- Recommend that the incubation duration for NTM cultures should be for a minimum of 6 weeks.

# How should respiratory tract samples from individuals with CF be decontaminated to optimize the detection of NTM?

- Recommend that respiratory tract samples should be decontaminated using the standard N-Acetyl L-cysteine (NALC) – NaOH (2%) method.
- In CF patients, recommend that if a sample remains contaminated with Gram negative bacteria after standard NALC-NaOH decontamination, it should be further treated with either 5% oxalic acid or 1% chlorhexidine.

# Molecular methods for the rapid characterization of NTM

- PCR-restriction fragment length polymorphism analysis (PRA)
  - *16S* ribosomal DNA (rDNA), *hsp65*, *dnaJ*, *groES*, *rpoB*...
  - Mainly (even exclusively) on culture positive specimens
  - 60% sensitivity on smear positive specimens (Kim et al, 2008)
- DNA probes (Gen-Probe, Innogenetics, Hain):
  - Incomplete identification
  - Highest number of problems with MAC, and *M. fortuitum* complex probes
  - Confusions like *M. abscessus*/*M. chelonae*; *M. marinum*/*M. ulcerans*; *M. haemophilum*/*M. malmoense*...
  - 69% sensitivity for direct detection in clinical specimens
- And finally the key is misidentification with regard to the diversity of mycobacterial taxa (Tortoli et al., 2010)
- Disagreements among commercialized assays: sequencing for confirmation
- None performed in the context of CF

# Consequences: How should NTM isolates from individuals with CF be identified?

- **Recommend against the use of non-culture based methods for detecting NTM in respiratory tract samples.**
- Recommend that all NTM isolates from individuals with CF should undergo molecular identification.
  - PCR and sequencing
  - *hsp65*; *rpoB*; 16S-23S internal transcribed spacer (ITS) offer high discriminatory power and can identify up to the subspecies level
  - whereas *16S rRNA* gene sequencing allows discrimination to the species level for most species, or at least to the complex level, particularly among the rapid growers (*M. fortuitum* complex, *M. chelonae*–*M. abscessus* complex)
  - But more than one target for several NTM complex
  - Impact of Mass Spectrometry with Maldi-Tof apparatus

## Consequences: How should NTM isolates from individuals with CF be identified?

- **Recommend against the use of non-culture based methods for detecting NTM in respiratory tract samples.**
- Recommend that all NTM isolates from individuals with CF should undergo molecular identification.
- **Recommend that all NTM isolates from individuals with CF should be identified to the species level, except for *M. abscessus* complex which should be sub-speciated.**



# NTM and antimicrobials

- The most frustrating aspect of NTM therapy remains the generally poor correlation between *in-vitro* antibiotic susceptibility and *in-vivo* response to antimicrobials. ( DE Griffith 2010; Brown-Elliott et al.; 2012 )
- A possible insight into this phenomenon is the recent discovery of an inducible macrolide resistance (*erm*) gene in *M. fortuitum* and *M. abscessus*, but not *M. chelonae* (Nash KA et al. 2009)
- It has been almost 20 years since the macrolides were recognized as an integral element in successful treatment regimens for multiple NTM species, but especially MAC.

# Antimicrobial activity and mechanisms of drug resistance

- Inhibition of mycobacterial cell wall synthesis
  - **Carbapenems and cephalosporins**
    - Inhibitor of mycobacterial D-D and D-L transpeptidases
    - No clinically acquired resistance
    - Permeability, beta-lactamases, affinity for their target
  - **Ethambutol**
    - Inhibition of the synthesis of arabinogalactan
    - Change in the permeability of the cell wall to other antimycobacterial agents
    - Mutations in *embB* gene
  - **Glycopeptides**
    - Natural resistance against vancomycin
    - permeability
  - **Isoniazid**
    - Major antituberculous agent; active against *M. kansasii* and *M. xenopi*
    - Prodrug, KatG, isoniazid-NAD adduct, InhA (one of the FASII enzymes)
    - Lack of catalase-peroxydase activity: intrinsic resistance of majority of NTM

# Antimicrobial activity and mechanisms of drug resistance

- Inhibition of Protein synthesis
  - All target the ribosome and interfere with the native peptide chain formation
  - **Aminoglycosides**
    - Modification of the 30S subunit (mutations in 16SRNA gene, or *rpsL* gene)
    - Intrinsic resistance: acetyltransferase enzymes, aminoglycoside-phosphotransferases
  - **Tetracyclines and glycylicyclines**
    - Ribosome protection (elongation factors) and drug efflux (Tet, Otr, Tap efflux pumps)
  - **Macrolides and ketolides**
    - Modification of the drug-binding site
    - One or two copies of rRNA (*rrn*); acquired resistance by mutation of the 23S RNA gene
      - Resistance acquired during therapy for *M. abscessus*, *MAC*, *M. chelonae*, *M. fortuitum* and *M. kansasii*
    - Primary mechanism of clinically acquired resistance
      - *erm* methylase genes
      - described for nearly 10 NTM species, the major being *M. fortuitum* [*erm*(39)] and *M. abscessus* [*erm*(41)]
  - **Oxazolidinones**
    - Reduction of drug-binding, 23SrRNA gene; in addition to *rplC* and *rplD*
    - Intrinsic resistance of *M. abscessus*

# Antimicrobial activity and mechanisms of drug resistance

## ■ Inhibition of Nucleic acid synthesis

### ○ Fluoroquinolones

- DNA gyrase, type II and type IV topoisomerases
- High level of resistance primarily associated with mutations in *gyrA*
- LfrA efflux pump; regulation by LfrR

### ○ Rifamycins

- $\beta$ -subunit of the RNA polymerase
- Variability between NTM species in impermeability of the mycobacterial cell wall; or ADP-ribosylation (*arr* gene)
- 80% of the acquired resistance is located in an 80-bp region of the *rpoB* gene
- Similar as in *M. tuberculosis*

### ○ Trimethoprim and Sulfonamides

- Inhibitors of the microbial folate metabolism

# NTM and antimicrobials

- Recommendations and criteria for antimicrobial susceptibility testing (AST) (CLSI, IDSA and ATS) best applicable for the MAC, *M. kansasii*, *M. marinum* and RGM isolates like *M. abscessus*, *M. chelonae* and *M. fortuitum*.
- The gold standard method for AST of NTM is broth microdilution
- AST patterns provide useful taxonomic help for commonly encountered RGM like *M. chelonae*, *M. fortuitum* group and *M. abscessus* complex (disk diffusion)
  - Polymyxin B (S for *M. fortuitum*)
  - Cefoxitin and tobramycin (R and S respectively for *M. chelonae* / *M. abscessus*)

# Treatment proposals

- Multidrug therapy: the key of success!
- Treatment is well established for *M. avium - intracellulare* and *M. kansasii*, with combination of macrolide-rifamycin-ethambutol and isoniazid-rifampicin-ethambutol respectively.
- For *M. xenopi*, the optimal treatment is not known and a combination of clarithromycin-rifampicin-ethambutol, with moxifloxacin as an alternative, is currently recommended.
- In general, treatment is prolonged and often associated with problems of tolerance.

# Treatment proposals: *M. abscessus*

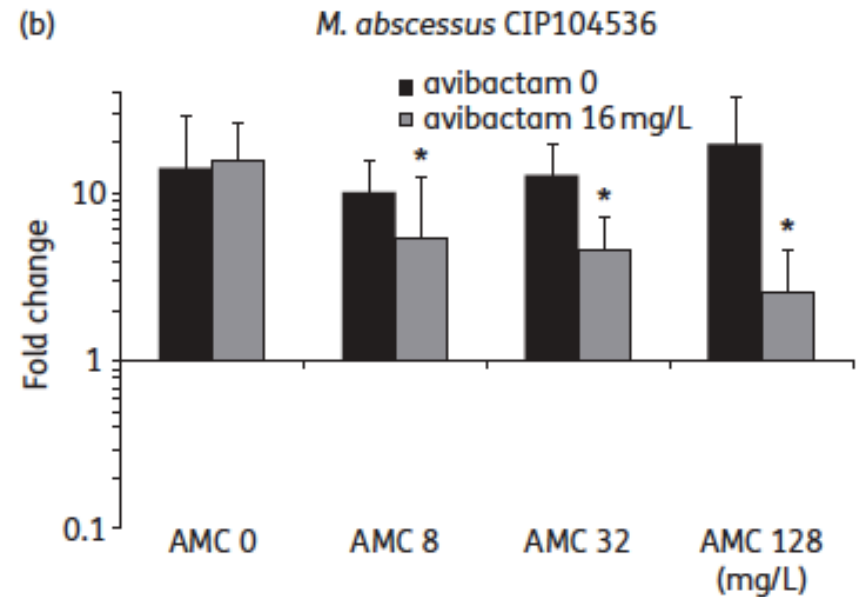
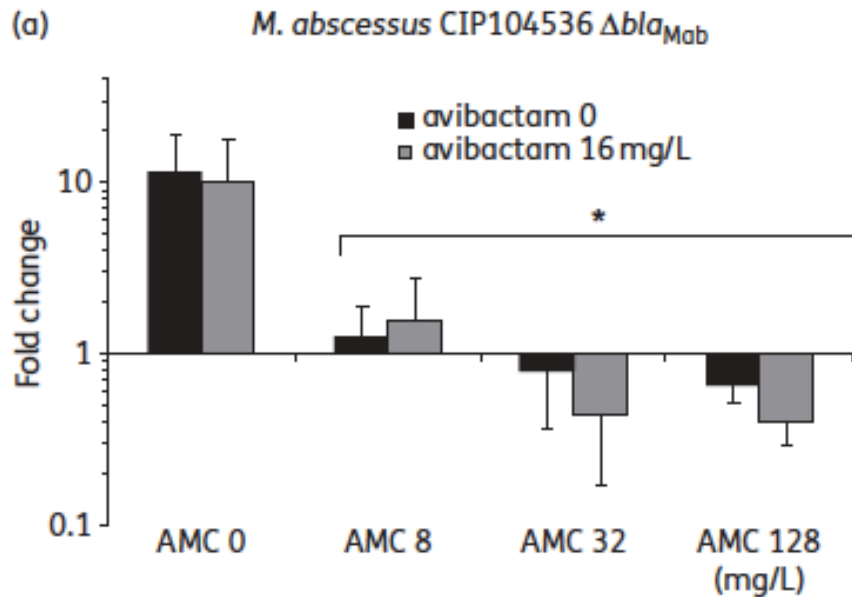
- The *in-vitro* susceptibility pattern of *M. abscessus* previously reported and its subsequent use *in-vivo* (with 0% isolates susceptible *in vitro* to fluoroquinolones, and less than 5% isolates susceptible *in vitro* to doxycycline) (Jeon K et al., 2009; Griffith DE et al., 2007)
- The most active drug is amikacin
- Multidrug regimen of ceftazidime (or imipenem) and amikacin
- 6 to 12 months of sputum cultures negative as a reasonable endpoint for *M. abscessus ssp. bolletii* infection
- No antimicrobial regimens to achieve this endpoint for patients with *M. abscessus ssp. abscessus* infection

# *M. abscessus* MICs against β-lactams +/-Avibactam

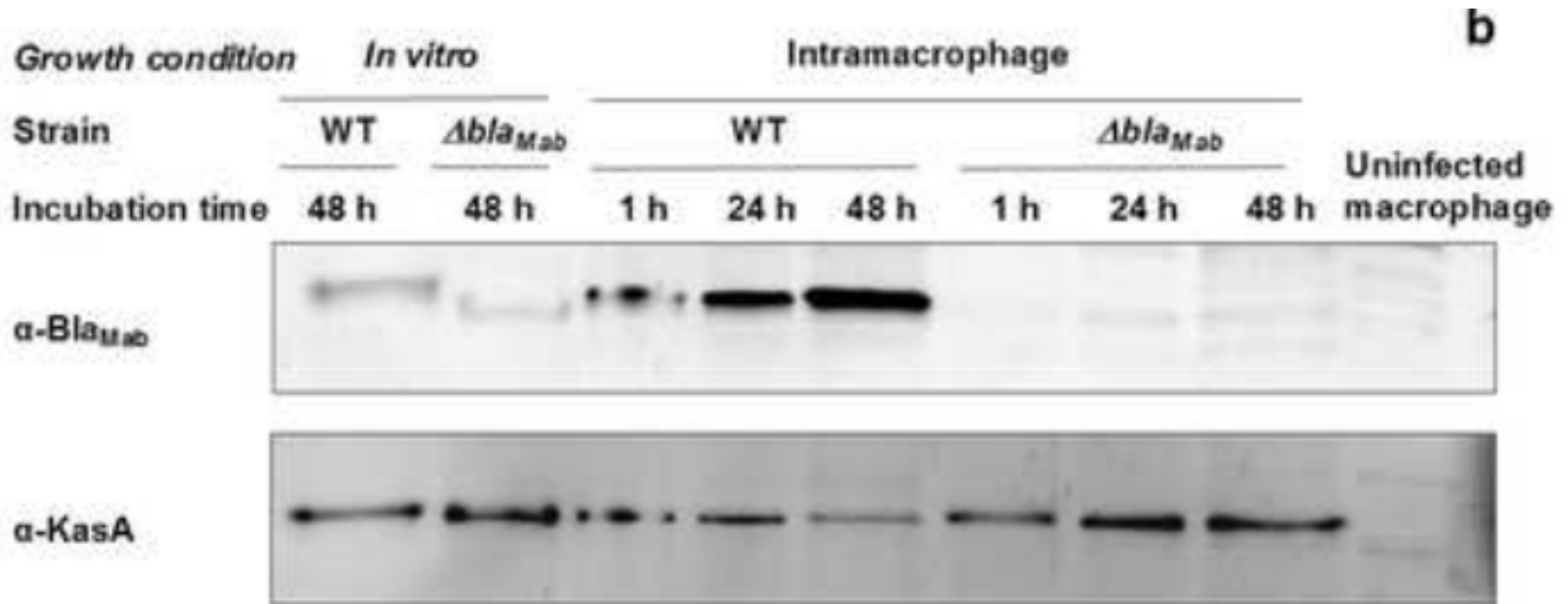
β-Lactam	7H9sB medium			
	CIP104536		Δbla <sub>Mab</sub>	
	-avibactam	+avibactam	-avibactam	+avibactam
Amoxicillin	>256	8	4	4
Cefalotin	>256	8	4	8
Cefuroxime	32	8	4	8
Cefamandole	128	8	4	4
Ceftriaxone	64	8	8	8
Ceftazidime	>256	>256	>256	>256
Cefoxitin	16	8	8	16
Imipenem	4	2	2	2
Meropenem	4	4	4	4
Aztreonam	>256	>256	>256	>256



# Intracellular activity of amoxicilline + avibactam

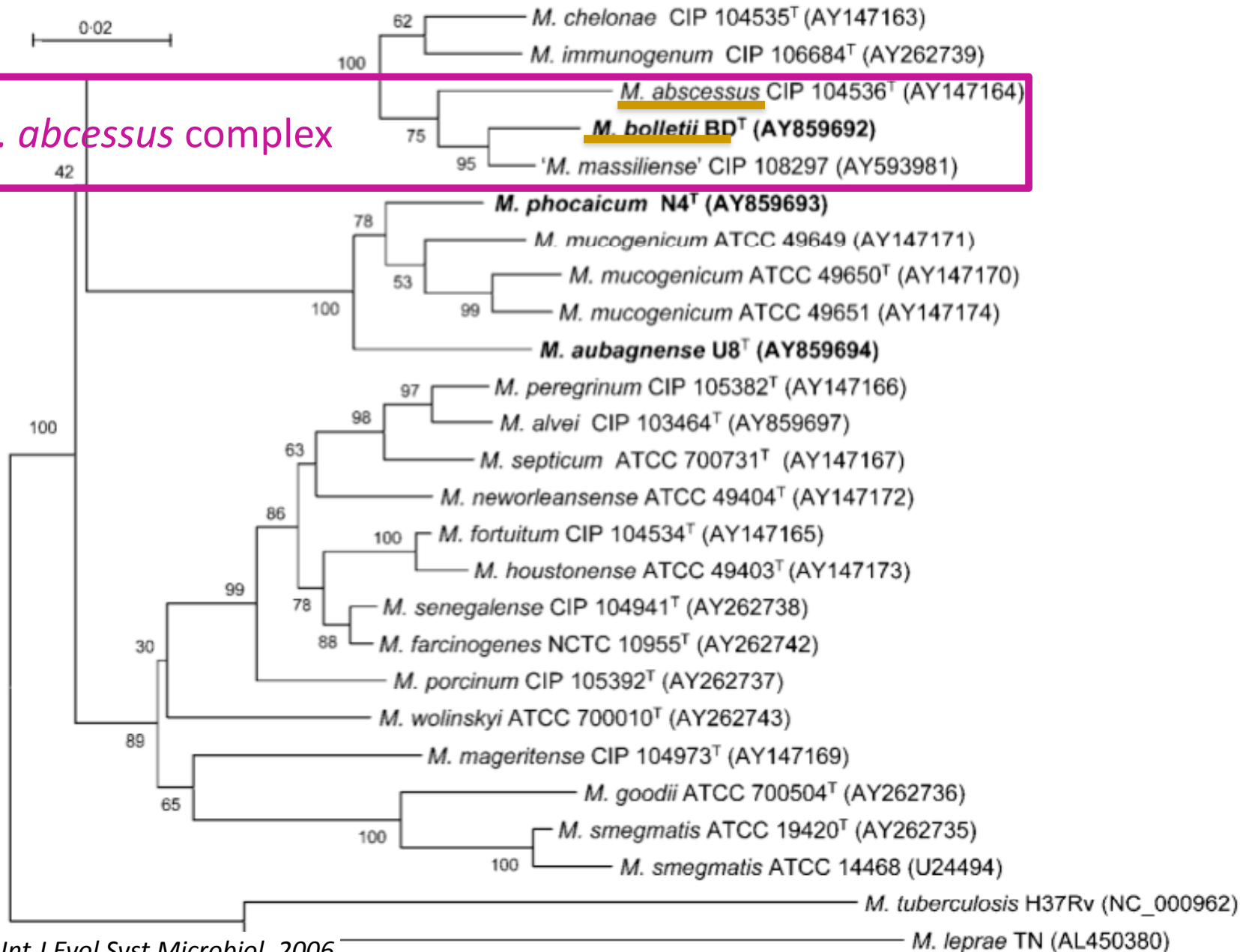


# *In vivo* induction of the *M. abscessus* $\beta$ -lactamase



0.02

*M. abscessus* complex

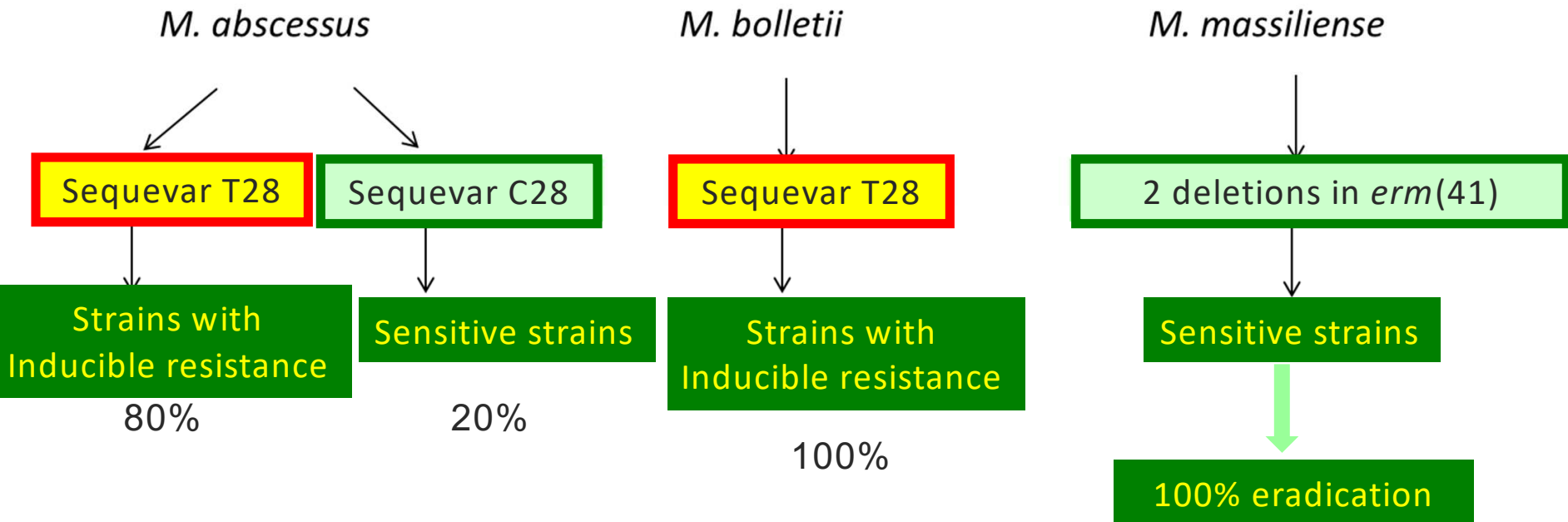


# *M. abscessus* complex and *erm(41)*

- Taxonomic considerations may have very real implications for the clinician and can either impede or facilitate the management of patients with NTM lung disease.
- *M. abscessus* has recently been subdivided into three new closely related species,
  - *M. abscessus* ssp. *abscessus* (formerly *M. abscessus*),
  - *M. abscessus* ssp. *bolletii*
    - comprises *M. massiliense*, and *M. bolletii*

**2 subspecies**

# erm(41) heterogeneity in the *M. abscessus* complex



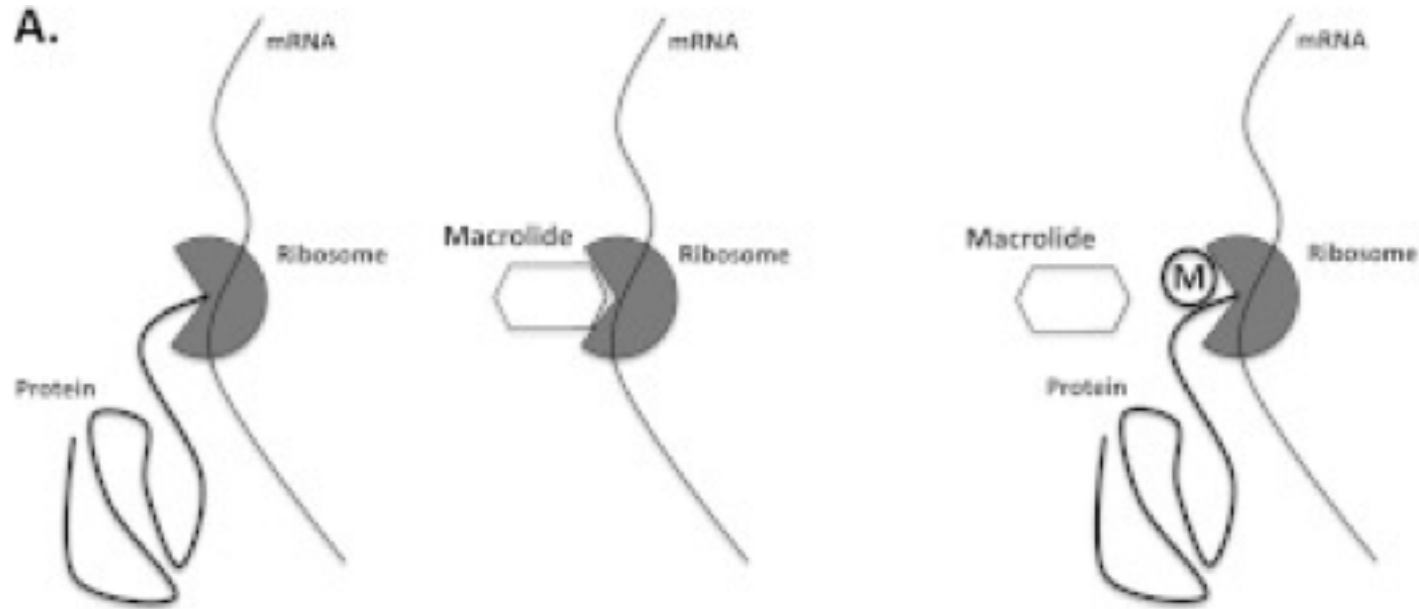
Importance to identify 'species' in *M. abscessus* complex

Increase duration of incubation up to 14 days for MICs

# Consequences of *erm*(41) knowledge

- A clinician who knows that a clinical NTM isolate is *M. abscessus* ssp. *bolletii* (previously *M. massiliense*) knows that this organism has an inactive macrolide resistance gene or *erm* gene so that macrolides would likely be clinically effective.
- Conversely, if the clinician knows that the clinical NTM isolate is *M. abscessus* ssp. *abscessus* (formerly *M. abscessus*), then the organism likely has an active *erm* gene with inducible macrolide resistance and therefore macrolides will not be reliably effective for treating this organism.

# Azythromycin vs. clarythromycin



B. *M. abscessus* *M. massiliense*



# Should drug susceptibility testing be performed on NTM isolates from individuals with CF?

- **Recommend that for *M. avium* complex, clarithromycin susceptibility testing should be performed on an isolate recovered prior to initiation of treatment.**
  
- **Clarithromycin susceptibility testing should also be performed on subsequent isolates if the patient:**
  - a) fails to culture convert after six months NTM treatment;
  - b) re-cultures *M. avium* complex after initial culture conversion while on NTM treatment;
  - or c) re-cultures *M. avium* complex after completion of NTM treatment.



Should drug susceptibility testing be performed on NTM isolates from individuals with CF?

- **Recommend that for *M. abscessus* complex, susceptibility testing should include at least clarithromycin, ceftazidime and amikacin (and preferably also tigecycline, imipenem, minocycline, moxifloxacin and linezolid).**
- **Recommend that drug susceptibility testing should be performed in accordance with CLSI guidelines.**

# Take home message

- NTM are everywhere; which renders the involvement of NTM in the infectious process pretty tedious
- EDUCATION within amoeba
- Prevalence increases, specifically in developed countries
- Peculiar susceptible populations, for which NTM infections is deleterious
- Tools in the lab are insufficient, and lack sensitivity
- In vitro AST do not correlate to in vivo activity
- New and promising anti-NTM drugs
- New and promising biomarkers for treatment success

# [ Future outlook ]

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- Many, many weaknesses and gaps in our understanding of NTM disease (lung)
- Markers of disease activity
- NTM organism virulence
- To predict progressive disease that requires therapy