Critically Appraised Topic

Transmission of nontuberculous Mycobacteria (NTM) between patients with cystic fibrosis: is there evidence for person-to-person transmission? Which techniques are available to investigate the NTM transmission?

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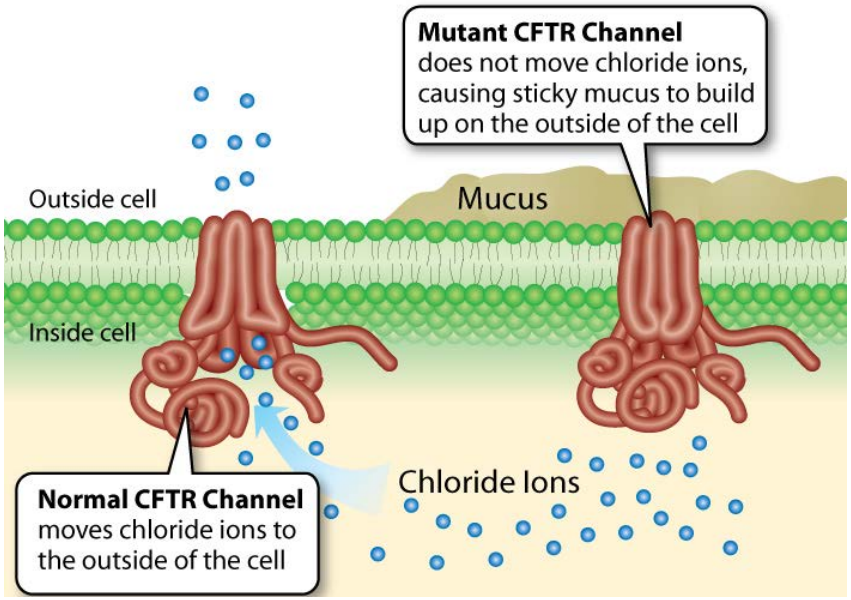
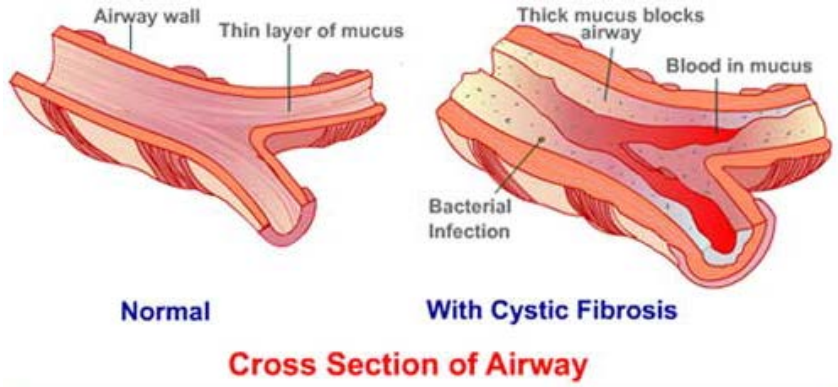
Introduction: cystic fibrosis (CF)

- Most common genetic disorder in Europe (autosomal recessive)
- Cause: mutations in CF transmembrane conductance regulator (CFTR) gene





- Symptoms: respiratory (cough, wheezing, …) and digestive due to viscous respiratory and gastrointestinal secretions
- Prevalence: 1/8.000 – 1/10.000
- Life expectancy: 37 years

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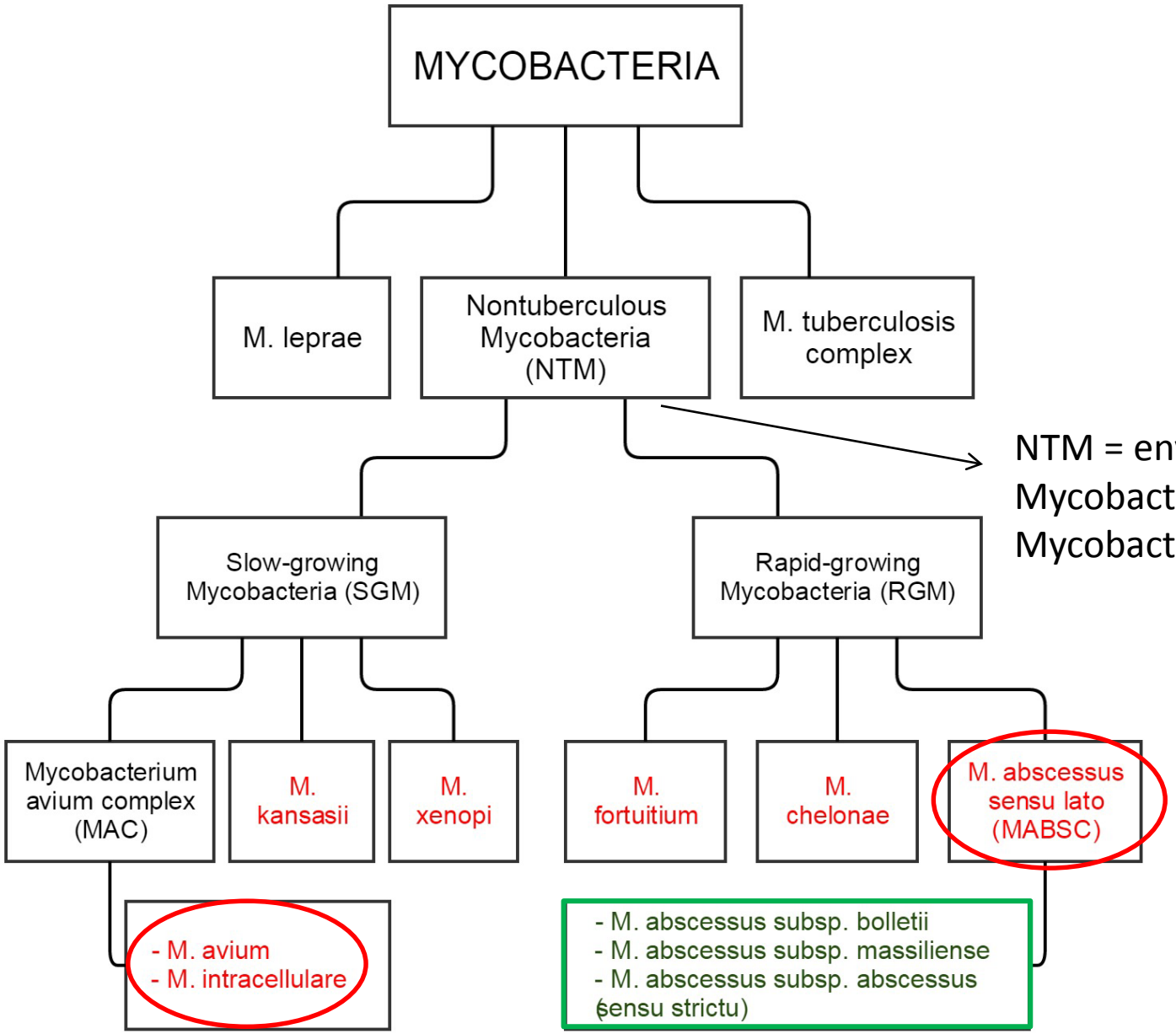
Introduction: taxonomy of NTM



NTM = environmental Mycobacteria = atypical Mycobacteria = MOTT

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Introduction: NTM infections

- Skin and soft tissue infection
- Disseminated disease: in immunocompromised patients
- Superficial lymphadenitis: in children (mostly cervical lymphadenitis)
- Lung infection

Introduction: NTM lung infection

- Most important NTM in CF lung infection:

  M. avium complex (MAC) >> *M. abscessus complex* (MABSC)

  USA

Introduction: NTM lung infection

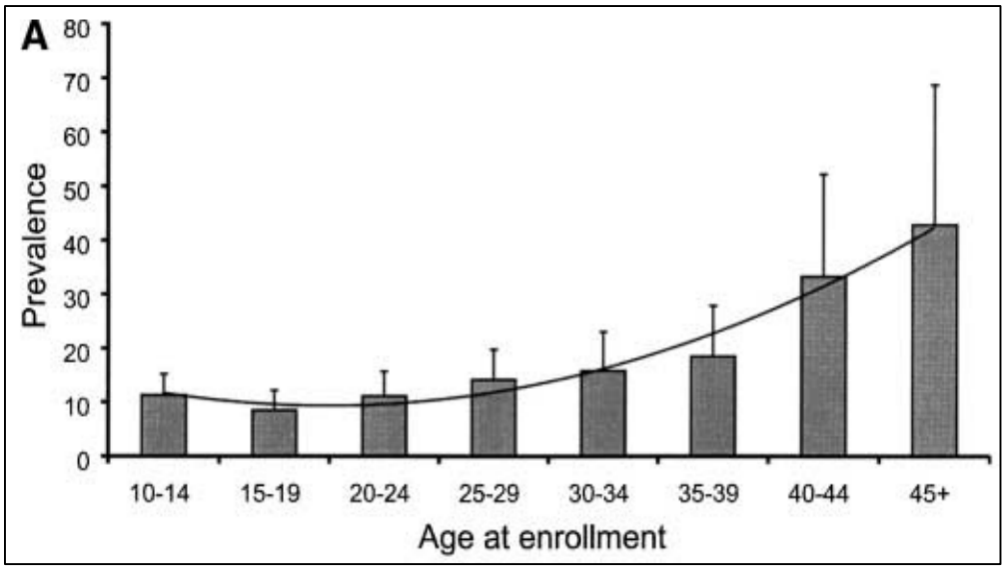
- Most important NTM in CF lung infection:

  M. avium complex (MAC) << *M. abscessus complex* (MABSC)

  Europe

- Prevalence ⬆: depending on studies = 6-15% (~ age)
  - Improved laboratory practices
  - Improved patient survival
  - Inhaled antibiotic usage

- Symptoms: nonspecific
  - Blood in sputum
  - Cough
  - Fever
  - Nausea
  - Night sweats
  - Weight loss



Olivier K. et al. Nontuberculous Mycobacteria. Am. J. Respir. Crit. Care Med. 2003

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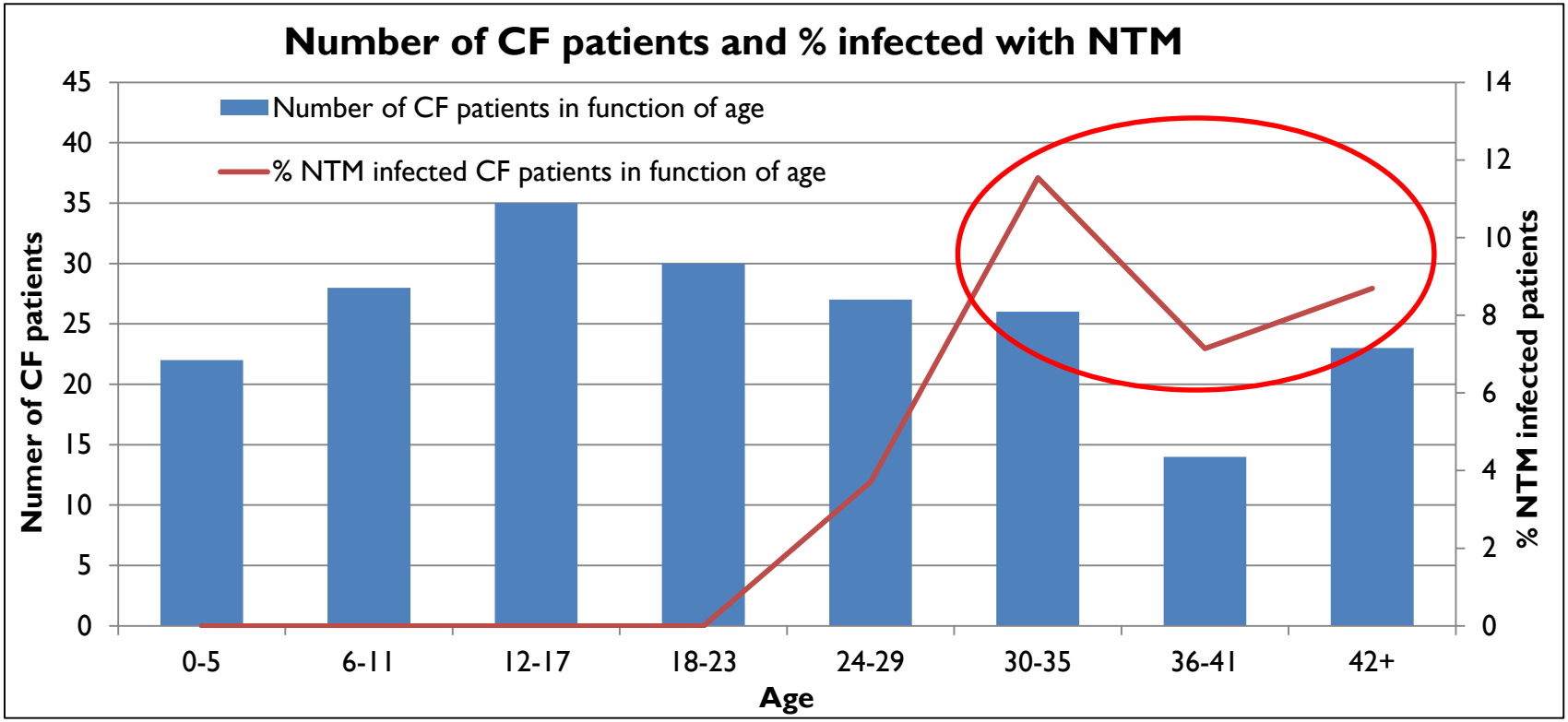
Introduction: NTM lung infection

- *Prevalence in the CF center of the university Hospital Saint-Luc Brussels*



Data CF center university Hospital Saint-Luc Brussels

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Introduction: diagnosis of NTM lung infection

Etiologic Agents	Diagnostic Procedures	Optimum Specimens	Transport Issues; Optimal Transport Time
Bacteria			
Staphylococcus aureus *Haemophilus influenzae* *Streptococcus pneumoniae* Enteric bacilli *Pseudomonas aeruginosa* *Stenotrophomonas maltophilia* *Achromobacter* spp	Culture	Expectorated sputum; throat swabs[a]; other respiratory samples	Sterile container, RT, 2 h; >2–24 h, 4°C
Burkholderia cepacia complex	Culture using *Burkholderia cepacia* selective agar	Throat swabs[a], expectorated sputum; other respiratory cultures	Sterile container, RT, 2 h; >2–24 h, 4°C
Opportunistic glucose nonfermenting gram-negative rods *Burkholderia gladioli* *Ralstonia* spp *Cupriavidus* spp *Pandorea* spp	Culture	Expectorated sputum; throat swabs[a]; other respiratory samples	Sterile container, RT, 2 h; >2–24 h, 4°C
***Mycobacterium* spp**			
Mycobacterium abscessus *Mycobacterium avium* complex	Mycobacteria culture Mycobacteria culture	Expectorated sputum, bronchoscopically obtained cultures; other respiratory cultures	Sterile container, RT, 2 h; >2–24 h, 4°C
Fungi			
Aspergillus spp *Scedosporium* spp *Trichosporon*	Calcofluor -KOH or other fungal stain Fungal culture	Expectorated sputum, bronchoscopically obtained cultures; other respiratory cultures	Sterile container, RT, 2 h; >2–24 h, 4°C
Viruses			
RSV Influenza Adenovirus Rhinovirus Coronavirus Parainfluenza virus Human metapneumovirus	Rapid antigen detection DFA Viral culture methods NAAT[b]	Nasal aspirates, nasal washes, NP swabs, throat washes, throat swabs; bronchoscopically obtained specimens	Transport in viral transport media, RT or 4°C, 5 d; −70°C, >5 d

IDSA guideline: A Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases: 2013 Recommendations by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM)

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Introduction: diagnosis of NTM lung infection

Clinical (both required)

1. Pulmonary symptoms, nodular or cavitary opacities on chest radiograph, or a high-resolution computed tomography scan that shows multifocal bronchiectasis with multiple small nodules (A, I)*

and

2. Appropriate exclusion of other diagnoses (A, I)

Microbiologic

1. Positive culture results from at least two separate expectorated sputum samples (A, II). If the results from (1) are nondiagnostic, consider repeat sputum AFB smears and cultures (C, III).

or

2. Positive culture result from at least one bronchial wash or lavage (C, III)

or

3. Transbronchial or other lung biopsy with mycobacterial histopathologic features (granulomatous inflammation or AFB) and positive culture for NTM or biopsy showing mycobacterial histopathologic features (granulomatous inflammation or AFB) and one or more sputum or bronchial washings that are culture positive for NTM (A, II)
4. Expert consultation should be obtained when NTM are recovered that are either infrequently encountered or that usually represent environmental contamination (C, III)
5. Patients who are suspected of having NTM lung disease but do not meet the diagnostic criteria should be followed until the diagnosis is firmly established or excluded (C, III)
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 (C, III)

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

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Introduction: treatment of NTM lung infection

J Antimicrob Chemother 2012; **67**: 810–818
doi:10.1093/jac/dkr578 Advance Access publication 30 January 2012

Journal of Antimicrobial Chemotherapy

Mycobacterium abscessus: a new antibiotic nightmare

Rachid Nessar[1]†, Emmanuelle Cambau[2]†, Jean Marc Reyrat[1]‡, Alan Murray[3,4]† and Brigitte Gicquel[3]*†

Curr Pulmonol Rep. 2015 Sep 1;4(3):152-161. Epub 2015 Jul 12.

The Challenge of Pulmonary Nontuberculous Mycobacterial Infection.

Novosad S[1], Henkle E[2], Winthrop KL[3].

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Introduction: treatment of NTM lung infection

- NTM intrinsically resistant to classical anti-tuberculous drugs (rifampicin, isoniazide and ethambutol)
- Antibiotics used in NTM lung infections (very often resistant):

Antibiotic	Frequency	Route
Amikacin	7 - 10 mg/kg once daily	IV
	250 – 500 mg twice daily	nebulized
Azithromycin	250 – 500 mg once daily	oral
Clarithromycin	500 mg twice daily	oral
Cefoxitin	4 g twice daily	IV
Imipenem	750 – 1000 mg twice daily	IV
Linezolid	300 – 600 mg once daily	oral
Tigecycline	25 – 50 mg daily	IV

- Duration of treatment: several months/years!

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Question 1: What is the importance and clinical significance of NTM in CF patients?

- Until 30-40 years ago: “NTM = a group of rather benign environmental bacteria associated with random colonization and only rarely with genuine infection of the airway”

- Nowadays: ”pulmonary disease caused by NTM may occur as a component of disseminated infection, but often the disease only affects the lungs”

Until recently, *M. abscessus* infection = contra-indication for lung transplantation among CF patients

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Question 1: What is the importance and clinical significance of NTM in CF patients?

- Whole population

# positive NTM samples (M. avium)	progressive radiographic abnormalities
1	2%
2	90%
3	98%

Tsukamura, M. Diagnosis of disease caused by Mycobacterium avium complex. CHEST J, 1991

- CF population:

Clinical Significance of a First Positive Nontuberculous Mycobacteria Culture in Cystic Fibrosis

Stacey L. Martiniano[1], Marci K. Sontag[2], Charles L. Daley[3], Jerry A. Nick[3], and Scott D. Sagel[1]

38.5% active NTM lung infections

Martiniano, S. L. & Nick, J. a. Nontuberculous Mycobacterial Infections in Cystic Fibrosis. Clin. Chest Med, 2015.

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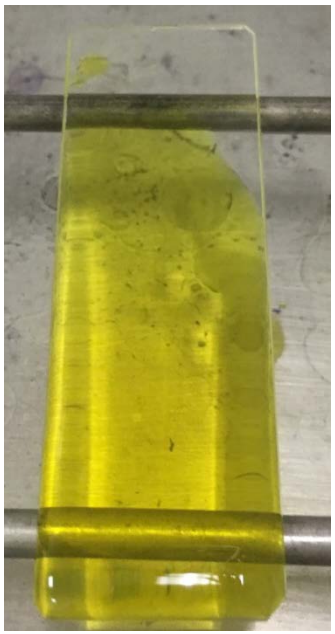
Question 2: How should NTM screening in CF patients be performed?

- Frequency of NTM screening in CF patients:
  - Routinely: 1x/year
  - In patients receiving NTM therapy: 1x/1-2 months
  - → Culture (liquid + solid media) + smear (auramine)



- Samples:
  - For NTM lung diagnosis: early-morning sputum specimens or BAL fluid
  - No oro-pharyngeal swabs (too little material)

Infection Prevention and Control Guideline for Cystic Fibrosis: 2013 Update

US Cystic Fibrosis Foundation and European Cystic Fibrosis Society consensus recommendations for the management of non-tuberculous mycobacteria in individuals with cystic fibrosis (2015)

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Question 2: How should NTM screening in CF patients be performed?

- Decontamination of sputum samples:
  - N-Acetyl-L-Cysteine sodium hydroxide (NALC-NaOH) method: 1st choice
    - NALC: digestant
    - NaOH: digestant + decontaminant
  - NaOH method
  - NALC-NaOH-OxA method:
    - Cave: OxA toxic for Mycobacteria (especially *M. abscessus* in low concentration)
  - Chlorhexidine method
    - Pro: high NTM yield
    - Contra: chlorhexidine is incompatible with the Mycobacterial growth indicator tube (MGIT) culture

→ *Laboratory of the university Hospital Saint-Luc Brussels*

- *non CF-patients: NALC-NaOH method (15 min)*
- *CF patients: NALC-NaOH method (45 min)*

Prolongation

US Cystic Fibrosis Foundation and European Cystic Fibrosis Society consensus recommendations for the management of non-tuberculous mycobacteria in individuals with cystic fibrosis (2015)

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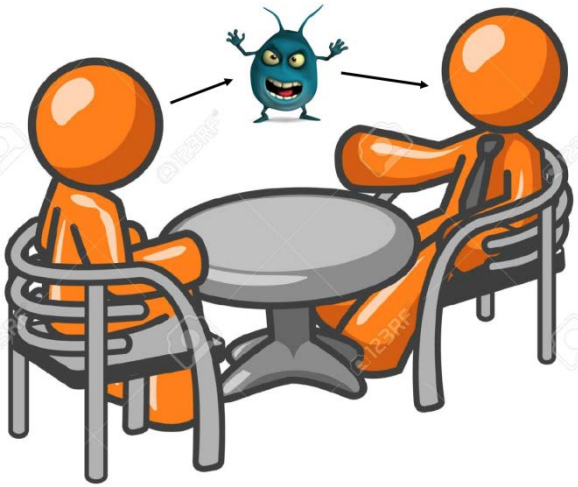
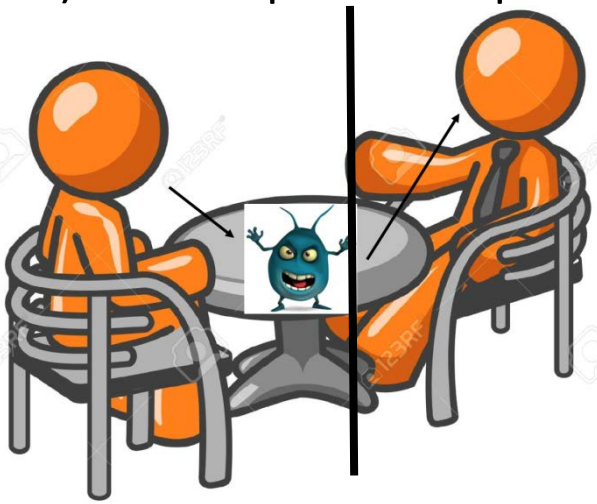
Question 3: Is there evidence for person-to-person transmission in CF patients?

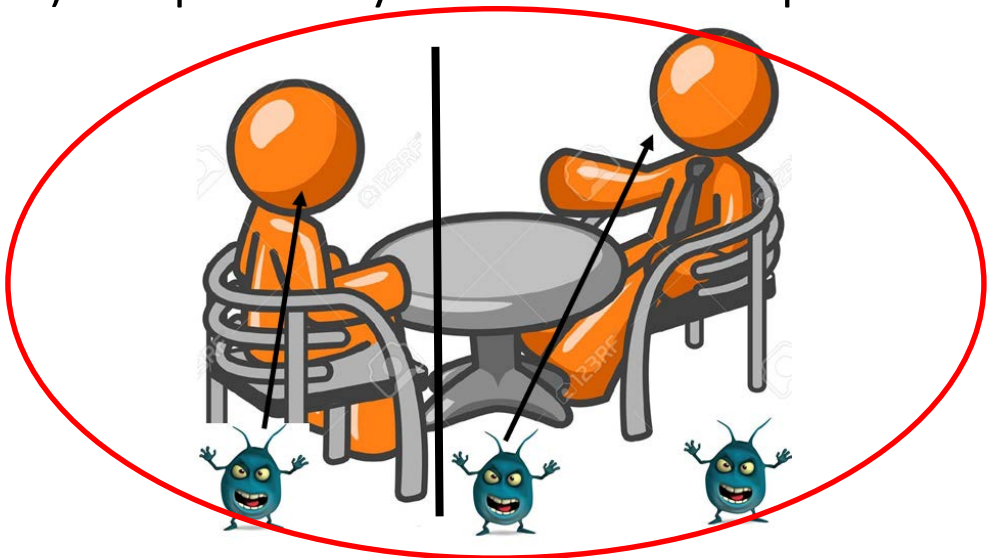
1) Direct person-to-person



2) Indirect person-to-person



3) Independently environmental exposure



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Question 3: Is there evidence for person-to-person transmission in CF patients?

- Until recent years: no evidence of person-to-person transmission of NTM → acquired from environmental exposure

- Nowadays:

Respiratory Outbreak of *Mycobacterium abscessus* Subspecies *massiliense* in a Lung Transplant and Cystic Fibrosis Center

Moira L. Aitken M.D. , Ajit Limaye M.D. , Paul Pottinger M.D. , Estella Whimbey M.D. , Christopher H. Goss M.D., B.S. , Mark R. Tonelli M.D., M.A. , Gerard A. Cangelosi Ph.D. , M. Ashworth Dirac B.S., B.A. , Kenneth N. Olivier M.D., M.P.H. , Barbara A. Brown-Elliott M.S., M.T., S.M. , Steven McNulty B.S. , and Richard J. Wallace, Jr. M.D.

- 2012
- 5 CF patients with *M. abscessus subsp. massiliense*

→ 5 strains indistinguishable by rep-PCR and PFGE

Aitken, M. L. et al. Respiratory Outbreak of Mycobacterium abscessus Subspecies massiliense in a Lung Transplant and Cystic Fibrosis Center. Am. J. Respir. Crit. Care Med.,2012

Whole-genome sequencing to identify transmission of *Mycobacterium abscessus* between patients with cystic fibrosis: a retrospective cohort study

Josephine M Bryant, Dorothy M Grogono*, Daniel Greaves, Juliet Foweraker, Iain Roddick, Thomas Inns, Mark Reacher, Charles S Haworth, Martin D Curran, Simon R Harris, Sharon J Peacock, Julian Parkhill, R Andres Floto*

Bryant, J. M. et al. Whole-genome sequencing to identify transmission of Mycobacterium abscessus between patients with cystic fibrosis: a retrospective cohort study. Lancet, 2013

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Question 3: Is there evidence for person-to-person transmission in CF patients?

Whole-genome sequencing to identify transmission of *Mycobacterium abscessus* between patients with cystic fibrosis: a retrospective cohort study

- 31 CF patients (UK) with 168 *M. abscessus* isolates: WGS

  → difference in SNP's

Bryant, J. M. et al. Whole-genome sequencing to identify transmission of Mycobacterium abscessus between patients with cystic fibrosis: a retrospective cohort study. Lancet, 2013

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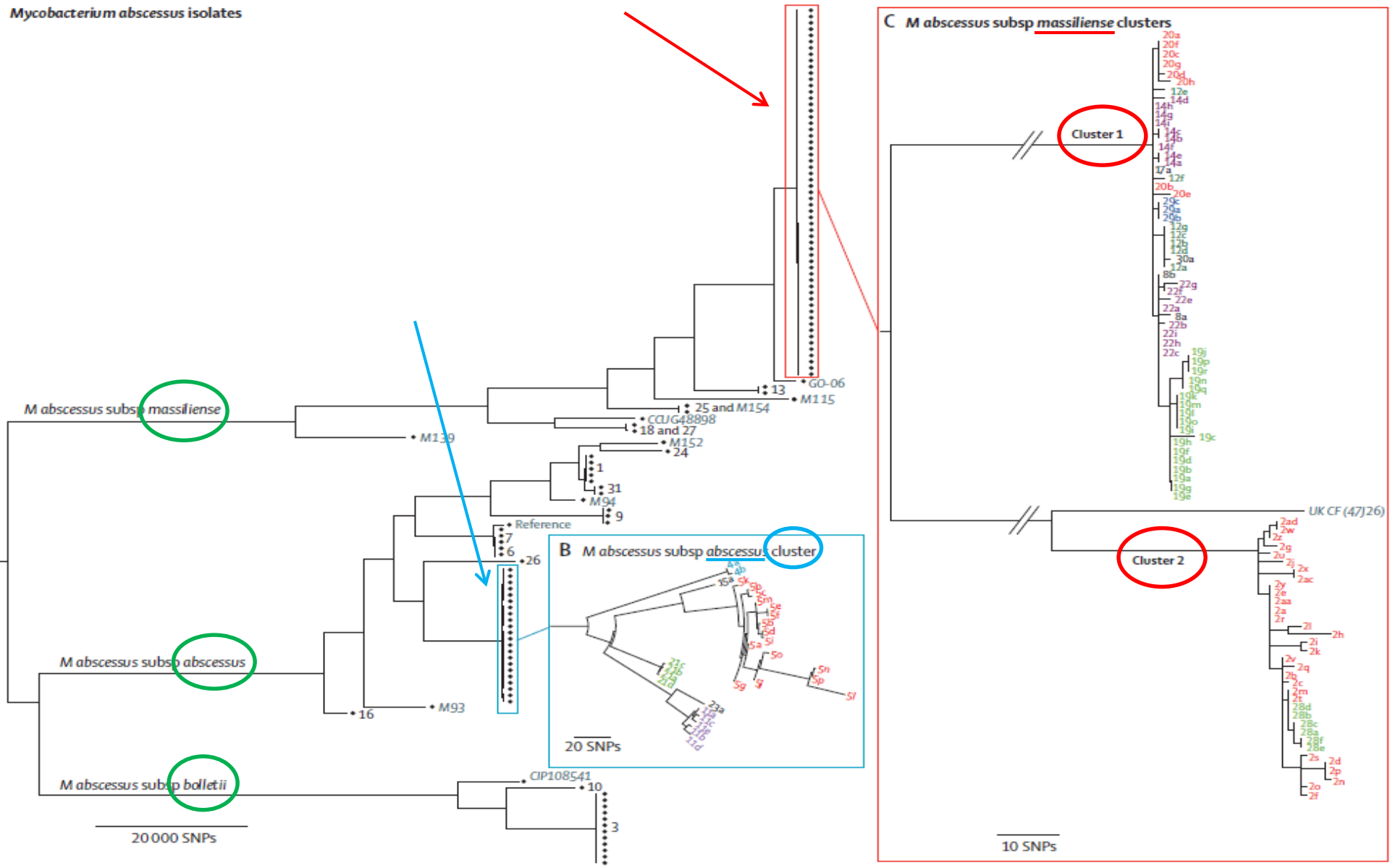
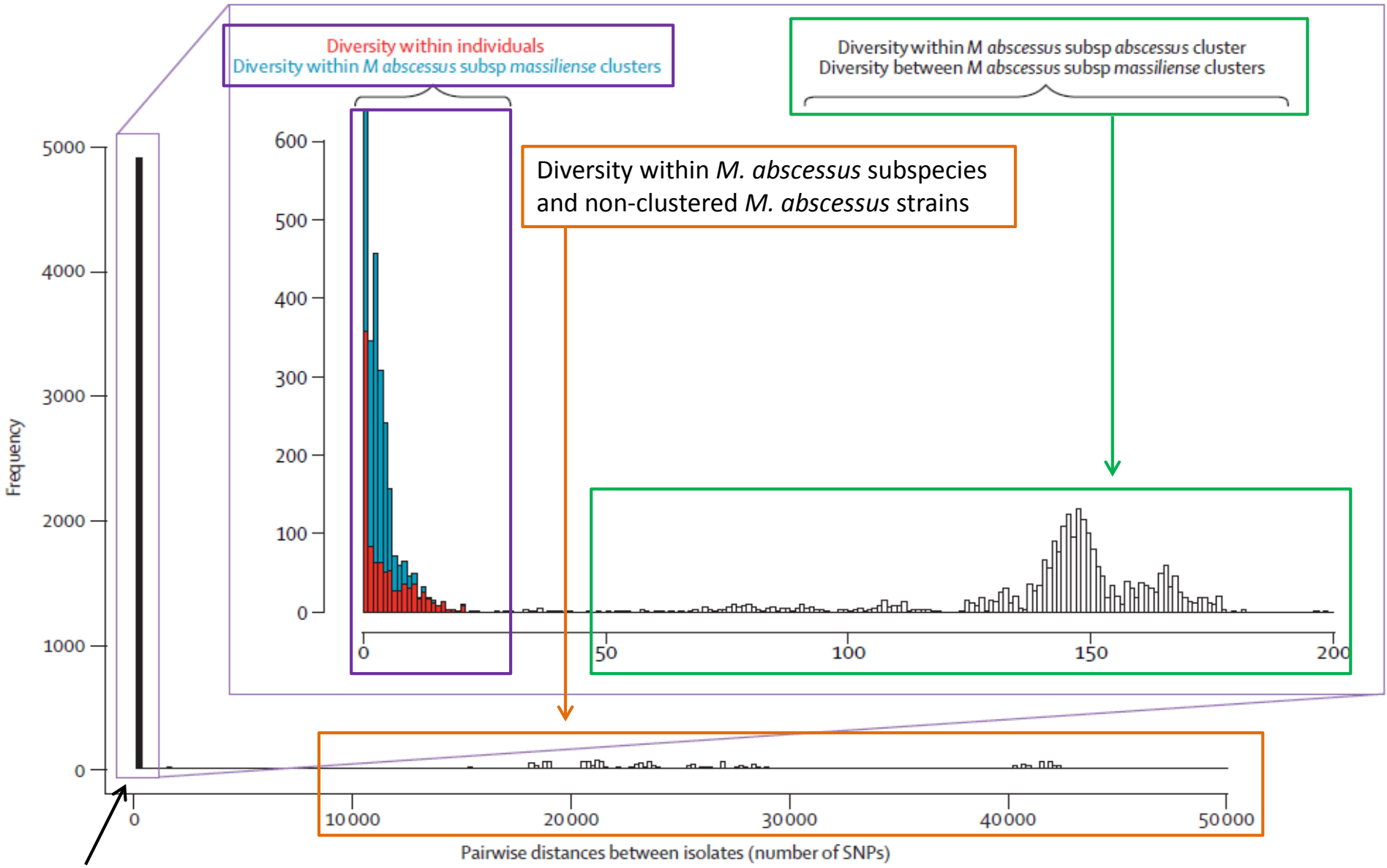
Question 3: Is there evidence for person-to-person transmission in CF patients?



Question 3: Is there evidence for person-to-person transmission in CF patients?



Question 3: Is there evidence for person-to-person transmission in CF patients?

- Conclusions:
  - *M. abscessus subsp. massiliense*: genetic difference between isolates from different individuals often less than variation of isolates seen within one person

    → person-to-person transmission very **likely**; probably **indirectly**
  - *M. abscessus subsp. abscessus*: independently acquired either genetically diverse strains (non-clustered isolates) or a dominant circulating clone

    → **no** person-to-person transmission

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Question 3: Is there evidence for person-to-person transmission in CF patients?

Whole-Genome Sequencing and Epidemiological Analysis Do Not Provide Evidence for Cross-transmission of *Mycobacterium abscessus* in a Cohort of Pediatric Cystic Fibrosis Patients

Kathryn A. Harris,[1,2] Anthony Underwood,[3] Dervla T. D. Kenna,[4] Anthony Brooks,[5] Ema Kavaliunaite,[6] Georgia Kapatai,[3] Rediat Tewolde,[3] Paul Aurora,[6] and Garth Dixon[1,2]

- 20 pediatric CF patients (UK) with 27 *M. abscessus* isolates → WGS and VNTR
  - 12/20 CF patients acquired *M. abscessus* the first time after initial contact with the hospital

1 patient: *M. abscessus subsp. massiliense*

11 patients: *M. abscessus subsp. abscessus*

- 3 patients: VNTR cluster I → **minimal** exposure to other patients from VNTR cluster **1** + **several** times exposed to patients from VNTR cluster **2**
- 3 patients: VNTR cluster II → 2/3 patients = siblings: **multiple** exposure + same **environment**…
- 5 patients: unique VNTR profiles

- Conclusion: person-to-person transmission was unlikely and these individuals must have independently acquired highly genetically related strains

Harris, K. A. et al. Whole-Genome Sequencing and Epidemiological Analysis Do Not Provide Evidence for Cross-transmission of Mycobacterium abscessus in a Cohort of Pediatric Cystic Fibrosis Patients. Clin. Infect. Dis, 2014.

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Question 3: Is there evidence for person-to-person transmission in CF patients?

- Answer to question?
  - Some studies (Aitken *et al*. and Bryant *et al*.) suggest person-to-person transmission
  - However, this could not be confirmed in the most recent study (Harris *et al.*)
  - Reasons for this discrepancy?
    - *M. abscessus subsp. massiliense* (Aitken *et al*. and Bryant *et al*.) is more transmissible than other *M. abscessus* subspecies (Harris *et al*.)?
    - Adults (Aitken *et al*. and Bryant *et al*.) experience more intense exposures or shed a higher load of NTM into the environment compared to children (Harris *et al*.)?
    - Difference in infection control practices between different CF centers?
    - Three types of transmission do co-exist?
- Limitations:
  - Some studies samples of the environment have not been taken
  - Limited number of CF patients and positive NTM cultures in each CF center
  - Retrospective studies

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Question 4: Which techniques are available to investigate the NTM transmission in CF patients?

- Identification up to species level:
  - **Commercial reverse hybridization DNA probe assays**:
    - INNO-LiPA Mycobacteria v2 (Innogenetics, Ghent, Belgium): 16 Mycobacteria species
    - Genotype Mycobacterium CM/AM kit (HAIN Lifescience GmbH, Nehren, Germany) 23 Mycobacteria species
  - **MALDI-TOF**: simple, suitable, reliable, and fast technique for identification of NTM
  - ***rpoB* gene**: until recently used for identification of the 3 subspecies
  - **16S rRNA gene**
- Identification up to subspecies level:
  - ***hsp65* and *erm* genes**
- Further differentiation: to investigate transmission events and/or outbreaks
  - **Whole-genome sequencing (WGS):** used in studies of Harris *et al*. and Bryant *et al.*
    - Sequence data of the entire genome
  - **Multiple Loci Variable number tandem repeat Analysis (MLVA) = VNTR**: used in study of Harris *et al.*
    - Tandemly repeated sequences (= loci): repetitions of one or more nucleotides

      → number of repetitions is hyper variable (4-50)

TGATGCATACATACATACATACATACATACATAGGACT

Question 4: Which techniques are available to investigate the NTM transmission in CF patients?

- Further differentiation: to investigate transmission events and/or outbreaks
  - **Whole-genome sequencing (WGS):**
  - **Multiple Loci Variable number tandem repeat Analysis (MLVA) = VNTR**
    - Tandemly repeated sequences (= loci): repetitions of one or more nucleotides
      → number of repetitions is hyper variable

      TGATGCATACATACATACATACATACATACATAGGACT
    - 1st step: PCR of the VNTR loci
    - 2nd step: separation of the amplification products on agarose gels or by capillary electrophoresis
    - 3rd step: size of the amplification products → calculation of the number of repetitions
    - Example: “14-4-6-4-15-7-12-6” (8 loci) → ”VNTR 1”

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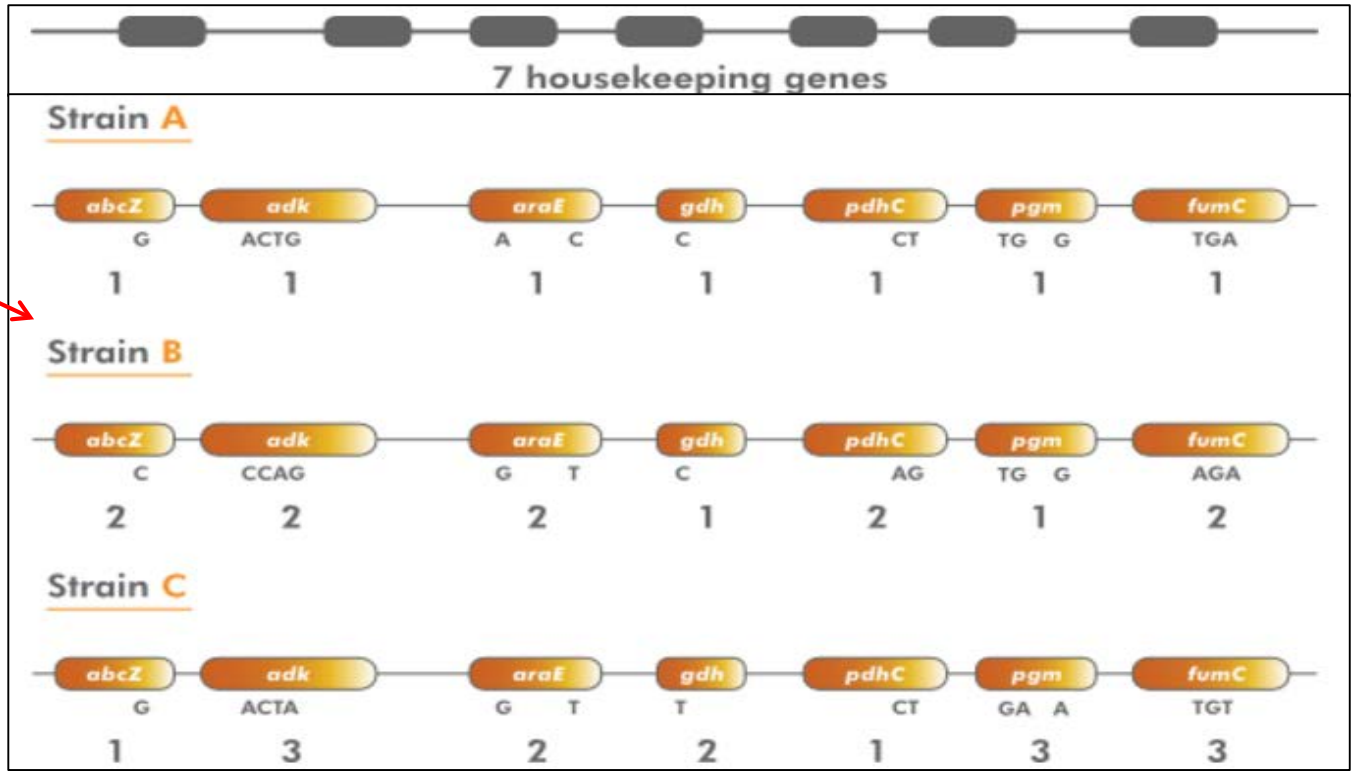
Question 4: Which techniques are available to investigate the NTM transmission in CF patients?

- Further differentiation: to investigate transmission events and/or outbreaks
  - **Multi Locus Sequence Typing (MLST)**
    - 1st step: PCR amplification of 6-8 housekeeping genes (MLST loci)
    - 2nd step: sequencing of the amplification products
    - Each different sequence is assigned a distinct allele number
    - Example: "2-2-2-1-2-1-2" → sequence type 2 (ST2) or strain B



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Question 4: Which techniques are available to investigate the NTM transmission in CF patients?

	WGS	MLVA (VNTR)	MLST/MLSA
ADVANTAGE	- PCR amplification isn't required - high degree of resolution	- rapid - inexpensive	- rapid
DISADVANTAGE	- price	- only a small part of the entire genome is analyzed	- only a small part of the entire genome is analyzed - high cost
REQUIREMENTS	sequencing system	standard PCR and gel electrophoresis/capillary electrophoresis	sequencing system
REQUIRED TIME	< 24h	5h (6 loci)	4-10h
COST	$100	$6 (6 loci)	$40 (7 loci)
PRACTICAL INFORMATION	most used device: Illumina Hiseq platform	primers for loci: attachment 1 and 2	primers for housekeeping genes: attachment 3
REFERENCE	Bryant *et al.* and Harris *et al.*	Wong *et al.* and Harris *et al.*	Kim *et al.*

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Question 4: Which techniques are available to investigate the NTM transmission in CF patients?

- Answer to question?
  - Variety of techniques available which allows the detection of epidemiological analysis of NTM transmission within CF patients
  - No 'gold standard' yet
  - Most studies: WGS and/or MLVA (VNTR)
  - *Laboratory of the university Hospital Saint-Luc Brussels: soon evaluation of the MLVA method*
- Questions for the future and to do actions:
  - IF person-to-person transmission really happens: what is the proportion?
  - Creation of database: Belgian (European) CF centers should create a database with information about *M. abscessus* strains within CF patients to better comprehend the situation

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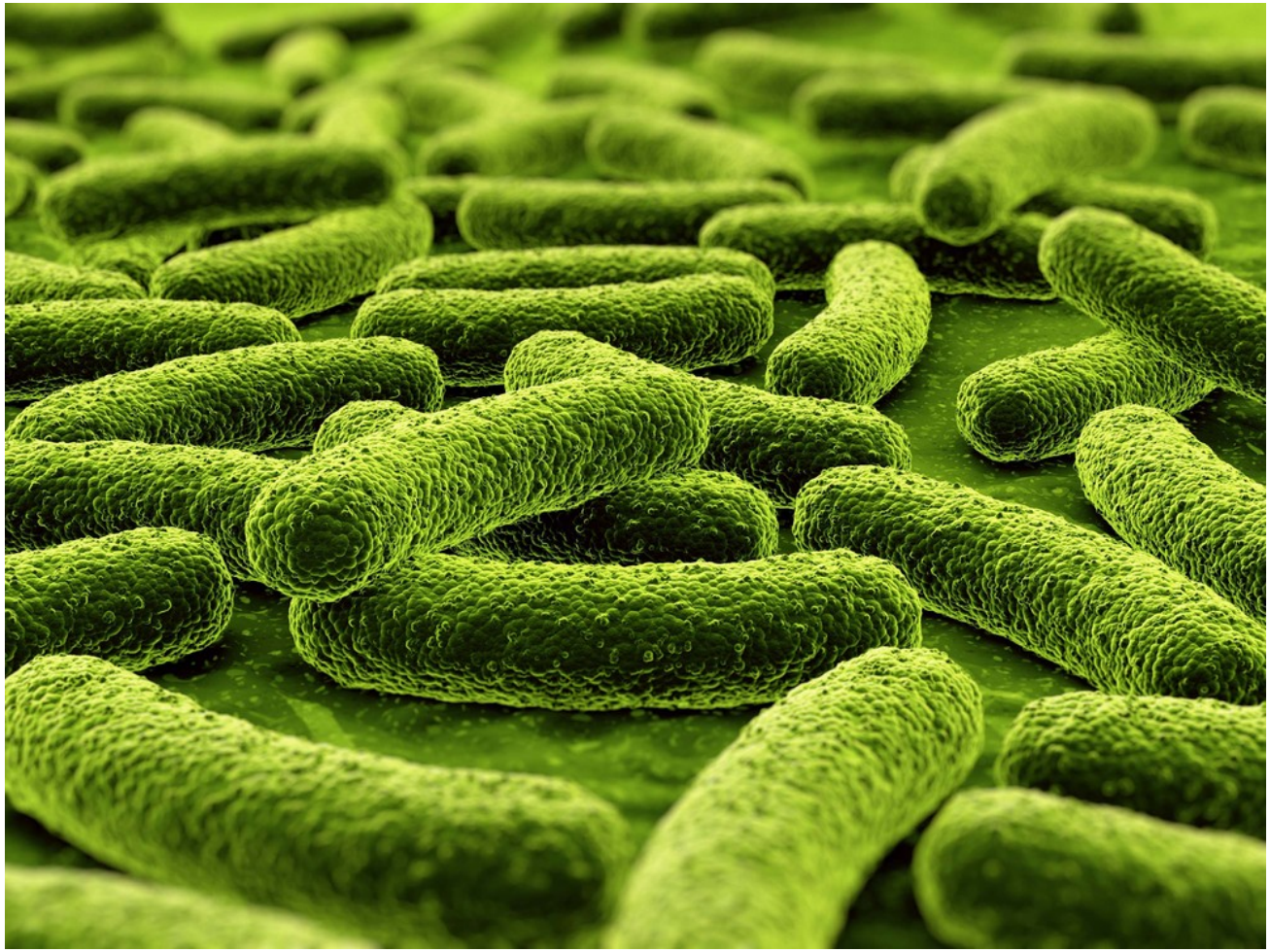
Thank you for your attention!!



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