

The role of the laboratory

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NTM teamplay

- ‘Dekkerswald’ sanatorium
- Multidisciplinary NTM team
 - Pulmonologists
 - Infectious Diseases
 - Pharmacists
 - Radiologists
 - Clinical microbiologists
- NTM research laboratory



Take home messages

The role of the laboratory:

- Detection & identification of NTM
- Drug susceptibility testing
- Treatment effect monitoring

How does it help:

Establishing the diagnosis

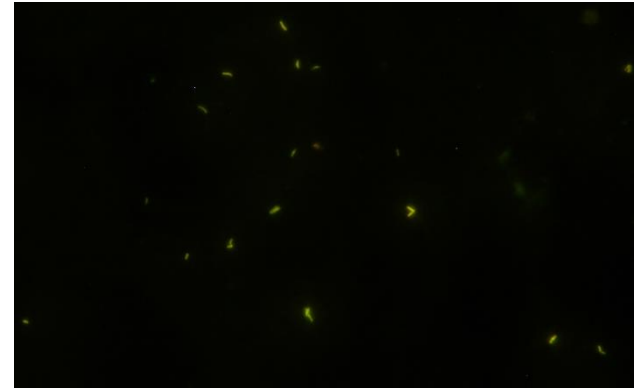
Aid in antibiotic regimen design

Monitor the effect of treatment

Detection and identification

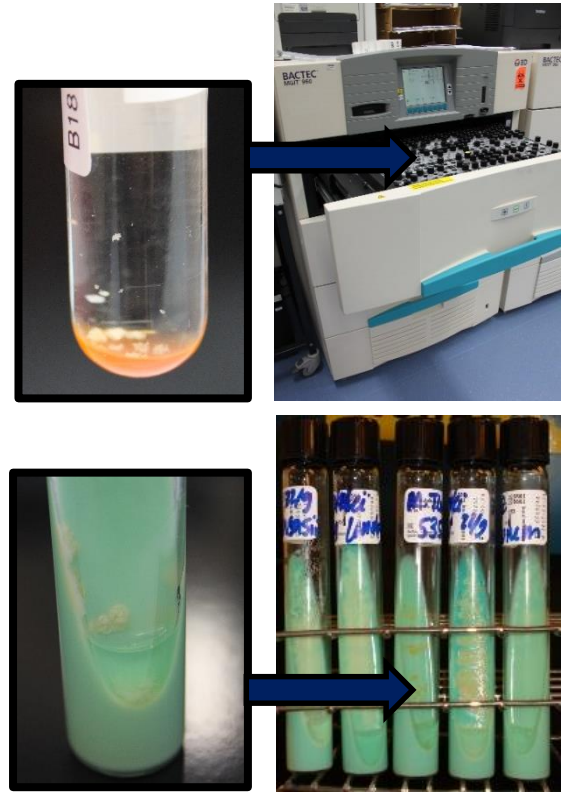
Sample preparation

- Sample quality / purulence
- Decontamination
- Monitor contamination rate (5%)
- Smear microscopy (auramine stain)
- PCR for detection of NTM DNA



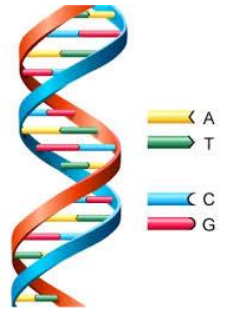
Culture – the long wait

- Liquid medium (broth)
- Solid medium
 - Löwenstein-Jensen
 - Middlebrook 7H10/7H11
- Combination is 10% more sensitive
- Qualitative assessment (pos / neg)
- Quantitative assessment (TTP / SQS)

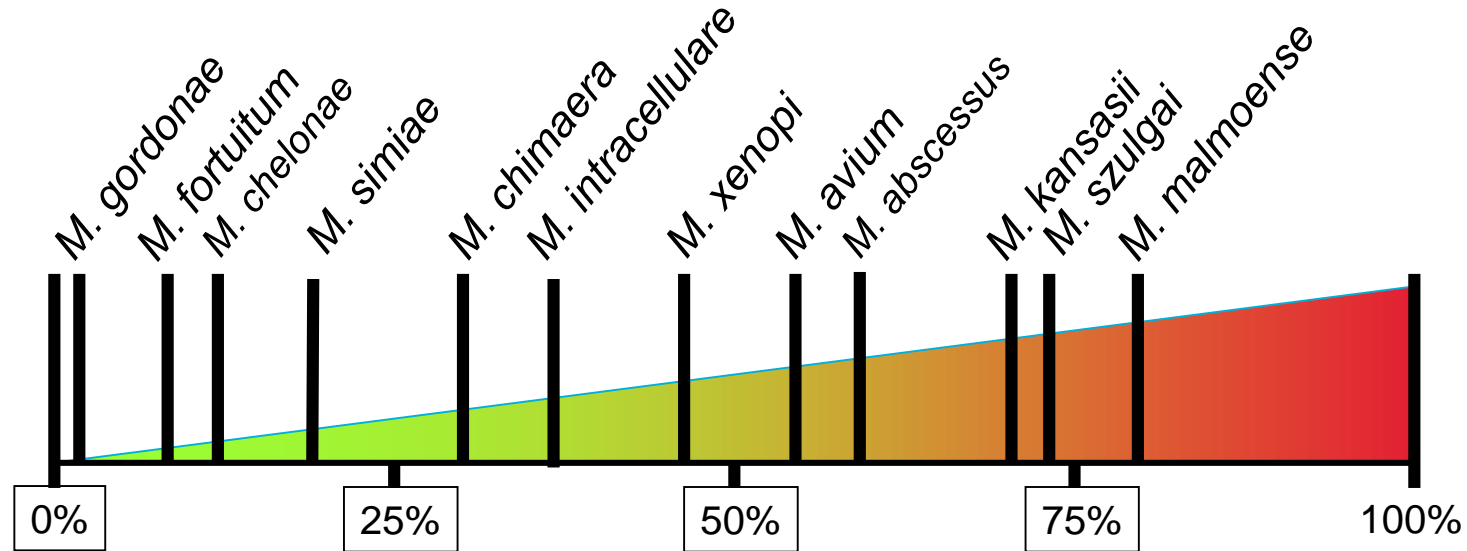


Identification – *nomen est omen*

- Molecular techniques preferred
 - **Probes / line probe assays**
 - (multi)gene sequencing
 - Whole genome sequencing
- ‘New’ kid on the block: **MALDI-TOF-ms**
 - Time-of-flight mass spectrometry
 - (ribosomal) protein content
- Difficult: distinguish MAC species and *M. abscessus* subspecies



Clinical relevance of pulmonary NTM isolates in NL



Clinical relevance differs by species!

(% of patients who met ATS/IDSA diagnostic criteria, per species)

Drug susceptibility testing

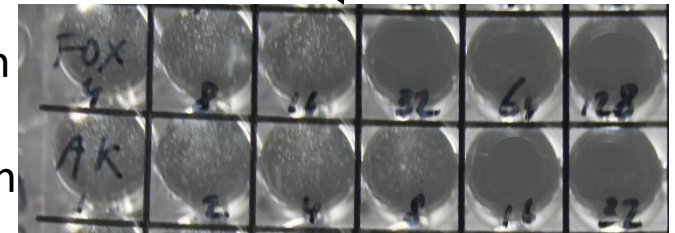
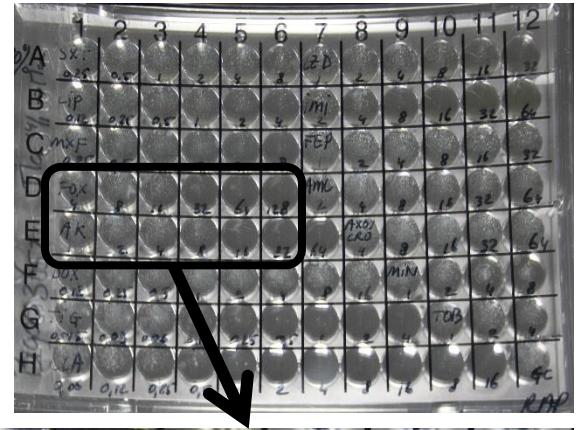
Basic criteria for DST

- DST is useful if:
 1. There is an infection/disease that needs antimicrobial therapy
 2. Effective antimicrobial drugs are available to the patient
 3. The activity of the drugs *in vitro* (in the lab) is related to their effect *in vivo*
 4. The *in vitro* activities of the drugs vary (e.g. resistance can emerge)

Drug susceptibility testing of NTM

- The recommended method: broth microdilution
- Which concentration of drug X kills my NTM?
- Minimum inhibitory concentration: MIC

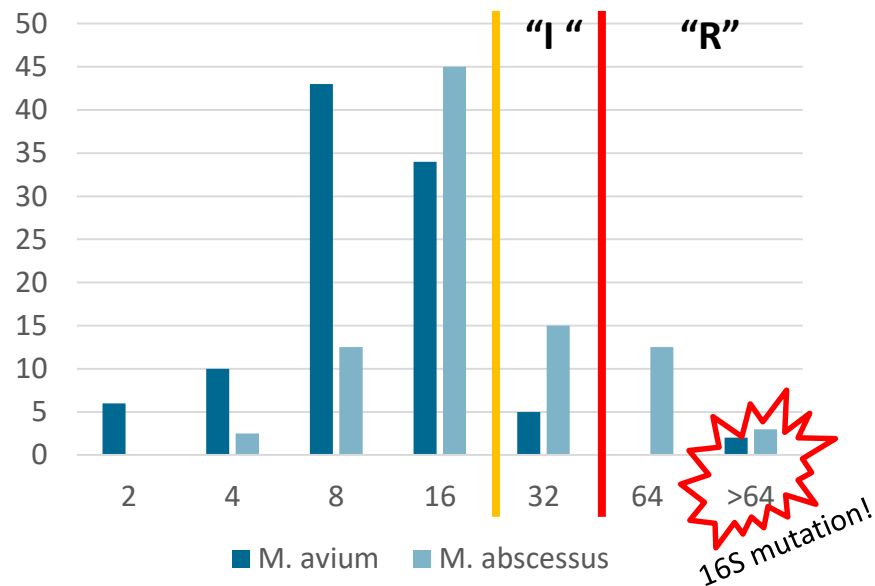
- MIC = 'susceptible' or 'resistant'
 - Dictated by guidelines



What does 'resistant' really mean?

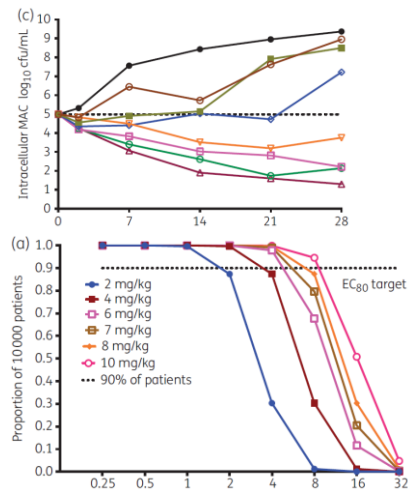
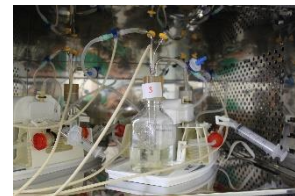
- Historically: abnormal high MIC
- Today: PK/PD science
 - Drug exposure/MIC ratio
 - Exposure-outcome relationships
 - MIC-outcome relationships
- Known for macrolides and amikacin
- Evolving science in NTM disease

Amikacin MIC distribution



What does resistance really mean (2)

- Enter: the hollow fiber model
- Build the human lung environment
 - Macrophages infected with NTM
- Deliver antibiotic as in real life
 - Using different daily doses
- Examine the survival of NTM
- *C/ Dose drug X (MIC Y) at dose Z work?*

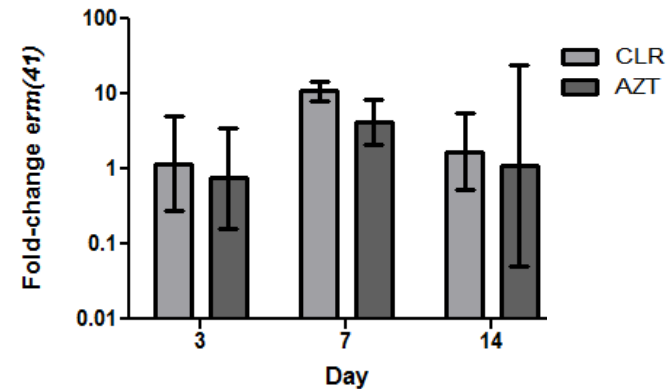


Focus: *M. abscessus* and macrolides

- *M. abscessus* has the *erm(41)* gene
 - Erythromycin Resistance Methylase
- *Inducible* macrolide resistance
- Induced equally by clarithro and azithro
- *M. abscessus* subsp. *massiliense*: *erm* deletion
- Rare: *M. a.* subsp. *abscessus*: *erm* mutation
- Can *develop* 23S mutational resistance on top



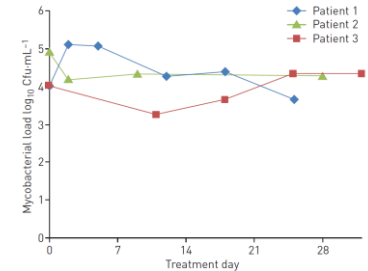
<i>M. a. abscessus</i>	MIC (mg/L)		
	Day 3	Day 7	Day 14
Azithromycin	4	64	128
Clarithromycin	0.5	>16	>16



Monitoring the effect of treatment

Monitoring the effect of treatment

- **Time-to-culture-positivity** / semi-quantitative scale
 - Follow the bacterial load over time during treatment
- **Culture conversion**
- Definition: ≥ 3 consecutive negative cultures from samples 4 weeks apart
 - sampling date of the first negative culture is date of culture conversion
- **Relapse or reinfection?**
- DNA fingerprinting -> whole genome sequencing
- Acquired drug-resistance?



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Thank you very much for your attention

Acknowledgements

Radboud University Medical Center NTM team

Wouter Hoefsloot

Martin Boeree

Cecile Magis

Sanne Zweijpfenning

Saskia Kuipers

Rob Aarnoutse

Reinout van Crevel

Frank van de Veerdonk

Mike Ruth

Jodie Schildkraut

