

Non-tuberculous mycobacteria

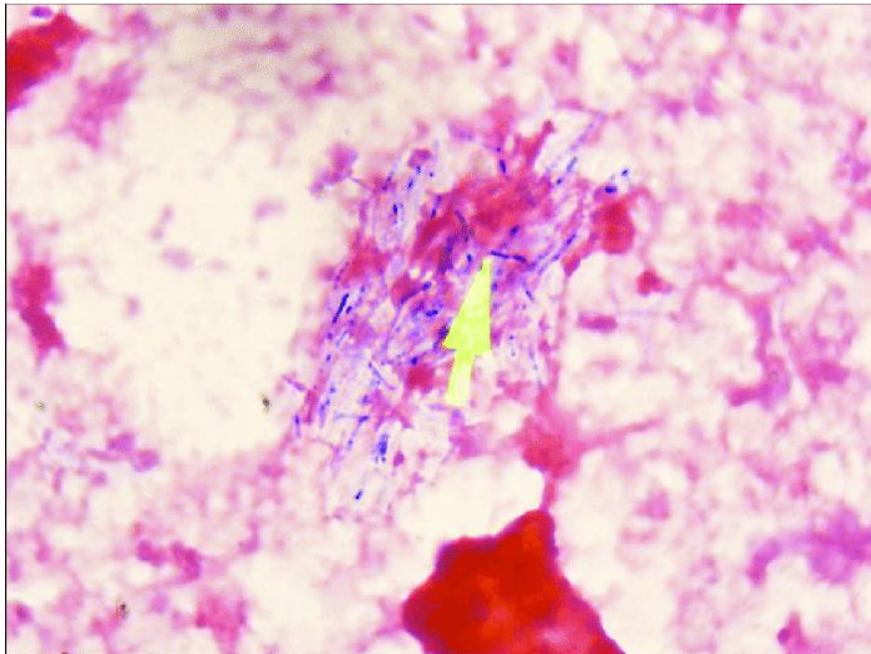
Gabrielle Fröberg

MD, PhD

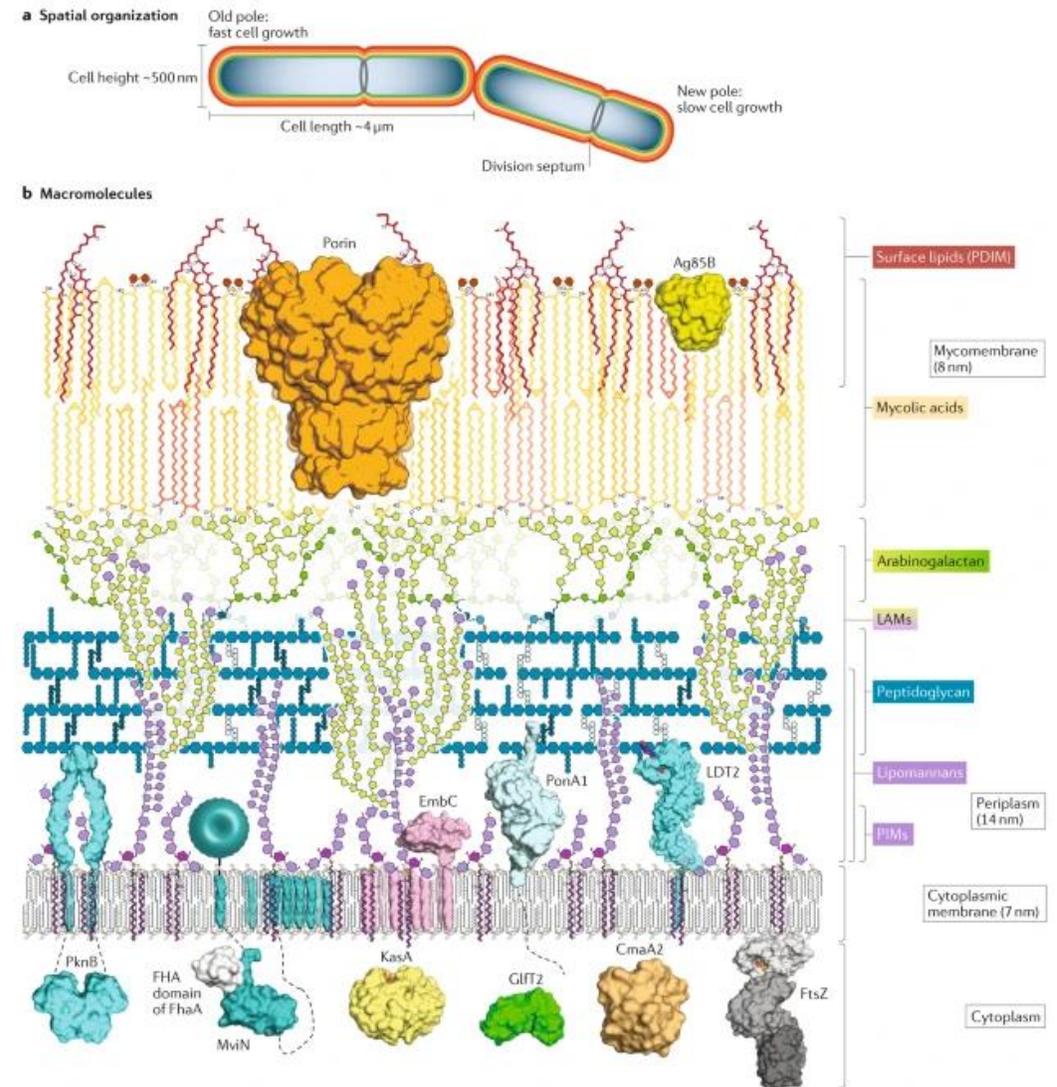
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Mycobacteria

- Aerobic rods
- Cell wall rich in lipids incl mycolic acid and lipoarabinomannan (LAM)
- Gram neutral (or weakly stained)
- Resistant to many cell-wall antibiotics



Rathor N et al. IJCCM. 19. 10.4103/0972-5229.158277.



Dulberger, C.L. et al. Nat Rev Microbiol 18, 47–59 (2020).

Mycobacteria classification

***M. tuberculosis* complex (MTBC)**

- Slowly growing
- Tuberculosis: *M. tuberculosis*, (*M. africanum*, *M. canettii* *mfl*)
- Bovine tuberculosis: *M. bovis*
- Vaccine: *M. bovis* Bacillus Calmette–Guérin (BCG)

M. leprae

- Leprosy

Non-tuberculous mycobacteria (NTM)

- More than 200 species
- Environmental bacteria (soil, water, aerosol)
- Rapidly growing (< 7 days) e.g. *M. abscessus*, *M. chelonae*, *M. fortuitum*
- Slowly growing e.g. *M. avium*, *M. marinum*, *M. ulcerans*



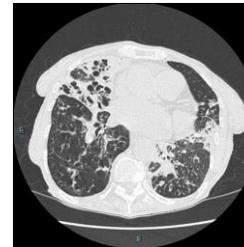
Tuberculosis
M. tuberculosis



Vaccine
M. bovis BCG



Leprosy
M. leprae



Lady Windemere
M. avium



Avium lymphadenitis
M. avium



"Fish tank granuloma"
M. marinum

Why are NTM increasing?



Exposure

- Aerosols e.g. air-condition, sauna, spa
- Lower hot water temps



Diagnostics

- More sensitive diagnostics
- More CT-scans



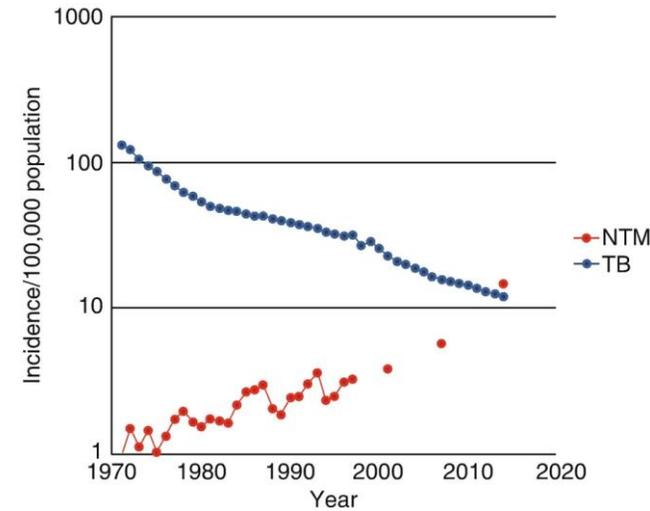
Host factors

- Aging population, more lung disease
- More immunosuppressed patients
- Decreased cross-immunity with M.tb?

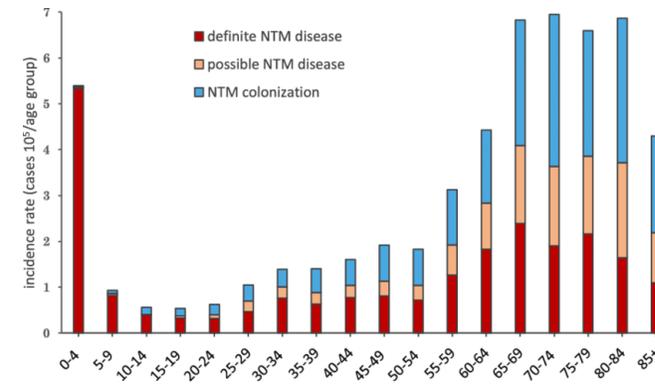


Organism

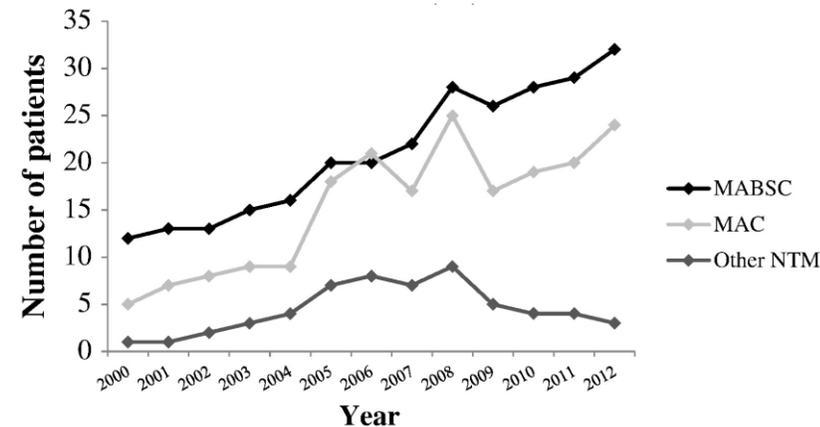
- Increased virulence?



Griffith, D.E. (2019). Nontuberculous Mycobacterial Disease. In: Griffith, D. (eds) Respiratory Medicine. Humana Press, Cham.

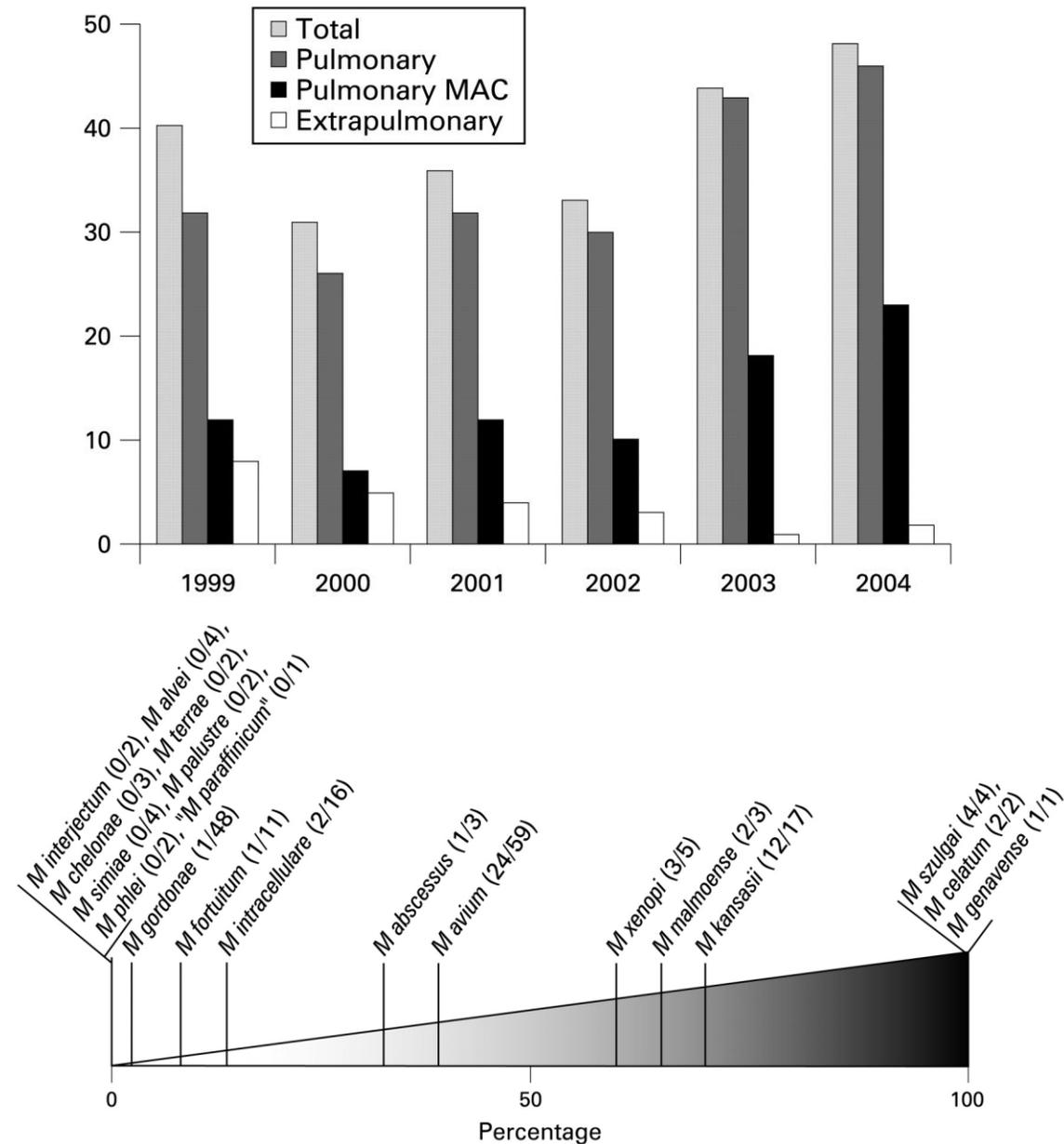
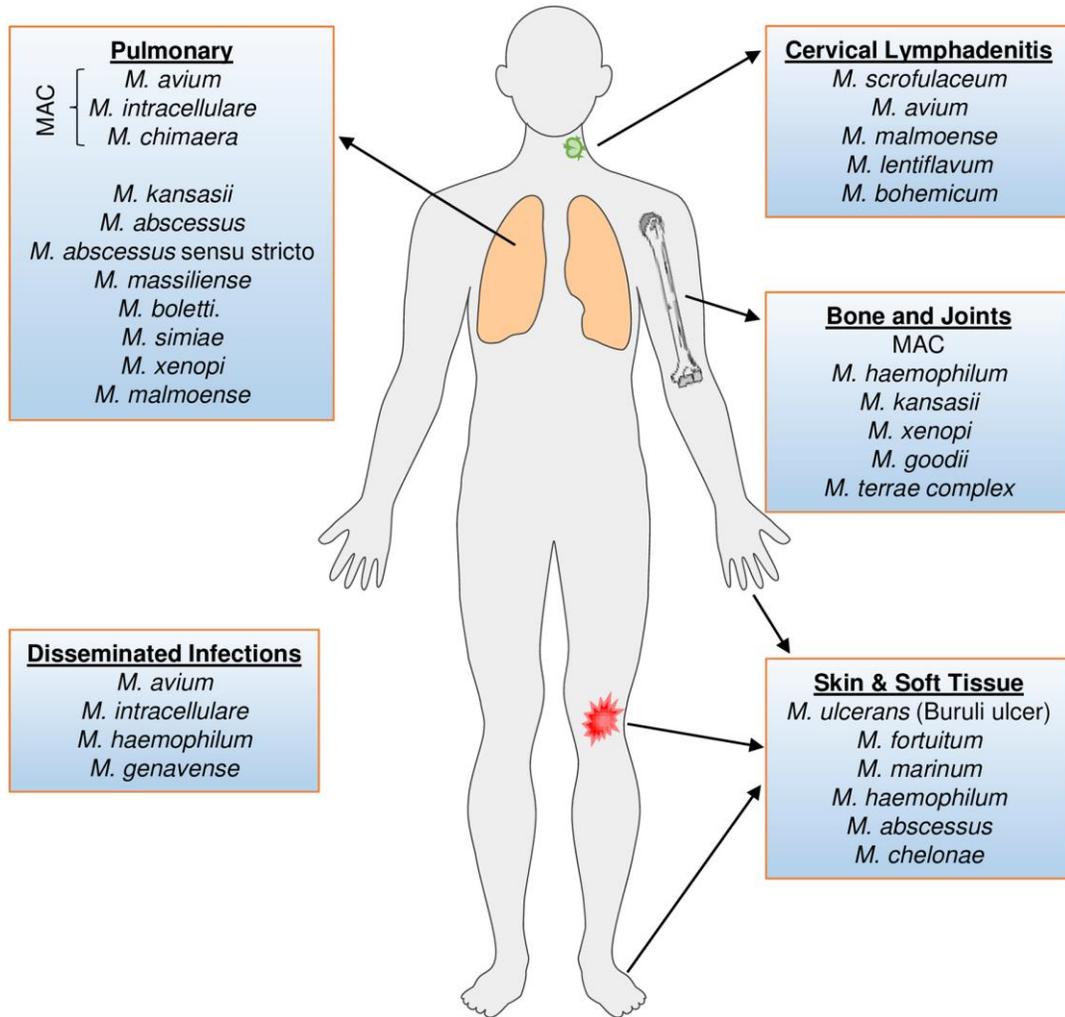


Hermansen, T.S et al. NTM in Denmark, incidence and clinical importance during the last quarter-century. Sci Rep 7, 6696 (2017).



Qvist, Tavs et al. "Epidemiology of NTM among CF patients in Scandinavia. Journal of cystic fibrosis : vol. 14,1 (2015): 46-52.

NTM - clinical presentation and relevance



Baldwin SL et al. The complexities and challenges of preventing and treating NTM . PLoS Negl Trop Dis. 2019 Feb 14;13(2):e0007083.

van Ingen J, Bendien SA, de Lange WCM, et al. Clinical relevance of NTM isolated in the Nijmegen-Arnhem region, The Netherlands Thorax 2009;64:502-506.

Mycobacterium avium complex (MAC)

- Most common slowly growing NTM
- *M. avium*, *M. intracellulare*, *M. chimaera* and several other species
- Pulmonary infections
 - Predisposed e.g. CF, COPD
 - Women >50 years (Lady Windemere syndrome)
 - 30-50% spontaneously sputum conversion
 - High risk of recurrence/re-infection (20-70%)
- Disseminated infections
 - HIV, immune deficiencies/therapies
- First-line treatment
 - Clarithromycin, rifampicin, ethambutol and possibly amikacin
 - Duration 6-12 months
 - Cure rates 50-70%, macrolide resistant 20-50%
- Drug resistance
 - Mutational: macrolides (*rrl*), aminoglycosides (*rrs*)
- Lymphadenitis
 - Children
 - Most often spontaneous cure, possibly surgery



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Mycobacterium abscessus complex (MABC)

- Most common rapidly growing NTM
- Three sub-species: *ssp abscessus*, *ssp massiliense*, *ssp bolletii*
- Two colony morphologies
 - Smooth and rough
 - Rough express less levels of glycopeptidolipids (GPL) in the cell wall and the ability to form cords. Virulence factor? Immunogenic driven?
 - Rough has been associated with more severe disease and less chance of clinical cure¹
 - Rough has *not* been associated with drug resistance²
- Pulmonary infections in predisposed e.g. CF, COPD
- Disseminated infections in HIV, immune deficiencies/therapies
- Skin infections after surgery, trauma (Tsunami)
- First line treatment:
 - Clarithromycin, clofazimine, linezolid, amikacin, imipenem, tigecycline (3-5 drugs)
 - Duration 2 +12 months after culture conversion
 - Cure rates 40-50%, macrolide sensitive 60-80%, macrolide resistant 25-40%
- Drug resistance
 - Inducible: Macrolides (*erm*), *ssp bolletii* och 70-80% av *ssp abscessus*
 - Mutational: Macrolides (*rrl*), aminoglycosides (*rrs*)

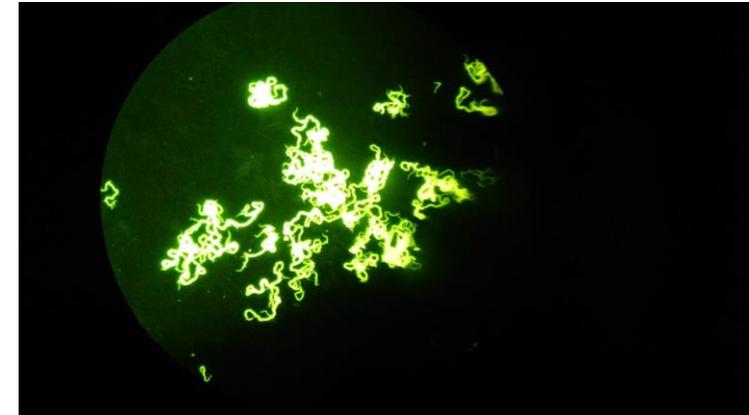


Photo: Fröberg G. 2023

NTM Pulmonary Disease – Diagnostic Criteria

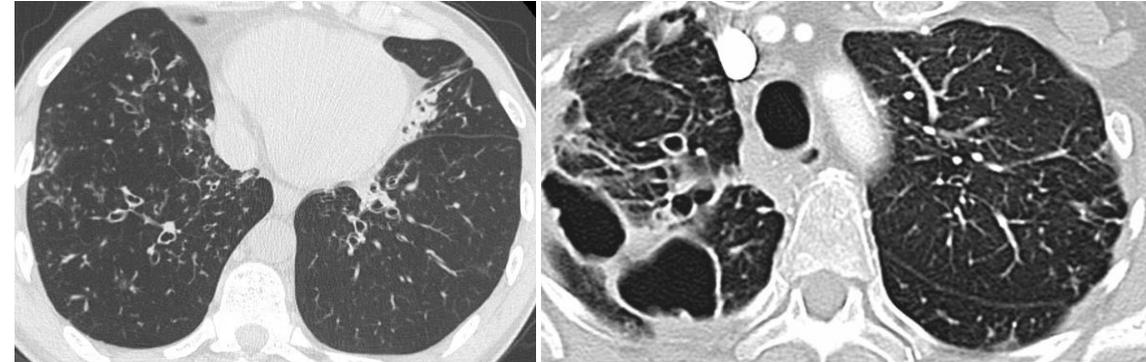
Clinical

- Cough
- Fatigue
- Weight loss



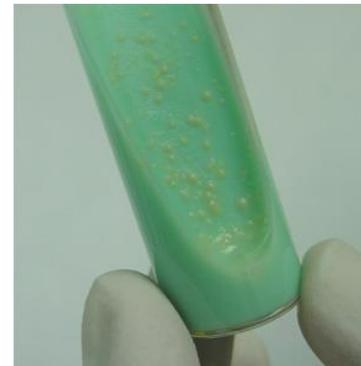
Radiology

- Cavitations
- Bronchioectasis
- Fibronodular, tree-in-bud
- Pleural thickening



Microbiology

- ≥ 2 positive sputum cultures
- ≥ 1 positive cultures from bronchoscopy



NTM Pulmonary Disease – Whom to treat?

Risk of severe disease

- Underlying diseases e.g. chronic pulmonary disease, immunosuppression?
- Degree of symptoms?
- Progression on radiology?
- Species? Microscopy positive? Morphology (MAB)?

Risk of treatment

- Several severe conditions? Prognosis?
- Drug-interactions? Side effects?
- Adherence to treatment?

Goal of treatment

- Cure? Relief of symptoms? Prevention of progression?
- Patients' choice?



M. marinum and *M. ulcerans*

M. marinum

- "Fish tank granuloma"; slowly growing blue/red, sometimes painful noduli -> wounds on hands.
- Transmission via aquarium tanks
- Incubation 2-3 weeks
- Treatment: clarithromycin, rifampicin, ethambutol, possibly surgery



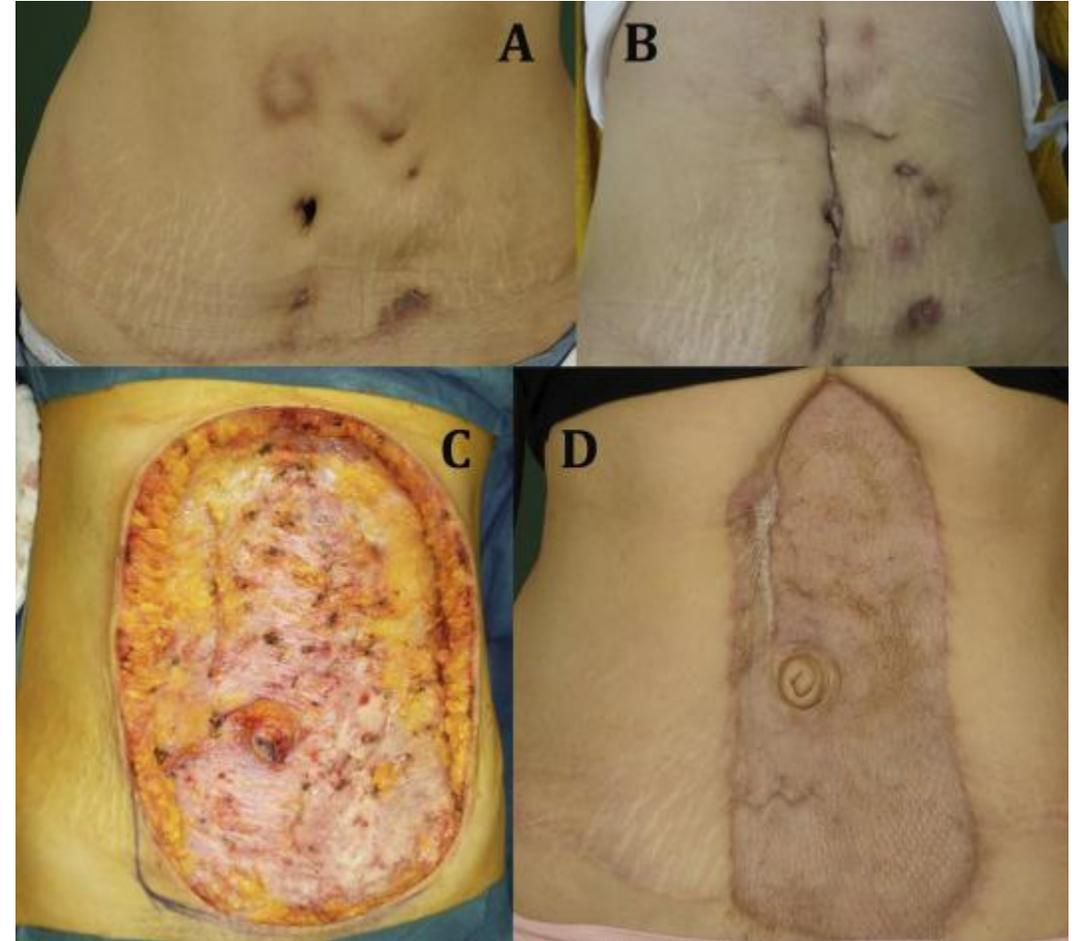
M. ulcerans

- Buruli ulcer: expanding, deep, deforming wounds on feet/ankles.
- West- and Central Africa, rural, close to still water ponds
- Tsunami wounds 2004
- Incubation 8-12 weeks
- High risk bacteria (P3), as *M. tuberculosis*
- Treatment: clarithromycin, rifampicin, quinolones, amikacin, surgery



Iatrogenic NTM infections

- *M. abscessus*, *M. fortuitum*, *M. chelonae*, *M. smegmatis*
- Post-surgery after e.g. plastic surgery, trauma abroad
- Non-sterile equipment, water, ventilation
- Biofilm on catheters, drainages etc
- Hard to treat
- Often 1-2 years of treatment, re-surgery, skin transplant



Schlarb, D et al. *New microbes and new infections* vol. 8 21-3. 10 Sep. 2015

NTM – diagnostics

Smear microscopy

- Acid fast bacilli

PCR

- MTBC PCR negative and microscopy positive = most likely NTM

Culture

- Liquid broth (MGIT) and solid media (Löwenstein-Jensen)
- May also grow on standard blood agar
- 30+37C
- Microscopy: Most often no cord formation

Line probe assay (Hain Lifesciences)

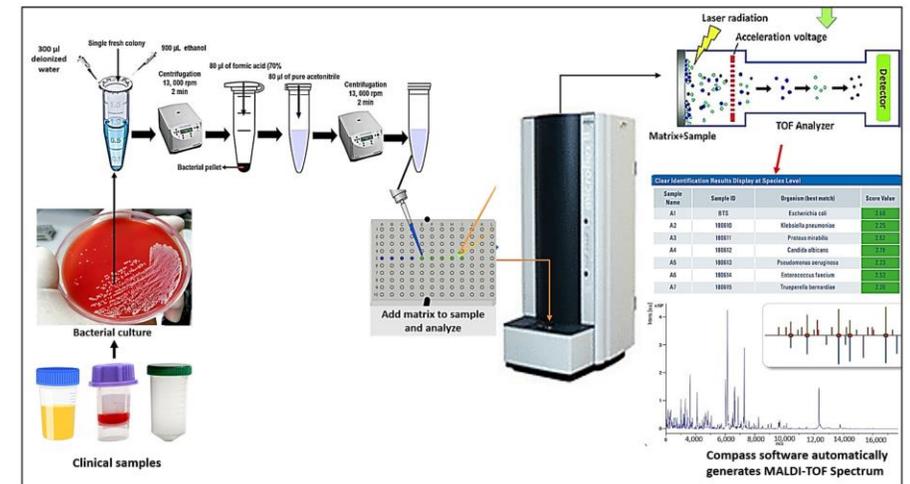
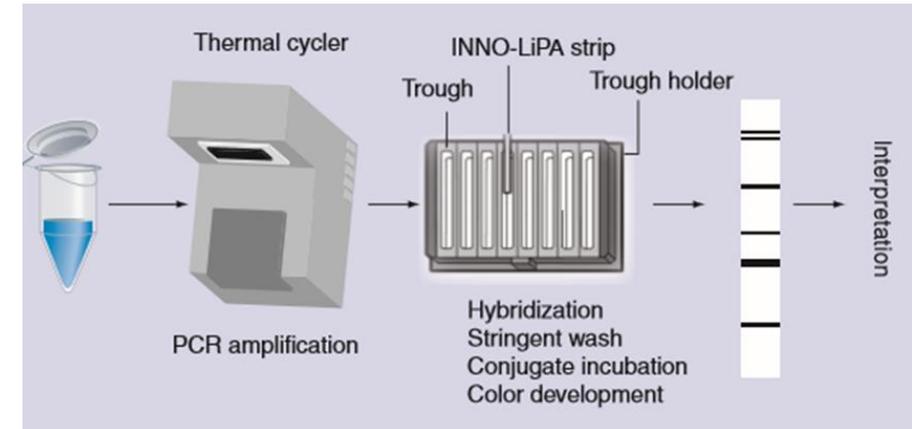
- Performed on isolate
 - CM (common mycobacteria), 27 species
 - AS (additional species), 19 species
- Performed on sample material
 - CM *direct*

MALDI (Biotyper®)

- MBT Mycobacteria module (Bruker), 182 species

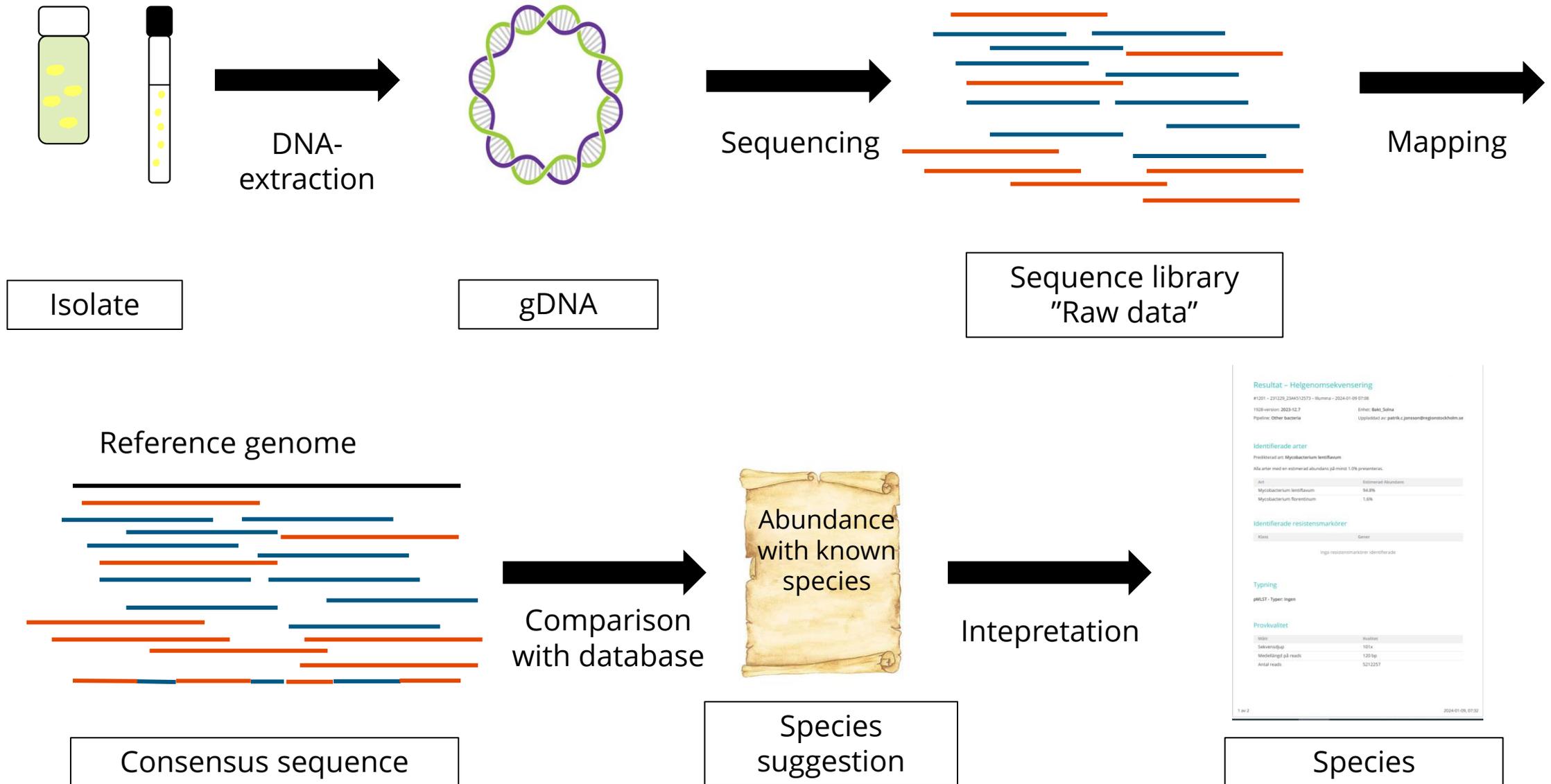
Whole genome sequencing (WGS)

- NGS (Next generation sequencing e.g. Illumina, Ion Torrent)



Elbehiry, A et al. Vaccines vol. 10,11 1881. 8 Nov. 2022.

NTM NGS



Resultat - Helgenomsekvensering

#1201 - 231225_23M312573 - Runna - 2024-01-09 07:08
150k-version: 2023-12.7 Enhet: Bakt_Solna
Pipeline: Other bacteria Upprättad av: pat@k.kj.se@regionstockholm.se

Identifierade arter
Predikterad art: Mycobacterium lentiflavum
Alla arter med en estimatorabundans på minst 1,0% presenteras.

Art	Estimatorabundans
Mycobacterium lentiflavum	94,8%
Mycobacterium fortuitum	1,6%

Identifierade resistensmarkörer

Klass	Gener
Inga resistensmarkörer identifierade	

Typpning
pMLST: Typen: Ingen

Provkvalitet

Yield:	Acidfast
Sekvensdjup:	107x
Medellängd på reads:	120 bp
Antal reads:	5212257

1 av 2 2024-01-09, 07:32

NTM – drug susceptibility testing

Relevant isolates

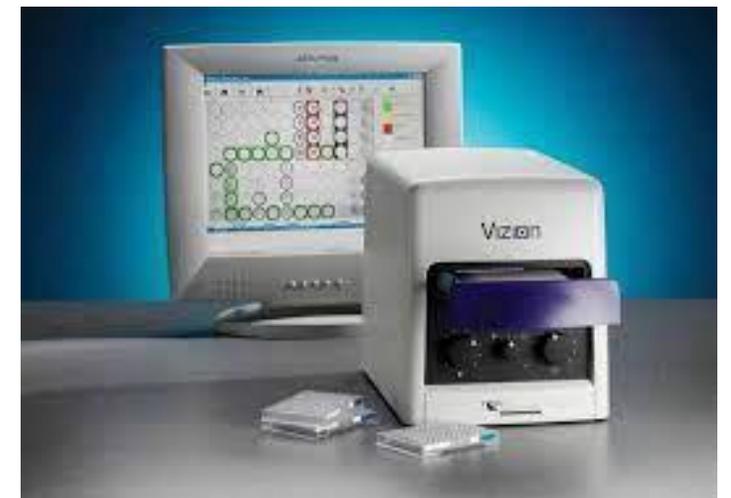
- Clinical infection where treatment is planned for or ongoing (on demand or information given)
- Samples from invasive infection e.g. blood, csf, biopsies/drainage
- Treatment failures e.g. repeated positive cultures during treatment > 3 months
- First NTM in CF-patients

Line probe assay (Hain Lifesciences)

- NTM-DR:
 - sub-species of *M. avium* complex (MAC) and *M. abscessus* complex (MABC)
 - resistance to clarithromycin (*erm* , *rrl*)
 - Resistance to aminoglycosides (*rrs*)

Broth micro dilution (BMD) (Sensititre, Thermo Fisher)

- SLOMYCO, QC *M. avium*, incubation 7 days
- RAPMYCO, QC *M. peregrinum*, incubation 3-5 days
- 2-fold serial dilution
- Technical variability +/- dilution step
- Need of trained reader



NTM ECOFFS and clinical breakpoints

- MIC distributions of NTM wild-type isolates have not been systematically evaluated despite their importance for establishing AST breakpoints.
- CLSI breakpoints are few, non-documented
- European study, 2023¹
 - 12 laboratories
 - *Mycobacterium avium* (n=1271) and *M. intracellulare* (n=415)
 - *Mycobacterium abscessus* complex (MABC) (n=1014)
- MIC determinations by SLOMYCOI and RAPMYCOI.
- Epidemiological cut-off values (ECOFFs) and tentative ECOFFs (TECOFFs) determined by EUCAST methodology incl quality controls.

¹Fröberg, Gabrielle et al. "Towards clinical breakpoints for non-tuberculous mycobacteria - Determination of epidemiological cut off values for the *Mycobacterium avium* complex and *Mycobacterium abscessus* using broth microdilution." *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* vol. 29,6 (2023): 758-764.

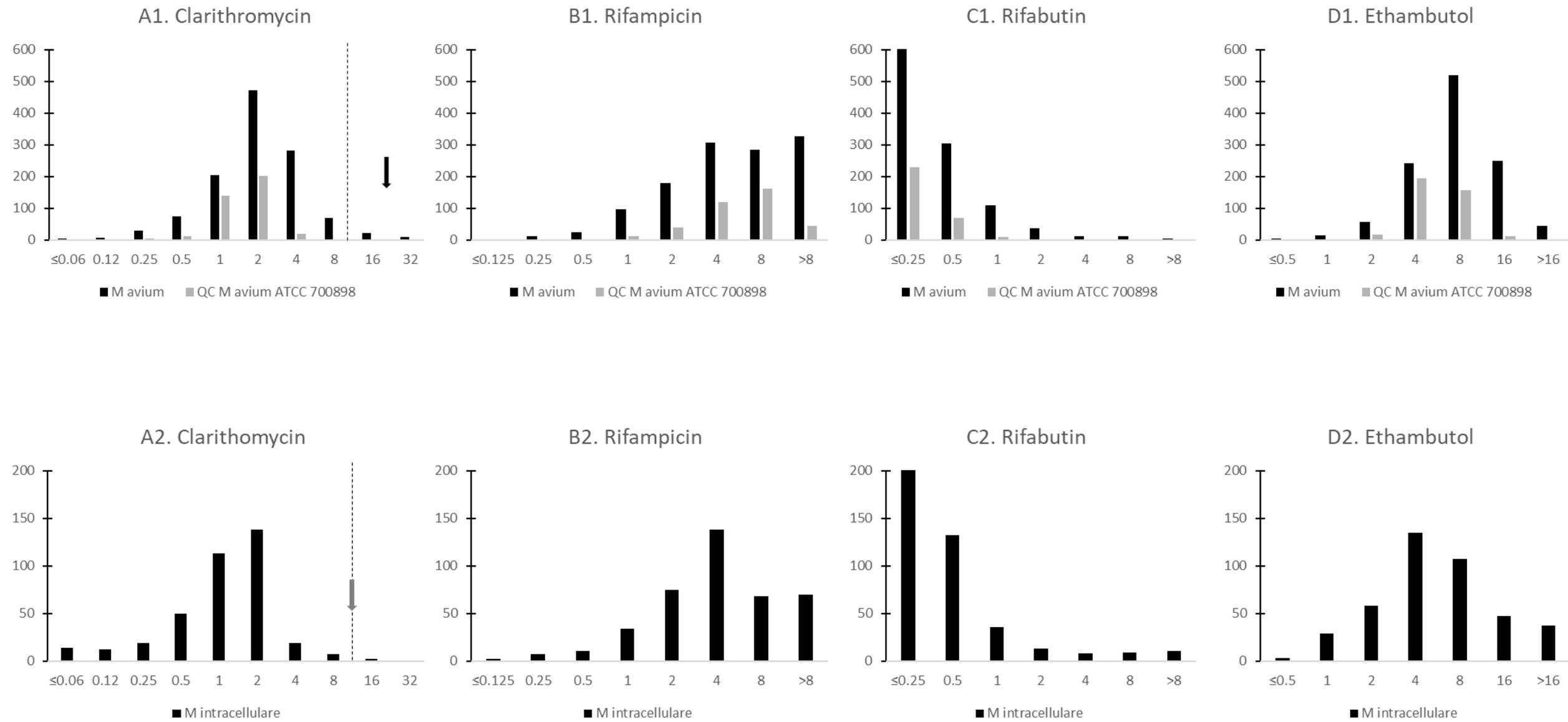


Figure 1. Wild-type MIC distributions for *M. avium* and *M. intracellulare* (black bars), as well as QC *M. avium* ATCC 700898 (grey bars). Arrows indicate ECOFFs/TECOFFs (black/grey) set on valid distributions and according to EUCAST criteria. Dotted vertical lines indicate current CLSI breakpoints.



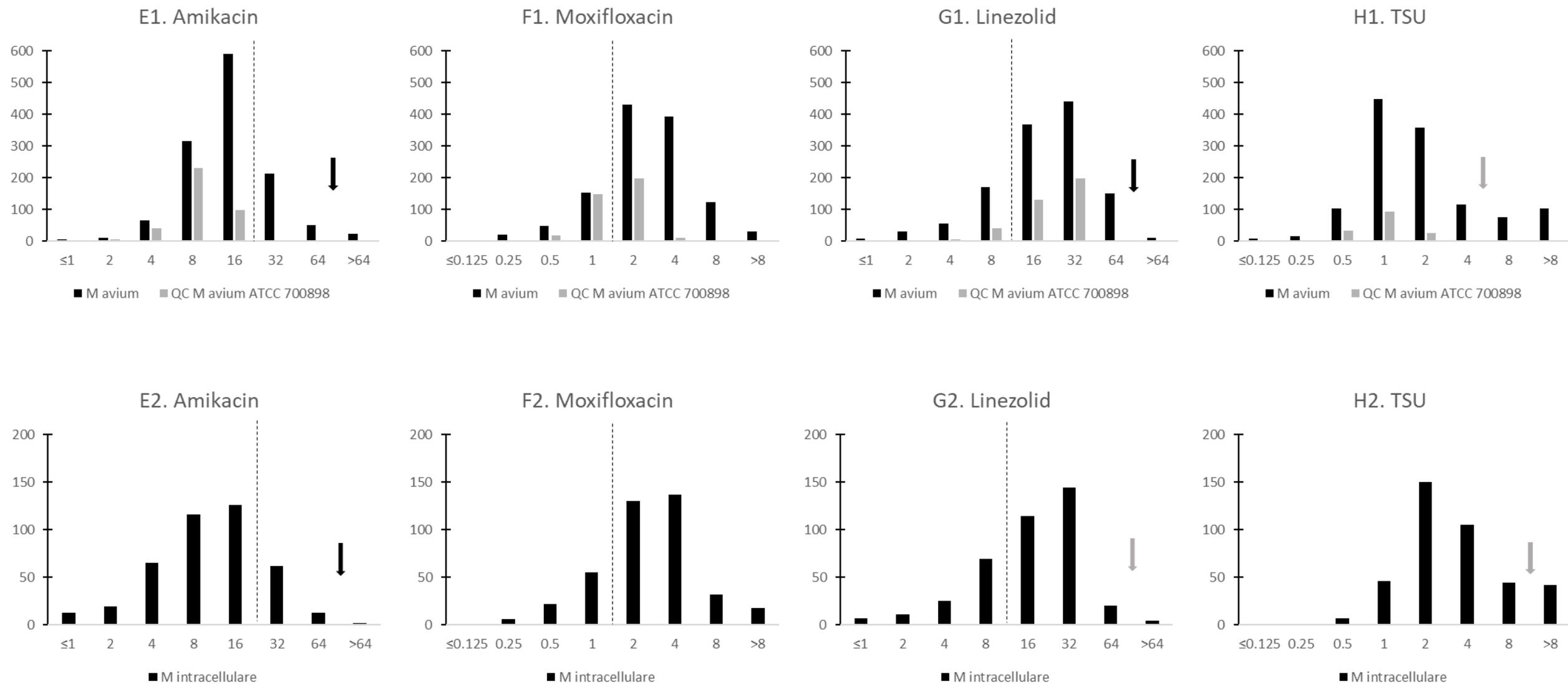


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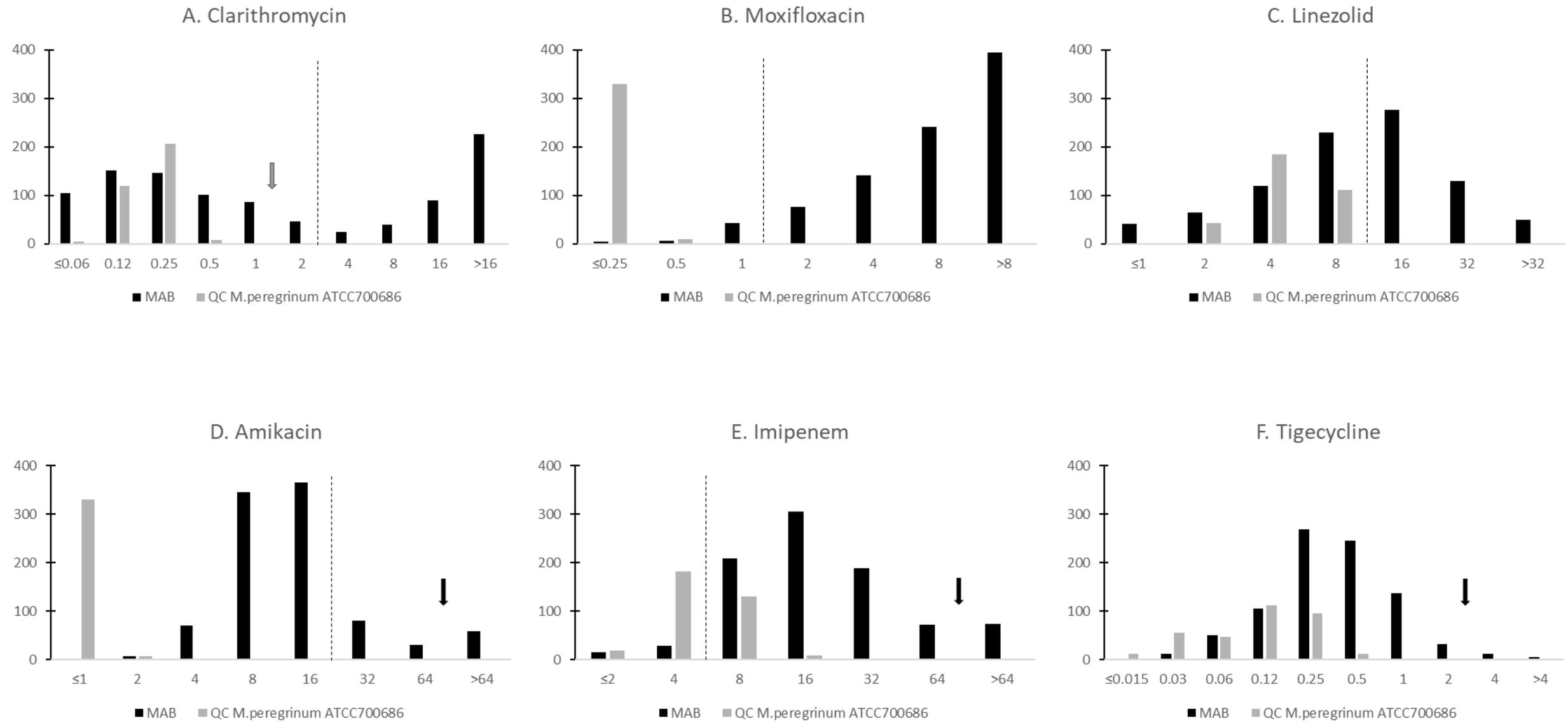


Figure 2. Wild-type MIC distributions for MAB (black bars) and QC *M. peregrinum* ATCC 700686 (grey bars). Arrows indicate ECOFFs/TECOFFs (black/grey) set on valid distributions and according to EUCAST criteria. Dotted vertical lines indicate current CLSI breakpoints.

NTM ECOFFS and clinical breakpoints

(T)ECOFFs were defined for several antimicrobials against MAC and MAB.
Generally high MIC-values for most antimicrobials

- Need for new drugs
- Need for combination therapies

Broad wild-type MIC distributions

- Need for more precise definition of species and sub-species
- Need for further refinement of method and QC
- Need for molecular methods

Several CLSI NTM breakpoints cut the wildtype distribution

- Uncertain results (technical variability)
- Risk of over/under treatment e.g. moxifloxacin, linezolid and imipenem

Correlation to PK/PD and clinical outcome?

- Need for further data/studies

How to report MIC-results?

- Values only?
- Comment about wildtype e.g. \leq ECOFF?
- Breakpoints in brackets (combination therapy)?
- Comment according to “Where there are no breakpoints”?