

First Insights into Irish *Mycobacterium chimaera* isolates



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Introduction

Mycobacterium chimaera is a slow-growing nontuberculous mycobacterium recently recognised as a novel species within the *Mycobacterium avium* complex (MAC). Diagnosing infections as being caused by *M. chimaera* is difficult as it is often misidentified as *M. intracellulare*.

Identification of this environmental pathogen is important as it is emerging as a respiratory pathogen and recent outbreaks of invasive *M. chimaera* infections following cardiac surgery have been reported in Europe through the ECDC. Following these reports, the Irish Mycobacteria Reference Laboratory (IMRL) is facilitating a national investigation in Ireland by identifying clinical and environmental isolates of *M. chimaera*.

Aims

- To reidentify a collection of clinical *M. intracellulare* isolates collected from the IMRL
- To investigate the commercial assay GenoType NTM-DR (Hain Lifescience) as a confirmatory assay for the identification of *M. chimaera*
- To characterise clinical *M. chimaera* isolates further by whole genome sequencing (WGS)

Materials and Methods

***M. intracellulare* isolates (n=132):** were collected in the IMRL between 2007 and 2016, and recovered from clinical specimens of 111 patients. Species identification was previously performed using the GenoType CM assay for Common mycobacteria (Hain Lifescience) as part of routine diagnostics.

Identification of *M. chimaera*: was performed by amplifying and sequencing the 16S rRNA gene and 16S – 23S Internal Transcribed Spacer (ITS) region. Sequence results were compared using BLAST on NCBI website.

GenoType NTM-DR assay: was tested with 61 *M. chimaera*, 10 *M. avium* and 10 *M. intracellulare* clinical isolates. Hybridization of amplified products was performed on the GT-Blot 48 instrument (Hain Lifescience). Results were then interpreted using the Genotype NTM-DR interpretation chart.

Whole Genome Sequencing: of X *M. chimaera* respiratory isolates and *M. chimaera* strain FI-0169T was performed using a paired-end approach on an Illumina MiSeq instrument (TrinSeq, Trinity College Dublin, Ireland). Phylogenomic tree was constructed using

Acknowledgements

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References

1. Tortoli, E. et al. Proposal to elevate the genetic variant MAC-A, included in the *Mycobacterium avium* complex, to species rank as *Mycobacterium chimaera* sp. nov. *Int. J. Syst. Evol. Microbiol.* **54**, 1277–1285 (2004).
2. Aogáin, M. M. et al. Draft Genome Sequences of Three *Mycobacterium chimaera* Respiratory Isolates. *Genome Announc.* **3**, e01409-15 (2015).

Results

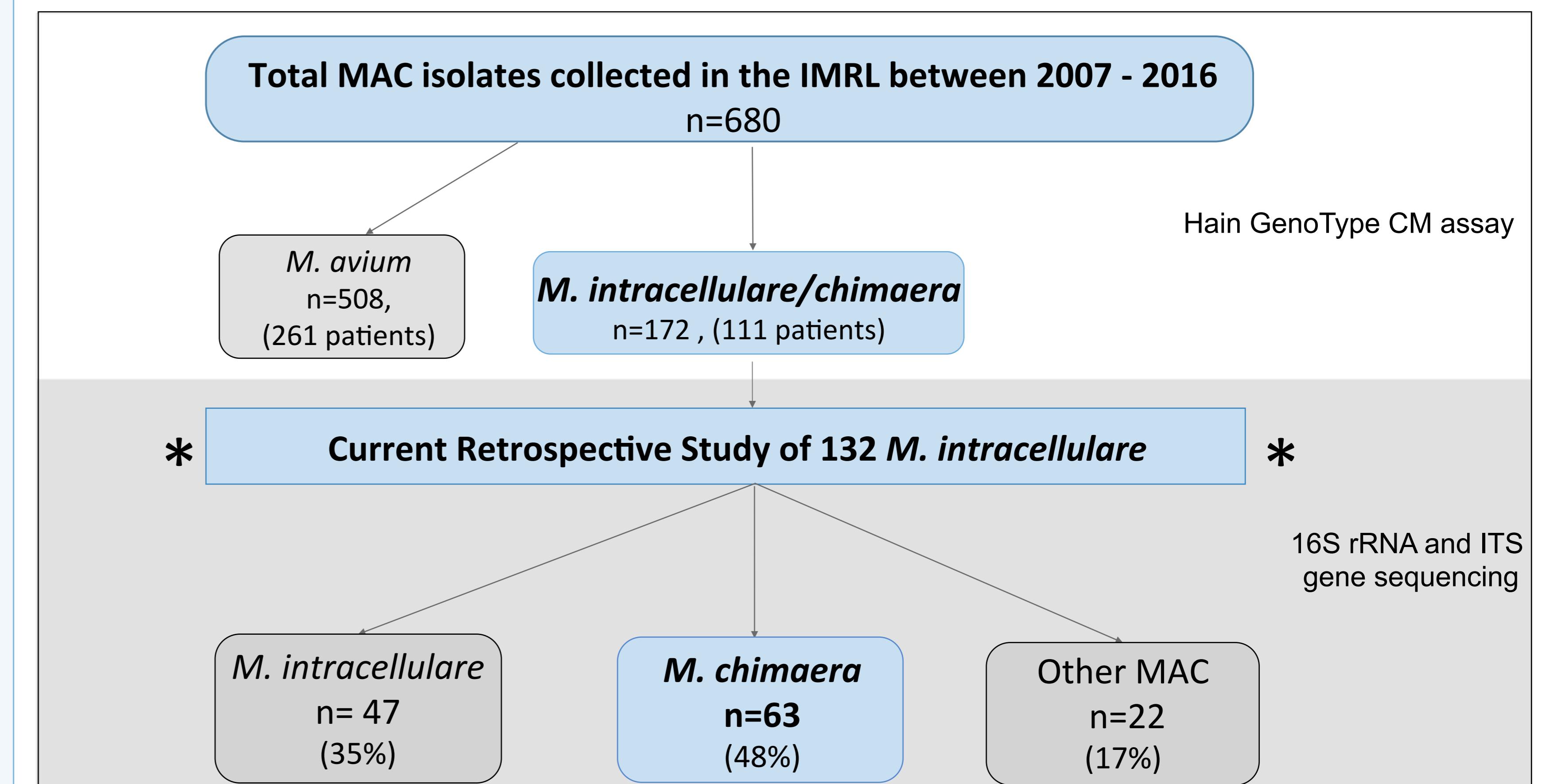


Figure 1. Retrospective identification of clinical *M. chimaera* isolates. A study of 132 clinical *M. intracellulare* isolates showed that 63 (48%) were identified as *M. chimaera* from 52 patients by 16S rRNA and 16S-23S ITS gene sequencing. Resultant ITS sequences shared 100% identity with *M. chimaera* FI-0169T as described by Tortoli et al (1).

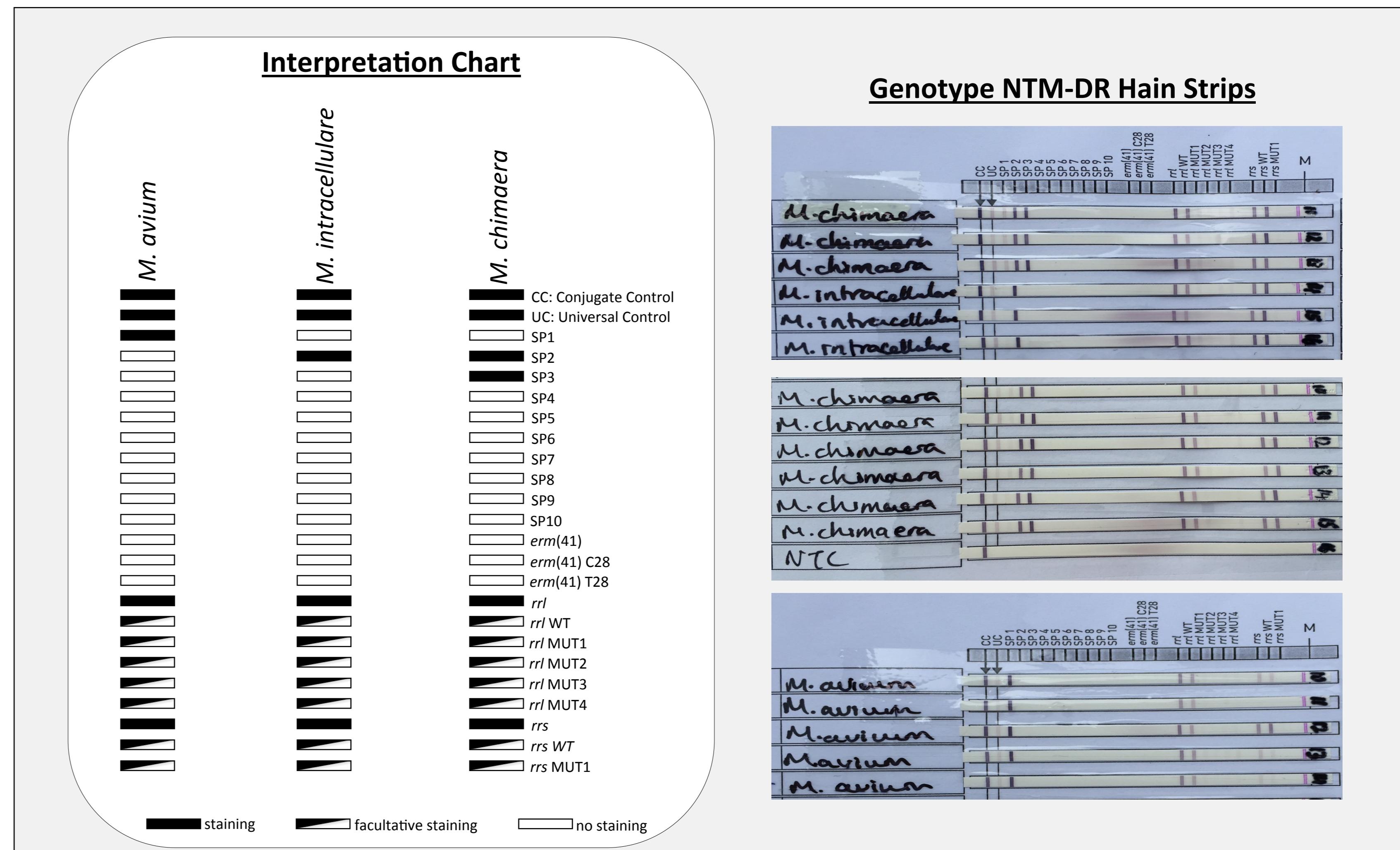
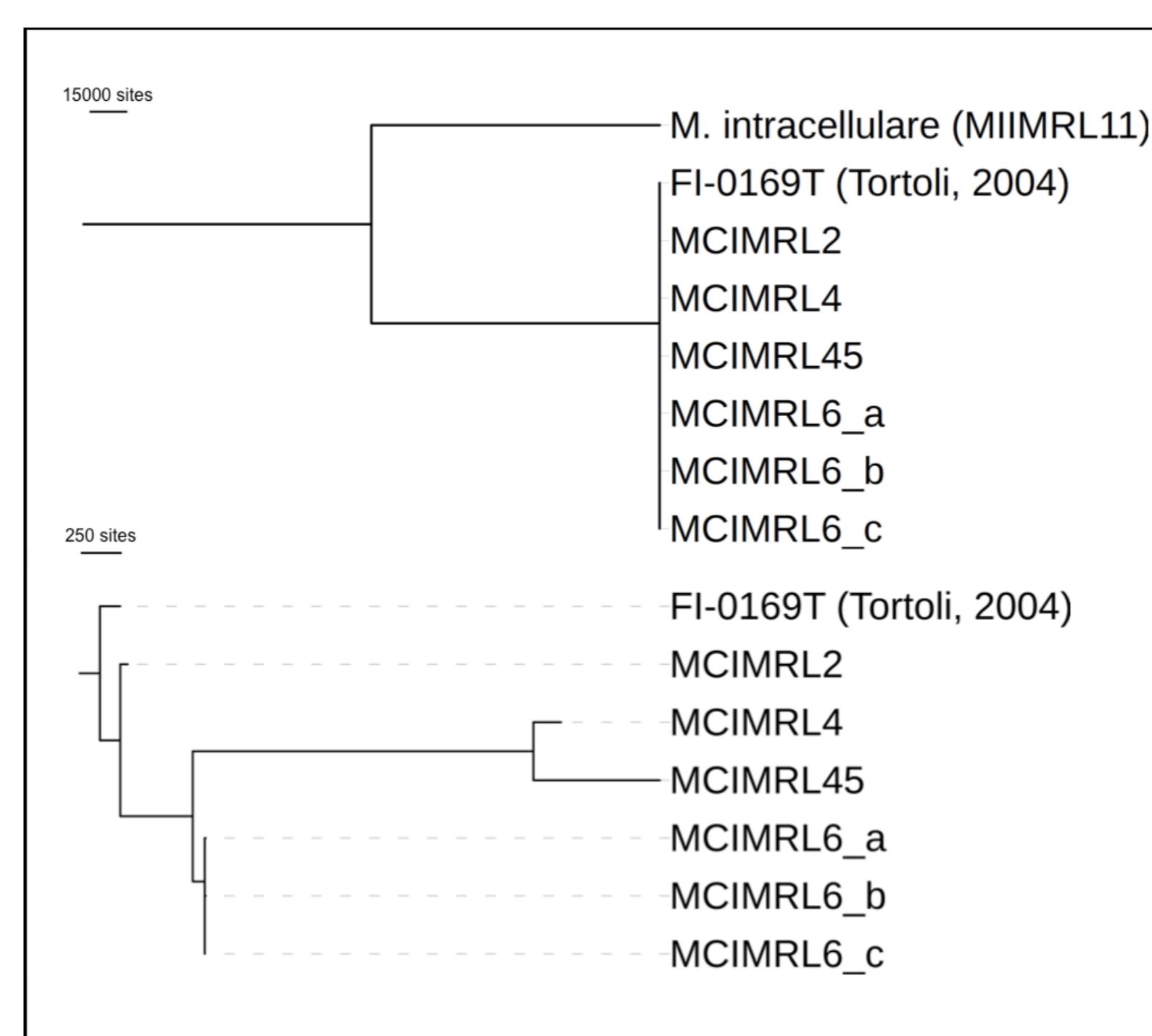


Figure 2. Evaluation and Interpretation of Results from Hain GenoType NTM-DR assay
All clinical isolates produced the expected banding pattern to the interpretation chart; *M. chimaera*: CC, UC, SP1 and SP2, *M. avium*: CC, UC, and SP1 and *M. intracellulare*: CC, UC and SP2. Drug resistance was not evaluated for MAC in the study.



Conclusions

- Differentiation of species within the MAC is important as *M. chimaera* is emerging as an important pathogen.
- Hain GenoType NTM-DR is a suitable commercial assay for the identification of *M. chimaera* for diagnostic laboratories.
- WGS of clinical *M. chimaera* isolates will be a useful reference as we are participating in the national investigation of *M. chimaera* infections associated with heater-cooler units in Ireland.....