

Identifying non-tuberculosis mycobacteria: Is it time to introduce new molecular assays?

To the Editor: Non-tuberculosis mycobacteria (NTM) are a group of >200 mycobacteria species and subspecies of acid-fast staining bacteria that are not members of the *Mycobacterium tuberculosis* complex (MTBC) or *M. leprae*.^[1] Although NTM species remain ubiquitous in the environment, their clinical significance as an emerging opportunistic infection is increasingly recognised, as they may cause pulmonary and extrapulmonary disease.^[2]

The Cape Town National Health Laboratory Service (NHLS) Tuberculosis (TB) laboratory received a sputum specimen collected in error in the community from a 69-year-old woman. On culture, this specimen had an unusual *Mycobacterium* line probe assay (LPA) banding pattern (Fig. 1). The microscopy (Ziehl Neelsen stain) showed acid-fast bacilli and no contamination. The presence or absence of bands at various sites on the LPA allows for NTMs to be identified and detected. Nevertheless, the banding pattern for this isolate could not be interpreted as the manufacturer did not specify an organism associated with the specific pattern observed.^[3]

To speciate this isolate, it was sent for Sanger sequencing (Central Analytical Facility, Stellenbosch University) of the *hsp65* (part of the heat shock protein family) and *rpoB* (encoding the β -subunit of RNA-polymerase) genes.^[4] The percentage sequence identities to *M. septicum* from the *hsp65* and *rpoB* genes were 98.88% and 100%, respectively.^[5] In addition, the clinical isolate's relationship to similar NTMs was shown in a phylogenetic tree based on the contigs of the *rpoB* gene (Fig. 2). Regardless of identifying the NTM, the finding remained coincidental, with no treatment or follow-up of the patient, since the NTM was not clinically relevant according to international criteria for pulmonary samples.^[10] This case demonstrates that distinguishing colonisation from NTM disease is challenging,^[10] and to our knowledge the literature is still awaiting a clinically significant *M. septicum* case from the African continent. A case report of *M. septicum* and a case series have been published in Asia^[11] and North America,^[12] respectively.

The assays currently used in South African public health laboratories to identify NTMs are the GenoType Mycobacterium CM and AS LPA (Hain Lifescience, Germany), which are commercial assays based on partially targeting the 23S rRNA sequence.^[4] The LPA technique is well received among laboratories, especially in resource-limited settings, owing to its simplicity in method and interpretation.^[4] However, these DNA hybridisation assays are limited in the spectrum of NTMs they can identify, and are not necessarily tailored for the most prevalent African species. Therefore

many NTMs are reported non-specifically as *Mycobacterium* species without further identification. A systematic review suggests that 29% of pulmonary NTMs from sub-Saharan Africa remained unidentified at the species level.^[13] This is also supported by unpublished data from the NHLS Cape Town TB laboratory, where 14% of 126 NTMs suspected to be clinically relevant in 2020 were documented as non-MTBC *Mycobacterium* species. Therefore, we conclude that new molecular methods, such as Sanger sequencing and whole genome sequencing,^[4] should be considered to overcome the current limitations in laboratory NTM identification that could impact clinical decision-making and patient outcome.

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Author contributions: CJO conceptualised the letter. TD interviewed the patient and requested additional investigations. SS and CJO reviewed and validated laboratory results. WG supervised the sequencing of the genes. All authors edited and approved the final letter.

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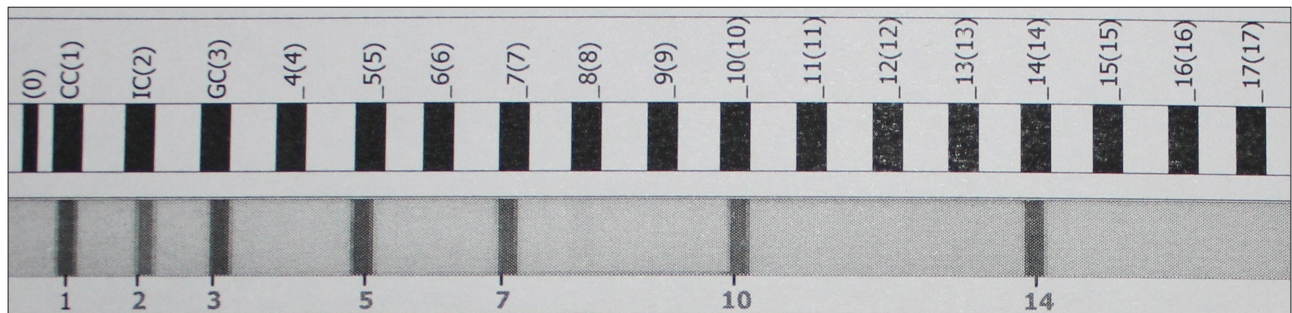


Fig. 1. Genotype Mycobacterium CM (Hain Lifescience, Germany) line probe assay based on a reverse hybridisation concept that allows amplicons to bind to probes linked to a nitrocellulose membrane strip. Bands are present for the conjugate (CC) and the internal control (IC), indicating the molecular assay performed correctly, and the genus control (GC) showing a member of the Mycobacterium group, as well as bands 5, 7, 10 and 14. Mycobacterium avium was used as the positive control, alongside a DNA template negative control. Sample processing, culturing, and the line probe assay were done at the National Health Laboratory Service, Green Point Tuberculosis Laboratory, Cape Town, South Africa.

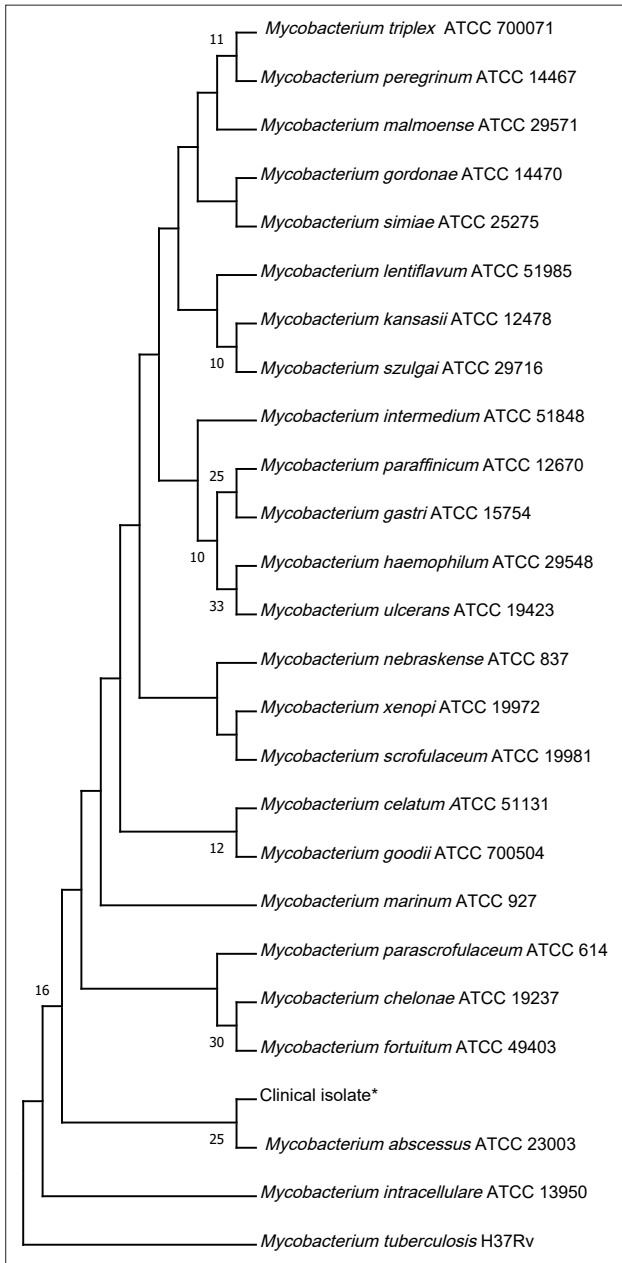


Fig. 2. The non-tuberculosis mycobacteria *rpoB* sequenced phylogenetic tree was inferred using the maximum likelihood method and Tamura-Nei model.^[6] Multiple sequence alignment was done with Multiple Sequence Comparison by Log-Expectation (MUSCLE).^[7] The bootstrap consensus was extrapolated from 500 replicates, and the analyses were conducted in Molecular Evolutionary Genetics Analysis (MEGA) version 11.^[8,9] Mycobacterium tuberculosis H37Rv was used to root the tree. (ATCC = American Type Culture Collection.)

*The position of the clinical isolate (*M. septicum*) is indicated among other non-tuberculosis mycobacteria found on the GenoType Mycobacterium CM and AS line probe assays.

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