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Combined approach to the identification of clinically infrequent non-tuberculous mycobacteria in Argentina

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SUMMARY

SETTING: Over 150 potentially pathogenic non-tuberculous mycobacteria (NTM) species have been described, posing an onerous challenge for clinical laboratory diagnosis.

OBJECTIVE: To evaluate different approaches for the identification of 40 clinically relevant NTM isolates whose species were not reliably identified using our routine diagnostic workflow comprising phenotypic tests and *hsp*65 polymerase chain reaction restriction analysis.

DESIGN: We used 1) sequencing analysis of four conserved gene targets: 16S rRNA, *rpo*B, *hsp*65 and *sod*A; 2) two commercial reverse hybridisation assays; and 3) protein analysis using matrix-assisted laser desorption/ionisation time of flight mass spectrometry (MALDI-TOF MS).

RESULTS: Combined, but not individual, sequence analysis allowed reliable species identification for 30/40 (75%) isolates, including species previously unknown to be circulating in Argentina. Commercial kits outperformed our routine identification in only 5/35 isolates, and misclassified many more. MALDI-TOF MS accurately identified species in 22/36 (61%) isolates and did not misidentify any.

CONCLUSIONS: Commercial kits did not resolve the problem of species of NTM isolates that elude identification. Combined DNA sequence analysis was the approach of choice. MALDI-TOF MS shows promise as a powerful, rapid and accessible tool for the rapid identification of clinically relevant NTM in the diagnostic laboratory, and its accuracy can be maximised by building up a customised NTM spectrum database.

KEY WORDS: mycobacteriosis; DNA sequence analysis; matrix-assisted laser desorption-ionization mass spectrometry (MALDI-TOF MS); DNA probes; nucleic acid hybridisation

CURRENT GENOMIC APPROACHES ALLOW increasing numbers of non-tuberculous mycobacteria (NTM) taxa to be identified, many of which are genetically close to each other, composing intricate groups or complexes. Over 150 mycobacterial species have been described that are potential human pathogens, posing a significant challenge for clinical laboratory diagnosis.[1,2] NTM infection, known as mycobacteriosis, is often associated with immunosuppression, comorbidity, or use of improperly sterilised substances or equipment in surgical and cosmetic procedures.[3,4] Given their environmental pervasiveness, occasional NTM isolation from non-sterile clinical specimens such as sputum samples does not allow us to presume the presence of mycobacteriosis. A comprehensive case-by-case approach is required, including clinical, imaging and microbiological assessment, to define the NTM responsible for a given medical condition.[5,6]

Accurate identification of NTM helps clinical management and is essential for assessing species distribution in a given epidemiological setting and elucidating the role of NTM in human disease. Most mycobacterial identification methods are now based on nucleic acid techniques. Sequencing of conserved genetic regions is the gold standard; widely used targets are 16S rDNA, *hsp65* and 16S–23S internal transcribed spacer (ITS). Other targets have also been proposed, such as *rpo*B, *gyr*A/B, *dna*J, *sod*A, *sec*A1 and *rec*A.[7,8] Due to the close links among mycobacteria, sequencing of a single DNA target does not always guarantee species discrimination.[2] Published

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data on the efficiency of individual targets are still limited and their performance has not been fully compared.[9–11] Commercial DNA hybridisation assays are available; however, these vary in accuracy and do not individually cover the whole spectrum of clinically relevant mycobacteria.[12–14] Matrix-assisted laser desorption ionisation–time-of-flight mass spectrometry (MALDI-TOF MS) has recently been introduced into clinical laboratories for microbial identification based on protein spectra.[15] This technology offers advantages for routine diagnostics, such as versatility, application in multiple pathogens, low cost of consumables and simple, rapid working protocols.[16]

Published data on the prevalence of NTM in Argentina are scarce.[17–19] According to our laboratory records, mycobacterioses represent ~1.3% of cases notified as having tuberculosis (TB). Following global trends, slowly growing mycobacteria (SGM) account for ~75% of diseases caused by NTM in humans in Argentina; members of the *Mycobacterium avium* complex and *M. kansasii* are the predominant species. Among rapidly growing mycobacteria (RGM), those most frequently associated with disease in humans are members of the *M. chelonae-abscessus* group and the *M. fortuitum* complex. Our laboratory (Instituto Nacional de Enfermedades Infecciosas–Administración Nacional de Laboratorios e Institutos de Salud [ANLIS]) acts as the reference laboratory for microbiological diagnosis of human TB and mycobacteriosis in Argentina. We perform mycobacterial identification based on phenotypic tests and polymerase chain reaction-restriction analysis (PRA) using the *hsp65* gene as target.[20,21] The former is time-consuming and insufficiently accurate, while the latter has limitations such as different species sharing identical restriction profiles. Approximately 20% of NTM isolates do not receive species assignation, and unidentified clinically relevant isolates are submitted for sequencing elsewhere.

The present study aimed to seek the best single or combined approach for the identification of a set of NTM clinical isolates whose species could not be unequivocally resolved in our laboratory. To this end, we used 1) sequencing of four genes, 16S rDNA, *rpo*B, *hsp65* and *sod*A; 2) two rapid commercial kits, Vircell Speed-Oligo® Mycobacteria (Vircell, Granada, Spain) and Hain GenoType Mycobacterium CM/AS (Hain Lifescience, Nehren, Germany); and 3) protein spectrum analysis using MALDI-TOF MS.

MATERIALS AND METHODS

Routine identification and isolate selection

Of ~1000 mycobacterial clinical isolates referred annually to our laboratory, ~200 are NTM. Phenotypic identification includes growth rate, growth temperature, growth in 5% sodium chloride Löwenstein-Jensen (LJ) medium, pigmentation and colony morphology, and the following biochemical tests: catalase (room temperature and 68°C), nitrate reductase, Tween 80 hydrolysis, arylsulfatase, urease, β-glucosidase, β-galactosidase, pyrazinamidase and iron uptake. PRA is based on polymerase chain reaction (PCR) amplification of a 441 base pair (bp) *hsp65* segment, followed by digestion using *Bst*EII and *Hae*III.[20,21] Restriction patterns are compared on PRAsite (http://app.chuv.ch/prasite/index.html).

We selected 40 NTM isolates representative of ambiguous phenotypic and/or PRA patterns, obtained from 39 clinically proven or highly suspicious cases of mycobacteriosis in 2002–2014. Culture, routine identification and the MALDI-TOF MS assay were performed at the Instituto Nacional de Enfermedades Infecciosas, ANLIS. Reverse hybridisation assays and gene sequencing were performed at Ghent University, Ghent, Belgium. Ethics committee approval and informed consent were not required because the work was retrospective and data were anonymised.

Sequencing

DNA was obtained by boiling lysis of bacilli freshly grown on LJ slants. Conventional PCRs were performed for partial amplification of 16S rRNA, *rpo*B, *hsp65* and *sod*A genes using primers depicted in Appendix Table A.1.*[9,20,22,23] Identical primers were used for sequencing PCR (BigDye Terminator Sequencing Kit, Applied Biosystems, Foster City, CA, USA), except for the 16S rRNA gene, in which case internal reverse primer BKL1 was used. Purified products (BigDye XTerminator kit, Applied Biosystems) were sequenced using the ABI Prism 3130 XL Genetic Analyzer (Applied Biosystems). Sequence assembly was performed with BioNumerics v. 7.0 (Applied Maths, Sint-Martens-Latem, Belgium). Targets were subjected to BLAST (Basic Local Alignment Search Tool) recognition in the following databases: National Center for Biotechnology Information (http://blast.ncbi.nlm.nih.gov/Blast.cgi), EzBioCloud (http://www.ezbiocloud.net/), and *Mycobacterium* 65 Kilodalton Heat Shock Protein Gene BLAST (http://hsp65blast.phsa.ca/blast/blast.html). To assign species, ⩾99.7% similarity to type strain sequences was required for 16S rRNA, ⩾97% for *rpo*B and ⩾99% for both *hsp65* and *sod*A.[9,23,24]

Reverse hybridisation assays

Speed-Oligo Mycobacteria (Vircell) consists of targeting 16S rDNA and the internal transcribed spacer in a double PCR, followed by reverse hybridisation with specific probes immobilised onto a dipstick and

* The appendix is available in the online version of this article, at http://www.ingentaconnect.com/content/iuatld/ijtld/2016/00000020/000000009/art00024

colour detection. The test reliably identifies *Mycobacterium* genus, *M. tuberculosis* complex and 13 NTM to species or complex level.[12,13]

GenoType® Mycobacterium CM/AS (Hain Lifescience), also based on DNA strip technology, uses 23S RNA as target. The procedure comprises multiplex amplification using biotinylated primers, reverse hybridisation and colour detection. The CM (common mycobacteria) component allows identification of *M. tuberculosis* complex and 13 NTM species or groups frequently isolated from clinical specimens. The AS (additional species) component allows identification of 16 less common NTM.[14]

All assays were performed following the manufacturers' instructions.

MALDI-TOF MS

Proteins were extracted as described, with minor modifications.[15] A loopful of bacilli freshly grown onto LJ slants was suspended in 0.5% Tween 80 distilled water, inactivated, and rinsed in 70% ethanol. The pellet was re-suspended in 100% formic acid and 0.1 mm zirconia-silica beads. After adding 100% acetonitrile, vortexing, and centrifuging, the supernatants were transferred into fresh tubes. Next, 1 µl was pipetted onto each spot on the MALDI-TOF plate and allowed to dry; then 1 µl of MALDI-TOF matrix was added and allowed to dry again. Spectra were analysed against the Bruker database v. 3.1 (Biotyper System; Bruker BioSciences Corporation, Billerica, MA, USA). Manufacturers recommend scores $\geqslant 2.0$ for optimal identification at the species level. On the basis of our experience with mycobacterial identification using MALDI-TOF MS, and that of others, we considered an isolate achieving a score $\geqslant 1.7$ to have been reliably identified, provided that the result was concordant with results of phenotypic tests and *hsp65* PRA.[25,26]

RESULTS

Sample characteristics and routine identification

Appendix Table A.2 shows characteristics of the 40 isolates. Phenotypic and PRA restriction profiles are presented in Appendix Table A.3.

Gene sequencing analysis

BLAST results are presented in Appendix Table A.4. Sequencing PCR failed to amplify one isolate in 16S rDNA, one in *rpo*B and three in *sod*A. As not all isolates attained the required proportion of similarities to type strain sequences, we asked for concordant results of at least two genes for species identification. Concordant species assignation of at least two gene sequences was achieved for 30/40 (75%) isolates, almost invariably involving 16S rDNA and *rpo*B, most *hsp65*, and less frequently all four gene targets. Only 11/37 (29.7%) isolates successfully sequenced for *sod*A were correctly identified to the species level. This gene was virtually unable to correctly identify *M. senegalense*, *M. lentiflavum*, *M. triplex* and *M. colombiense*. *M. senegalense* and *M. lentiflavum*, respectively, were the RGM and SGM species most frequently identified. *M. triplex* was initially identified using PRA in two sputum specimens obtained from the same patient 2 years apart and confirmed using combined gene analysis.

Reverse hybridisation assays

A total of 35/40 isolates were available for testing using both commercial kits. Speed-Oligo Mycobacteria identified 19 isolates up to the genus level and assigned mycobacterial species to 16. Only 4/16 were accurately classified according to consensus sequencing of two or more gene targets. GenoType Mycobacterium CM allowed species identification in 32 isolates, only five of which were correctly assigned according to consensus sequencing. GenoType AS contributed to the correct identification of six additional SGM isolates, and none of the RGM isolates. Overall, GenoType Mycobacterium CM/AS outperformed our identification routine in five isolates.

MALDI-TOF MS

Of 36 NTM isolates analysed, 22 (61%) were correctly identified, while 14 did not attain a reliable MALDI-TOF MS score. Using consensus sequence analysis, five isolates that were determined to be *M. senegalense*, a species not present in the Bruker database v. 3.1, were initially identified as the *M. farcinogenes*/*M. senegalense* group by MALDI-TOF MS. After introducing spectra of three sequencing-confirmed *M. senegalense* strains into our customised MALDI-TOF MS database, all five *M. senegalense* isolates in our study were correctly re-classified. MALDI-TOF MS did not discriminate between two pairs of species: *M. intracellulare*/*M. chimaera* and *M. mucogenicum*/*M. phocaicum*. The latter pair could not be differentiated using gene sequencing either.

Appendix Tables A.5 and A.6 show the results of consensus gene sequencing, hybridisation kits and MALDI-TOF MS for RGM and SGM, respectively, against our laboratory initial identification. The Figure summarises the main results for the 40 isolates.

DISCUSSION

Two commercial rapid assays with proven accuracy and ease of use performed poorly when applied to a set of clinically relevant NTM isolates eluding species assignation in our laboratory workflow. These kits cover a range of species representative of disease-related NTM that are globally prevalent.[12–14] How-

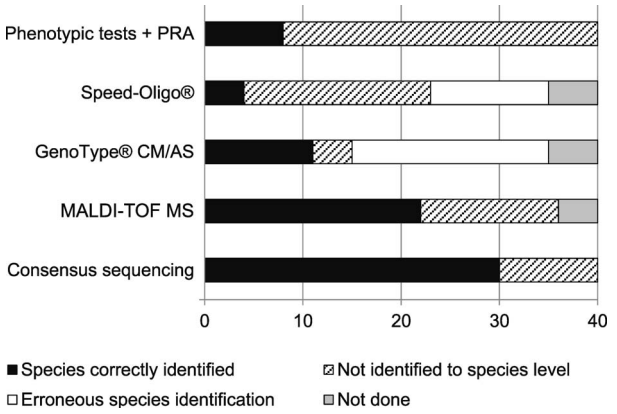

Figure Summary results of different identification approaches for 40 non-tuberculous mycobacterial isolates without definite species assignation in our laboratory, 2002–2014. PRA = polymerase chain reaction-restriction analysis; CM = common mycobacteria; AS = additional species; MALDI-TOF MS = matrix-assisted laser desorption/ionisation time of flight mass spectrometry.

ever, the mycobacteria accurately identified by these assays do not represent a diagnostic challenge to our medium-resource laboratory. As previously observed, these kits fail precisely when we challenge them to identify less frequent NTM species.[27] More importantly, they yield a number of erroneous species assignations.

When applied to our set of isolates, 16S rDNA, *rpo*B and *hsp65* were often in agreement in assigning species for both RGM and SGM. In line with previous reports, *sod*A sequence analysis did not add much certainty.[2,9] This is partially related to the fact that *sod*A sequences are not available for a number of NTM species in public databases (http://blast.ncbi.nlm.nih.gov/Blast.cgi). We failed to extend sequence analysis to other gene targets, in particular to ITS, due to difficulties in amplification (data not shown). A similar amplification failure might explain the poor performance of Speed-Oligo Mycobacteria in our isolates, as the assay is partly based on ITS.

Until whole-genome sequencing analysis becomes widely available for microbial identification, consensus sequencing results of at least two, and ideally three, conserved genetic regions seems to be a reliable approach for species determination in diagnostic settings such as ours. This criterion is intended not to yield strict taxonomic classifications, but to provide pragmatic, definite identification of clinical NTM isolates. It may, nevertheless, fail to distinguish between pairs of closely related species such as *M. phocaicum* and *M. mucogenicum*.

Most of the SGM in our study were associated with lung disease and belonged to three large, heterogeneous NTM groups that defy systematisation even today: *M. avium* complex, *M. simiae* complex and *M. terrae* complex. Only *M. simiae* and *M. sherrisii* were associated with disseminated disease in our series, associations that were previously recorded as infrequent in Argentina.[28] We were unable to assign a definite species to members of the *M. terrae* complex even after using combined sequencing analysis. We verified the presence of two SGM species previously unknown to be circulating in Argentina. One is *M. colombiense*, which we found to be associated with human immunodeficiency virus (HIV) infection; it is likely that this member of the *M. avium* complex[29] had not been previously reported in our laboratory due to the insufficient resolution of our diagnostic tools. The other is *M. triplex*, an emerging member of the *M. simiae* complex,[30] which was identified in two sputum specimens from a single HIV-negative patient with chronic lung disease. This is the only case of *M. triplex* definitely associated with human disease in our setting. In our PRA database, which contains over 10 000 patterns from Argentina recorded since 2003, only two other *M. triplex* PRA patterns are recorded, and both were occasional findings.

Our set of RGM isolates were more diverse in mycobacterial species and targeted more patient organs than SGM. Like the SGM, they included species previously unreported in Argentina, such as *M. mageritense* and *M. aubagnense*.[31,32] Four scotochromogens in this group were tenaciously refractory to identification by combined gene sequencing, even to the complex level. We hypothesise that these strains represent as yet unrecognised species which would have remained unidentified independently of the approach used. More than a third of the RGM in our study were involved in surgical complications, including *M. senegalense*, members of the *M. smegmatis* group, and the above-mentioned unclassified scotochromogens, which underlines the fact that NTM involved in surgical wound infections are not restricted to members of the *M. abscessus* complex. A remarkable finding is the isolation of *M. vanbaalenii*, an RGM closely related to *M. vaccae*,[33] in a patient with a rare recurrent meningeal syndrome. Regarding the involvement of *M. peregrinum* in human disease, in line with other authors, we were able to reliably assess its aetiological role in our pulmonary case but not in the case of urinary infection.[34] We did not detect any geographic association of the species identified in the study.

We have adapted the MALDI-TOF MS protocol to extract proteins directly from LJ slants to reduce costs and, most importantly, to detract as little as possible from our laboratory routine. The Bruker database v. 3.1, against which we analysed our spectra, is still deficient in terms of both the number of NTM species represented and the number of spectra per species. After incorporating *M. senegalense* into our customised spectra database, MALDI-TOF MS successfully identified species for >60% of the isolates in our study, and, most importantly, this method did not lead to any misclassification. This is a remarkable

performance for a particularly difficult-to-type set of isolates, second only to combined sequencing (Figure). Furthermore, MALDI-TOF MS analysis is open to continuous improvement. We are currently maximising its accuracy by building up a customised NTM database. Upon further examination, we are considering incorporating this tool into our diagnostic workflow. We envisage a modified algorithm to achieve a more accurate identification of NTM in Argentina. First, it will analyse PRA patterns and phenotypic characteristics such as pigmentation and growth rate. MALDI-TOF MS will then be used. Finally, in case of incongruent or undefined results, we will submit the isolate to 16S rDNA and *rpo*B sequencing. The increasing availability of NTM whole genome sequences will probably allow the selection of further conserved DNA regions suitable for species identification in the near future. If MALDI-TOF MS databases are expanded, requirements for gene analysis will eventually decrease in clinical laboratories, allowing faster and more accurate NTM species identification.

In conclusion, commercial DNA kits were not valid options for resolving the problem of specimens that elude NTM species identification in Argentina. Multiple, but not single, gene analysis is the approach of choice; however, this does not guarantee identification to the species level in every case. The systematic use of MALDI-TOF MS would allow swift and reliable species identification of clinically relevant NTM in the diagnostic laboratory, thereby substantially reducing the number of isolates requiring multiple gene sequencing or whole genome sequencing for species assessment.

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Conflicts of interest: none declared.

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APPENDIX

Appendix Table A.1 Primers used for PCR amplification and sequencing

Gene	Primers	Sequence (5′-3′)	PCR product size bp	Reference
16S rRNA				
	16S27F	AGAGTTTGATCMTGGCTCAG	921	22
	16S907R	CCGTCAATTCMTTTRAGTTT		
	BKL1*	GTATTACCGCGGCTGCTGGCA	860†	
*rpo*B				
	Myco-F	GGCAAGGTCACCCCGAAGGG	764	23
	Myco-R	AGCGGCTGCTGGGTGATCATC		
*hsp*65				
	Tb11	ACCAACGATGGTGTGTCCAT	441	20
	Tb12	CTTGTCGAACCGCATACCCT		
*sod*A				
	SodF	ACATCTCGGGTCAGATCAACGAGC	442	9
	SodR	GACGTTCTTGTACTGCAGGTA		

* Internal primer used for 16S rDNA sequencing PCR.
† Sequencing PCR product.
PCR = polymerase chain reaction; bp = base pair.

Appendix Table A.2 Characteristics of 40 non-tuberculous mycobacterial isolates in the study

Isolate no.	Year	Province	Specimen	Clinical condition, comorbidity	Consensus sequencing
53588	2002	Córdoba	Bronchial lavage	Lung disease	*M. lentiflavum*
53581	2002	Córdoba	Bronchial lavage	Lung disease	*M. lentilfavum*
53490	2003	Buenos Aires	Bronchial lavage	Lung disease	*M. lentilfavum*
53582	2003	Buenos Aires	Biopsy	Immunosuppression	*M. lentiflavum*
53465	2005	Buenos Aires	Ascitic fluid	Peritonitis, diabetes	Unclassified RGM
53466	2007	Buenos Aires	Skin nodule aspirate	Cosmetic surgery complication	*M. wolinskyi*
53467	2007	Córdoba	Bone marrow	Immunosuppression	*M. simiae*
53468	2008	Buenos Aires	Bronchial lavage	Immunosuppressive drug therapy	*M. intracellulare*
53469	2008	Buenos Aires	Cerebrospinal fluid	Recurrent meningeal syndrome	*M. vanbaalenii*
54466	2008	Buenos Aires	Bone marrow	Immunosuppressive drug therapy	Unclassified RGM
53491	2010	Corrientes	Bronchial lavage	Lung disease, SLE	*M. peregrinum*
53470	2010	Buenos Aires	Breast abscess aspirate	Breast implant infection	*M. senegalense**
53471	2010	Buenos Aires	Sputum	Chronic lung disease	*M. senegalense*
53472	2010	Córdoba	Wound drainage	Surgical wound infection	Unclassified RGM
53473	2010	Buenos Aires	Gut biopsy	Intestinal disorder	*M. terrae* complex
53474	2011	Buenos Aires	Breast abscess drainage	Breast implant infection	*M. wolinskyi*
53475	2011	Buenos Aires	Blood	Leukaemia	*M. neoaurum*
53494	2011	Buenos Aires	Dialysis AV fistula	Chronic renal failure	*M. aubagnense*
53476	2011	Córdoba	Abscess drain urine	Breast implant infection	Unclassified RGM
53477	2011	Córdoba	Urine	Urinary infection†	*M. peregrinum*
53493	2013	Buenos Aires	Leg ulcer	Immunosuppressive therapy	*M. chelonae*
54464	2012	Buenos Aires	Skin nodule aspirate	Subcutaneous nodule, HIV	*M. colombiense*
54465	2012	Córdoba	Bone marrow	Sepsis, HIV	*M. sherrisii*
53478	2012	Córdoba	Seroma	Ventral hernia repair complication	*M. senegalense*
53479	2012	Buenos Aires	Sputum	Chronic lung disease	*M. senegalense*
53480*	2012	Buenos Aires	Sputum	Chronic lung disease	*M. intracellulare*
53580	2012	Jujuy	Sputum	Lung disease	*M. intracellulare*
53483	2012	Córdoba	Sputum	Lung disease	*M. gordonae*
53492	2013	Buenos Aires	Abscess secretion	Breast implant infection	*M. senegalense*
53484	2013	Buenos Aires	Sputum	Lung disease	*M. simiae* complex
53485	2013	Buenos Aires	Bronchial lavage	Lung disease, HIV	*M. mageritense*
53587	2013	Salta	Sputum	Lung disease	*M. avium* complex
53495	2013	Córdoba	Wound drainage	Surgical wound infection	*M. smegmatis*
53496	2013	Buenos Aires	Blood	Sepsis, HIV	*M. mageritense*
53486	2013	Buenos Aires	Gut biopsy	HIV	*M. terrae* complex
53487*	2014	Buenos Aires	Sputum	Chronic lung disease	*M. triplex*
53497	2014	Buenos Aires	Sputum	Lung disease	*M. terrae* complex
53488	2014	Buenos Aires	Sputum	Lung disease, HIV	*M. colombiense*
54467	2014	Buenos Aires	Sputum	Lung disease, HIV	*M. phocaicum/mucogenicum*
53498	2014	Córdoba	Sputum	Lung disease	*M. terrae* complex

* Two of five isolates obtained from one patient between 2010 and 2015.
† Aetiological association not confirmed.
RGM = rapidly growing mycobacteria; SLE = systemic lupus erythematosus; AV = arteriovenous; HIV = human immunodeficiency virus.

Table A.3 Phenotypic/biochemical test and PRA identification

	PRA identification		
ID no	*Bst*EII fragments	*Hae*III fragments	Indenification according to PRA site (score)
M. fortuitum complex			
53470	235/120/85	140/125/100/55	(>10)
53471	235/120/85	140/125/100/55	(>10)
53478	235/130/85	140/125/100/55	*M. gordonae* type 6 (7)
53479	235/120/85	140/125/100/55	(>10)
53492	235/120/85	140/125/100/55	(>10)
53485	235/130/85	145/125/60	*M. goodii* type 1, *M. mageritense* type 1, *M. smegmatis* type 1 (0)
53496	235/130/85	145/125/60	*M. goodii* type 1, *M. mageritense* type 1, *M. smegmatis* type 1 (0)
53477	235/130/85	140/100/60	(>10)
53491	235/210/0	140/100/60	*M. kubicae* type 1 (0)
M. smegmatis group			
53466	235/130/85	140/125/60	*M. senegalense* type 2, *M. wolinskyi* type 1 (0)
53474	235/130/85	140/90/60	(>10)
53495	235/130/85	145/125/60	*M. goodii* type 1, *M. mageritense* type 1, *M. smegmatis* type 1 (0)
M. mucogenicum group			
53494	320/115	145/80/60	*M. aubagnense* type 1 (0)
54467	235/120/85	140/65/60	(>10)
M. chelonae-abscessus group			
53493			No amplification
Other RGM			
53469	235/210/0	160/60/55	*M. vanbaalenii* type 1, *M. astroafricanum* type 1 (5)
53475	320/115/0	170/140/0	*M. parafortuitum* type 2, *M. neoaurum* type 1 (0)
Unclassified RGM			
53465	440/0/0	140/90/75/55	(>10)
53472	440/0/0	140/90/75/55	(>10)
54466	235/120/85	140/90/60	(>10)
53476	440/0/0	145/85/60	*M. parafortuitum* type 1, *M. komosense* type 1 (7)
M. simiae complex			
53588	440/0/0	145/130/0	*M. lentiflavum* type 1 (0), *M. simiae* type 5 (0), *M. florentinum* type 1 (0)
53581	440/0/0	145/130/0	*M. lentiflavum* type 1 (0), *M. simiae* type 5 (0), *M. florentinum* type 1 (0)
53490	440/0/0	145/130/0	*M. lentiflavum* type 1 (0), *M. simiae* type 5 (0), *M. florentinum* type 1 (0)
53582	440/0/0	145/130/0	*M. lentiflavum* type 1 (0), *M. simiae* type 5 (0), *M. florentinum* type 1 (0)
53467	235/210/0	185/130/0	*M. genavense* type 2, *M. simiae* type 1 (0)
54465	235/210/0	145/130/40	*M. avium* type 3, *M. intracellulare* type 3, *M. simie* type 6, *M. interjectum* type 1, *M. intermedium* type 1 (0)
53480*	320/115/0	145/130/50	*M. triplex* type 1 (0)
53487*	320/115/0	145/130/50	*M. triplex* type 1 (0)
53484	440/0/0	130/115	(>10)
M. avium complex			
53468	235/120/100	145/130/0	*M. lentiflavum* type 3 (0)
53580	235/120/100	130/95/60/50	*M. intracellulare* type 5 (5)
54464	235/210/0	145/130/0	*M. saskatchewanense* type 1, *M. seoulense* type 1, *M. simie* type 6, *M. intracellulare* type 3, *M. interjectum* type 1, *M. intermedium* type 1, *M. avium* type 3 (0)
53488	440/0/0	130/105/0	(>10)
53587	235/210/0	145/130/0	*M. saskatchewanense* type 1, *M. seoulense* type 1, *M. simie* type 6, *M. intracellulare* type 3, *M. interjectum* type 1, *M. intermedium* type 1, *M. avium* type 3 (0)
M. terrae complex			
53473	320/115/0	140/100/85	(>10)
53486	325/120	140/110/100/90	(>10)
53497	320/115/0	140/100/55	(>10)
53498	320/115/0	185/140/0	*M. terrae* type 2 (0)
Other SGM			
53483	320/115/0	130/115/0	(>10)

* Two of five isolates obtained from the same patient in 2010–2015, all yielding identical phenotypical/PRA identification.
PRA = polymerase chain reaction-restriction enzyme analysis; NaCl = sodium chloride; RT = real time; RGM = rapidly growing mycobacteria; + = positive; ND = not determined; − = negative; SGM = slowly growing mycobacteria.

Table A.3 *(continued)*

				Biochemical tests				
Rate of growth	Pigment production	Colony morphology	Growth in the presence of 5% NaCl	Catalase RT	Catalase 68°C	Nitrate reductase	Tween 80 hydrolysis	Urease
RGM	Non-chromogen	Smooth	+	+	+	+	+	+
RGM	Non-chromogen	Smooth	ND	ND	ND	ND	ND	ND
RGM	Non-chromogen	Smooth	+	+	+	+	+	–
RGM	Non-chromogen	Smooth	–	+	+	+	+	+
RGM	Non-chromogen	Smooth	+	+	+	+	+	+
RGM	Non-chromogen	Smooth	+	+	+	+	–	+
RGM	Non-chromogen	Smooth	+	+	+	–	+	+
RGM	Non-chromogen	Smooth	–	+	+	+/–	+	+/–
RGM	Non-chromogen	Smooth	+	+	+	+	+/–	+
RGM	Non-chromogen	Smooth	ND	+	+	+	+	+
RGM	Non-chromogen	Smooth	ND	ND	ND	ND	ND	ND
RGM	Non-chromogen	Smooth	+	+	+	+	+	+
RGM	Non-chromogen	Smooth	–	+	–	–	+	+
RGM	Non-chromogen	Smooth	–	+	+	–	–	+
RGM	Non-chromogen	Smooth	–	+	+	+	+/–	–
RGM	Scotochromogen	Smooth	ND	+	+	+	+	+
RGM	Scotochromogen	Smooth	ND	+	+	–	+	+
RGM	Scotochromogen	Smooth	–	+	+	+	+	+
RGM	Scotochromogen	Smooth	ND	ND	ND	ND	ND	ND
RGM	Scotochromogen	Smooth	–	+	–	–	+	+
RGM	Scotochromogen	Smooth	ND	+	+	+	+	+
SGM	Scotochromogen	Smooth	ND	+	+	–	–	–
SGM	Scotochromogen	Smooth	ND	+	+	–	–	–
SGM	Scotochromogen	Smooth	ND	+	+	–	–	–
SGM	Scotochromogen	Smooth	–	+	+	–	–	–
SGM	Non chromogen	Smooth	ND	+	+	–	–	–
SGM	Non chromogen	Smooth	–	+	+	–	–	–
SGM	Non-chromogen	Smooth	–	+	+	–	–	+
SGM	Non-chromogen	Smooth	–	+	+	+	–	+
SGM	Non-chromogen	Smooth	ND	+	+	–	–	+
SGM	Non-chromogen	Smooth	ND	+	+	–	–	–
SGM	Non-chromogen	Smooth	–	+	+	–	+	–
SGM	Non-chromogen	Smooth	–	+	+	–	–	–
SGM	Non-chromogen	Smooth	ND	+	+	–	–	–
SGM	Non-chromogen	Smooth	ND	ND	ND	ND	ND	ND
SGM	Non-chromogen	Smooth	–	+	+	+/–	+	–
SGM	Non-chromogen	Smooth	–	+	+	+/–	+	–
SGM	Non-chromogen	Smooth	–	+	+	+	+	–
SGM	Non-chromogen	Smooth	–	+	+	+	+	–
SGM	Scotochromogen	Smooth	–	+	+	+	+	–

Table A.3 *(continued)*

Biochemical tests						
β-glucosidase	β-galactosidase	Acid phosphatase	Pyrazinamidase	Iron uptake	Arylsulfatase	Consensus phenotypic tests and PRA
+	+	ND	ND	−	+	Non-chromogen RGM
ND	ND	ND	ND	ND	ND	Non-chromogen RGM
+	−	ND	+	+	+	Non-chromogen RGM
+	+	ND	+	−	+	Non-chromogen RGM
+	+	ND	+	−	+	Non-chromogen RGM
+	ND	ND	ND	+	+	Non-chromogen RGM
+	ND	ND	+	+	+	Non-chromogen RGM
ND	+	ND	+	−	+	Non-chromogen RGM
ND	ND	ND	ND	+	+	*M. fortuitum* complex
+	+		+	+	−	Non-chromogen RGM
ND	ND	ND	ND	ND	ND	Non-chromogen RGM
+	+	ND	+	+	−	*M. smegmatis* group
ND	−	ND	+	−	+	*M. mucogenicum* group
−	+	ND	ND	−	+	*M. mucogenicum* group
ND	+	ND	+	−	+	*M. abscessus* group
−	−	ND	+	+	−	Scotochromogen RGM
ND	+	ND	+	+	+	*M. neoaurum/M. parafortuitum*
+	+	ND	+	−	−	Scotochromogen RGM
ND	ND	ND	ND	ND	ND	Scotochromogen RGM
ND	ND	ND	ND	+	+	Scotochromogen RGM
ND	−	ND	+	−	−	Scotochromogen RGM
−	ND	−	+	ND	ND	*M. lentiflavum*
−	−	−	+	ND	ND	*M. lentiflavum*
−	−	−	+	ND	ND	*M. lentiflavum*
−	−	−	−	ND	ND	*M. lentiflavum*
−	ND	ND	+	ND	ND	*M. avium* complex
−	−	ND	+	ND	ND	*M. avium* complex
ND	−	ND	ND	−	−	*M. triplex*
+	+	ND	ND	−	+	*M. triplex*
−	−	ND	+	−	−	Non-chromogen SGM
−	−	ND	+	ND	ND	*M. avium* complex
ND	−	ND	+	−	−	*M. intracellulare*
−	ND	ND	−	ND	ND	*M. avium* complex
−	−	ND	+	−	−	*M. avium* complex
ND	ND	ND	ND	ND	ND	*M. avium* complex
ND	+	ND	ND	ND	+	*M. terrae* complex
+	+	ND	+	−	−	*M. terrae* complex
−	+	ND	+	−	−	*M. terrae* complex
ND	+	ND	−	−	−	*M. terrae*
ND	−	ND	+	−	−	Scotochromogen SGM

Table A.4 BLAST results and consensus sequence identification

	NCBI BLAST analysis (% identity)	
ID n°	16S rDNA	*rpo*B
M. fortuitum complex		
53470	*M. senegalense* IEMVT 378T (99)	*M. senegalense* ATCC 35796T (99.4)
53471	*M. senegalense* IEMVT 378T (99)	*M. senegalense* ATCC 35796T (99.4)
53478	*M. senegalense* IEMVT 378T (99)	*M. senegalense strain* ATCC 35796T (99)
53479	*M. senegalense* IEMVT 378T (99)	*M. senegalense strain* ATCC 35796T (95), *M. conceptionense* CIP 108544T (95)
53492	*M. senegalense* IEMVT 378T (99)	*M. senegalense* ATCC 35796T (99)
53485	*M. mageritense* CIP 104973T (100)	*M. mageritense* ATCC 700351T (99)
53496	*M. mageritense* CIP 104973T (99.8)	*M. mageritense* ATCC 700351T (99)
53477	*M. peregrinum* CIP 105382T (100), *M. septicum* DSM 44393T (100)	*M. peregrinum* ATCC 14467T (98)
53491	*M. peregrinum* CIP 105382T (99.7)	*M. peregrinum* ATCC 14467T (99)
M. smegmatis group		
53466	*M. wolinskyi* ATCC 700010T (100)	*M. wolinskyi* ATCC 700010T (99)
53474	*M. wolinskyi* ATCC 700010T (100)	*M. wolinskyi* ATCC 700010T (99)
53495	*M. smegmatis* ATCC 19420T (100)	*M. smegmatis* ATCC 19420T (99)
M. mucogenicum group		
53494	*M. aubagnense* CIP 108543T (99.5)	*M. aubagnense* CIP 108543T (96)
54467	*M. mucogenicum* ATCC 49651T (99)	*M. phocaicum strain* CIP 108542T (99)
M. chelonae-abscessus group		
53493	*M. chelonae* JCM 6388T (100)	*M. chelonae* CIP 104535T (99)
Other RGM		
53469	*M. vanbaalenii* PYR-1T (99.8)	*M. vanbaalenii* PYR-1T (99)
53475	*M. neoaurum* ATCC 25795T (99.9)	*M. neoaurum* ATCC 25795T (99)
Unclassified RGM		
53465	*M. duvalii* ATCC 43910T (98.8)	*M. vanbaalenii* PYR-1T (93)
53472	*M. duvalii* 43910T (98.8)	*M. vanbaalenii* PYR-1T (93), *M. smegmatis* ATCC 19420 (93)
54466	*M. goodii strain* ATCC 700504T (98.7)	*M. vanbaalenii* PYR-1T (92)
53476	*M. moriokaense* CIP 105393T (98)	*M. flavescens* CIP 104533T (96)
M. simiae complex		
53588	*M. lentiflavum* ATCC 51985T (99.8)	*M. lentiflavum* ATCC 51985T (99.8)
53581	*M. lentiflavum* ATCC 51985T (100)	*M. lentiflavum* ATCC 51985T (100)
53490	*M. lentiflavum* ATCC 51985T (100)	*M. lentiflavum* ATCC 51985T (99)
53582	*M. lentiflavum* ATCC 51985T (100)	*M. lentiflavum* ATCC 51985T (100)
53467	*M. simiae* ATCC 25275T (99.5)	*M. simiae* ATCC 25275T (99)
54465	*M. sherrisii* 4773T (99.9)	*M. sherrisii* ATCC BAA823 (99.7)
53480*	ND	*M. triplex* ATCC 70071T (99)
53487*	*M. triplex* HF 8705T (100)	*M. triplex* ATCC 70071T (99)
53484†	*M. simiae* ATCC 25275T (99.7)	*M. parmense* CIP 107385T (92)
M. avium complex		
53468	*M. intracellulare* ATCC 13950T (99.8)	*M. intracellulare* ATCC 13950T (99)
53580	*M. intracellulare* ATCC 13950T (100)	*M. intracellulare* ATCC 13950T (99)
54464	*M. colombiense* CIP 108962T (99.8), *M. bouchedurhonense* 4355387T (99.8)	*M. colombiense* CIP 108962T (99)
53488	*M. colombiense* CIP 108962T (99.8), *M. bouchedurhonense* 4355387T (99.8)	*M. colombiense* CIP108962T (97)
53587	*M. colombiense* CIP 108962T (99.8), *M. bouchedurhonense* 4355387T (99.8)	*M. chimaera* CIP107892T (97) *M. intracellulare* ATCC 13950T (97)
M. terrae complex		
53473	*M. arupense* AR30097 T (99.3)	*M. nonhromogenicum* ATCC 19530T (97)
53486	*M. arupense* AR30097T (99.3)	*M. nonchromogenicum* ATCC 1 9530T (97)
53497	*M. kumamotonense* CST 7274T (99.1)	*M. senuense* DSM 44999T (94)
53498	*M. senuense* 05-832T (99.1)	Failed
Other SGM		
53483	*M. gordonae* ATCC 14470T (99.8)	*M. gordonae* ATCC 14470T (97)

* Two of five isolates obtained from the same patient in 2010–2015, all yielding identical phenotypical/PRA identification.
† 16S RNA is 100% identical to *M. shigaense*, a species not officially recognised; however, the other genes do not match this identification. 1 cut-off: >99,7%; 2 cut-off: >97%; 3 cut-off: >99%; 4 cut-off: >99%.
BLAST = Basic Local Alignment Search Tool; NCBI = National Center for Biotechnology Information; CM = common mycobacteria; AS = additional species; MALDI-TOF MS = Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; IEMVT = Institute for Tropical Animal Production and Veterinary Research, Maisons Alfort, France; ATCC = American Type Culture Collection; CIP = Collection Institut Pasteur; ND = not determined; RGM = rapidly growing mycobacteria; SGM = slowly growing mycobacteria.

Table A.4 *(continued)*

NCBI BLAST analysis (% identity)		Consensus sequence identification
hsp65	*sod*A	
M. senegalense ATCC 35796T (99), *M conceptionense* CIP 108544 (99)	*M. conceptionense* CIP 108544T (99)	*M. senegalense*
M. senegalense ATCC 35796T (99), *M. conceptionense* CIP 108544 (99)	*M. conceptionense* (99)	*M. senegalense*
M. senegalense ATCC BAA849 (100), *M. conceptionense* CIP 108544T (99)	*M. conceptionense* CIP 108544T (99)	*M. senegalense*
M. neworleansense ATCC 49404T (96)	*M. senegalense* ATCC 35796T (95)	*M. senegalense*
M. senegalense CIP 108544T (99)	*M. conceptionense* CIP 108544T (99)	*M. senegalense*
M. mageritense CIP 104973T (99)	*M. mageritense* CIP 104973T (100)	*M. mageritense*
M. mageritense CIP 104973T (100)	*M. mageritense* CIP 104973T (99)	*M. mageritense*
M. peregrinum CIP 105382T (99)	*M. peregrinum* CIP 105382T (99)	*M. peregrinum*
M. peregrinum CIP 105382T (100)	*M. peregrinum* CIP 105382T (99)	*M. peregrinum*
M. wolinskyi ATCC_700009T (99)	*M. wolinskyi* ATCC 700010T (99)	*M. wolinskyi*
M. wolinskyi CIP 106348T (99)	*M. wolinskyi* (99) ATCC 700009T	*M. wolinskyi*
M. smegmatis CIP 104444T (99)	*M. smegmatis* (99)	*M. smegmatis*
M. aubagnense CIP 108543T (99)	*M. aubagnense* CIP 108543T (98)	*M. aubagnense*
M. mucogenicum ATCC 49650T (100)	*M. mucogenicum* ATCC 49651T (97)	*M. phocaicum/mucogenicum*, *M. mucogenicum*
M. chelonae ATCC 19237T (100)	*M. chelonae* CIP 104535T (99)	*M. chelonae*
M. vanbaalenii PYR-1T (100)	*M. nonchromogenicum* DSM 44164T (92)	*M. vanbaalenii*
M. neoaurum ATCC 25795T (99)	*M. neoaurum* CIP 105387T (98)	*M. neoaurum*
M. boenickei CIP 107829T (94), *M. septicum strain* DSM 44393T (94), *M. peregrinum* CIP 105382T (94)	*M. avium subsp. avium strain* ATCC 25291T (99)	Unclassified RGM
M. pyrenivorans DSM 44605T (94)	*M. nonchromogenicum* DSM 44164T (94)	Unclassified RGM
M. flavescens CIP 104533T (96)	*M. immunogenum* CIP 106684T (95)	Unclassified RGM
M. novocastrense CIP 105546T (96)	*M. flavescens* CIP 104533T (92), *M. novocastrense* CIP 105546T (91)	Unclassified RGM
M. lentiflavum CIP 105465T (100)	*M. florentinum* DSM 44852T (94)	*M. lentiflavum*
M. lentiflavum CIP 105465T (100)	*M. florentinum* DSM 44852T (94)	*M. lentiflavum*
M. lentiflavum CIP 105465T (99)	*M. florentinum* DSM 44852T (92)	*M. lentiflavum*
M. lentiflavum CIP 105465T (100)	*M. florentinum* DSM 44852T (94)	*M. lentiflavum*
M. simiae ATCC 25275T (92)	*M. simiae* CIP 104531T (99)	*M. simiae*
M. avium ATCC 25291T (98), *M. colombiense* CIP 108962T (98)	*M. avium* ATCC 19698T (99)	*M. sherrisii*
M. triplex ATCC 70071T (100)	*M. florentinum* DSM 44852T (94)	*M. triplex*
M. triplex ATCC 70071T (100)	*M. florentinum* DSM 44852T (92)	*M. triplex*
M. nebraskense ATCC BAA-837T (96)	*M. gordonae* CIP 104529T (90)	*M. simiae complex*
M. intracellulare ATCC 13950T (100)	*M. intracellulare* ATCC 13950T (100)	*M. intracellulare*
M. intracellulare ATCC 13950T (99)	Failed	*M. intracellulare*
M. colombiense CIP 108962T (99)	Failed	*M. colombiense*
M. colombiense CIP108962T (99)	*M. intracellulare* ATCC 13950T (94)	*M. colombiense*
M. avium ATCC 25291T (99)	*M. nonchromogenicum SM 44164T (92)*	*M. avium* complex
M. engbaekii ATCC 27353T (97)	*M. nonchromogenicum* DSM 44164T (94)	*M. terrae* complex
M. engbaekii ATCC 27353T (98)	*M. nonchromogenicum* DSM 44164T (94)	*M. terrae* complex
M. senuense DSM 44999T (97)	Failed	*M. terrae* complex
M. algericum DSM 45454T (99)	*M. senuense* DSM 44999T (90)	*M. terrae* complex
M. gordonae MS460T (98)	*M. gordonae* CIP 104529T (99)	*M. gordonae*

Table A.4 *(continued)*

Speed-Oligo®	GenoType® CM/AS	MALDI-TOF MS	Clinical specimen
M. fortuitum	*M. fortuitum*	*M. senegalense**	Cosmetic surgery drainage
M. fortuitum	*M. fortuitum*	*M. senegalense**	Sputum
M. fortuitum	*M. fortuitum*	*M. senegalense**	Ventral hernia seroma
M. abscessus	*M. fortuitum*	*M. senegalense**	Sputum
M. fortuitum	*M. fortuitum*	*M. senegalense**	Cosmetic surgery drainage
M. fortuitum	*M. fortuitum*	*M. mageritense*	Bronchoalveolar lavage
M. fortuitum	*M. fortuitum*	*M. mageritense*	Blood
M. fortuitum	*M. peregrinum*	ND	Urine
M. peregrinum	*M. peregrinum*	*M. peregrinum*	Bronchoalveolar lavage
Mycobacterium sp.	*M. fortuitum*	*M. wolinskyi*	Cosmetic surgery drainage
Mycobacterium sp.	*M. intermedium*	*M. wolinskyi*	Cosmetic surgery drainage
Mycobacterium sp.	*M. fortuitum*	*M. smegmatis*	Surgical wound drainage
Mycobacterium sp.	*M. intermedium*	ND	Blood
ND	ND	*M. phocaicum/M. mucogenicum*	Sputum
M. chelonae	*M. chelonae*	ND	Leg ulcer
M. fortuitum	*M. intermedium*	*M. vanbaalenii*	Cerebrospinal fluid
Mycobacterium sp.	*M. intermedium*	*M. neoaurum*	Blood
Mycobacterium sp.	*Mycobacterium* sp.	Score <1.7	Ascitic fluid
Mycobacterium sp.	*M. intermedium*	Score <1.7	Surgical wound drainage
ND	ND	Score <1.7	Bone marrow
Mycobacterium sp.	*Mycobacterium* sp.	Score <1.7	Cosmetic surgery drainage
Mycobacterium sp.	*M. lentiflavum*	Score <1.7	Bronchoalveolar lavage
Mycobacterium sp.	*M. lentiflavum*	Score <1.7	Bronchoalveolar lavage
Mycobacterium sp.	*M. lentiflavum*	Score <1.7	Bronchoalveolar lavage
ND	ND	Score <1.7	Lymph node biopsy
Mycobacterium sp.	*M. simiae*	*M. simiae*	Bone marrow
ND	ND	*M. sherrisii*	Bone marrow
Mycobacterium sp.	*M. triplex/M. genavense*	*M. triplex*	Sputum
Mycobacterium sp.	*M. triplex/M. genavense*	*M. triplex*	Sputum
Mycobacterium sp.	*Mycobacterium* sp.	Score <1.7	Sputum
M. intracellulare	*M. intracellulare*	*M. chimaera/M. intracellulare*	Bronchoalveolar lavage
M. avium	*M. intracellulare*	*M. chimaera/M. intracellulare*	Sputum
ND	ND	Score <1.7	Subcutaneous abscess
M. intracellulare	*M. intracellulare*	*M. colombiense*	Sputum
M. intracellulare	*M. intracellulare*	ND	Sputum
Mycobacterium sp.	*M. intermedium*	Score <1.7	Gut biopsy
Mycobacterium sp.	*M. intermedium*	Score <1.7	Gut biopsy
Mycobacterium sp.	*M. intermedium*	Score <1.7	Sputum
Mycobacterium sp.	*M. intermedium*	Score <1.7	Sputum
M. gordonae	*M. xenopi*	*M. gordonae*	Sputum

Table A.5 Identification of 21 RGM according to different identification approaches

Group isolate no.	Consensus sequencing	Speed-Oligo®	GenoType® CM/AS	MALDI-TOF MS	Phenotypic tests and PRA
M. fortuitum complex					
53470	*M. senegalense*	*M. fortuitum*	*M. fortuitum*	*M. senegalense**	Non-chromogen RGM
53471	*M. senegalense*	*M. fortuitum*	*M. fortuitum*	*M. senegalense**	Non-chromogen RGM
53478	*M. senegalense*	*M. fortuitum*	*M. fortuitum*	*M. senegalense**	Non-chromogen RGM
53479	*M. senegalense*	*M. abscessus*	*M. fortuitum*	*M. senegalense**	Non-chromogen RGM
53492	*M. senegalense*	*M. fortuitum*	*M. fortuitum*	*M. senegalense**	Non-chromogen RGM
53485	*M. mageritense*	*M. fortuitum*	*M. fortuitum*	*M. mageritense*	Non-chromogen RGM
53496	*M. mageritense*	*M. fortuitum*	*M. fortuitum*	*M. mageritense*	Non-chromogen RGM
53491	*M. peregrinum*	*M. peregrinum*	*M. peregrinum*	*M. peregrinum*	*M. fortuitum* complex
53477	*M. peregrinum*	*M. fortuitum*	*M. peregrinum*	ND	Non-chromogen RGM
M. smegmatis group					
53466	*M. wolinsky*	*Mycobacterium* sp.	*M. fortuitum*	*M. wolinsky*	Non-chromogen RGM
53474	*M. wolinsky*	*Mycobacterium* sp.	*M. intermedium*	*M. wolinsky*	Non-chromogen RGM
53495	*M. smegmatis*	*Mycobacterium* sp.	*M. fortuitum*	*M. smegmatis*	*M. smegmatis group*
M. mucogenicum group					
53494	*M. aubagnense*	*Mycobacterium* sp.	*M. intermedium*	ND	*M. mucogenicum* group
54467	*M. phocaicum/ mucogenicum*	ND	ND	*M. phocaicum/ mucogenicum*	*M. mucogenicum* group
M. chelonae-abscessus group					
53493	*M. chelonae*	*M. chelonae*	*M. chelonae*	ND	*M. abscessus* group
Other					
53469	*M. vanbaalenii*	*M. fortuitum*	*M. intermedium*	*M. vanbaalenii*	Scotochromogen RGM
53475	*M. neoaurum*	*Mycobacterium* sp.	*M. intermedium*	*M. neoaurum*	*M. neoaurum/parafortuitum*
53465	Unclassified RGM	*Mycobacterium* sp.	*Mycobacterium sp.*	Score <1.7	Scotochromogen RGM
54466	Unclassified RGM	ND	ND	Score <1.7	Scotochromogen RGM
53472	Unclassified RGM	*Mycobacterium sp.*	*M. intermedium*	Score <1.7	Scotochromogen RGM
53476	Unclassified RGM	*Mycobacterium sp.*	*Mycobacterium sp.*	Score <1.7	Scotochromogen RGM

* Initially *M. farcinogenes/M. senegalense* group according to Bruker database v 3.1, subsequently adjusted to *M. senegalense* in our customised database.
RGM = rapidly growing mycobacteria; CM = common mycobacteria; AS = additional species; MALDI-TOF MS = matrix-assisted laser desorption/ionisation time of flight mass spectrometry; PRA = polymerase chain reaction-restriction analysis; ND = not determined.

Table A.6 Identification of 19 SGM according to combined gene sequence analysis, two commercial hybridisation kits, MALDI-TOF MS, and our laboratory workflow

Group isolate no.	Consensus sequencing	Speed-Oligo®	GenoType® CM/AS	MALDI-TOF MS	Phenotypic tests and PRA
M. simiae complex					
53588	*M. lentiflavum*	*Mycobacterium* sp.	*M. lentiflavum*	Score <1.7	*M. lentiflavum*
53581	*M. lentiflavum*	*Mycobacterium* sp.	*M. lentiflavum*	Score <1.7	*M. lentiflavum*
53490	*M. lentiflavum*	*Mycobacterium* sp.	*M. lentiflavum*	Score <1.7	*M. lentiflavum*
53582	*M. lentiflavum*	ND	ND	Score <1.7	*M. lentiflavum*
53467	*M. simiae*	*Mycobacterium* sp.	*M. simiae*	*M. simiae*	*M. avium* complex
54465	*M. sherrisii*	ND	ND	*M. sherrisii*	*M. avium* complex
53487*	*M. triplex*	*Mycobacterium* sp.	*M. triplex/genavense*	*M. triplex*	*M. triplex*
53480*	*M. triplex*	*Mycobacterium* sp.	*M. triplex/genavense*	*M. triplex*	*M. triplex*
53484†	*M. simiae* complex	*Mycobacterium* sp.	*Mycobacterium sp.*	Score <1.7	Non-chromogen SGM
M. avium complex					
53468	*M. intracellulare*	*M. intracellulare*	*M. intracellulare*	*M. chimaera/intracellulare*	*M. avium* complex
53580	*M. intracellulare*	*M. avium*	*M. intracellulare*	*M. chimaera/intracellulare*	*M. intracellulare*
54464	*M. colombiense*	ND	ND	Score <1.7	*M. avium* complex
53488	*M. colombiense*	*M. intracellulare*	*M. intracellulare*	*M. colombiense*	*M. avium* complex
53587	*M. avium* complex	*M. intracellulare*	*M. intracellulare*	ND	*M. avium* complex
M. terrae complex					
53473	*M. terrae* complex	*Mycobacterium* sp.	*M. intermedium*	Score <1.7	*M. terrae* complex
53486	*M. terrae* complex	*Mycobacterium* sp.	*M. intermedium*	Score <1.7	*M. terrae* complex
53497	*M. terrae* complex	*Mycobacterium* sp.	*M. intermedium*	Score <1.7	*M. terrae* complex
53498	*M. terrae* complex	*Mycobacterium* sp.	*M. intermedium*	Score <1.7	*M. terrae*
Other					
53483	*M. gordonae*	*M. gordonae*	*M. xenopi*	*M. gordonae*	Scotochromogen SGM

* Two of five isolates obtained from the same patient in 2010–2015, all yielding identical phenotypical/PRA identification.
† 16S RNA is 100% identical to *M. shigaense*, a species not officially recognised, but the other genes do not match this identification.
SGM = slowly growing mycobacteria; MALDI-TOF MS = matrix-assisted laser desorption/ionisation time of flight mass spectrometry; CM = common mycobacteria; AS = additional species; PRA = polymerase chain reaction-restriction analysis; ND = not determined.

RESUME

CONTEXTE : Il existe plus de 150 espèces de mycobactéries non-tuberculeuses (NTM) qui sont potentiellement pathogènes et posent un défi pour le diagnostic de laboratoire clinique.

OBJECTIF : Évaluer différentes approches pour l'identification de 40 isolats cliniquement significatifs de NTM dont les espèces ne sont pas résolues par notre méthode de diagnostic routinière consistant à des tests phénotypiques et à l'amplification de l'ADN par la réaction polymérase en chaîne–analyse de restriction du gène *hsp*65.

MÉTHODES : Nous avons appliqué 1) l'analyse de séquençage de quatre gènes cibles conservés : 16S rDNA, *rpo*B, *hsp*65 et *sod*A ; 2) deux tests commerciaux d'hybridation inversée ; et 3) l'analyse des protéines par spectrométrie de masse (MALDI-TOF MS).

RÉSULTATS : L'analyse combinée des séquences et non individuel a permis l'identification fiable de 30/40 (75%) isolats, y compris des espèces non reportées précédemment en Argentine. Les tests commerciaux ont surpassé notre routine d'identification pour seulement 5/35 des isolats et ont mal classé beaucoup d'autres. MALDI-TOF MS a correctement identifié 22/36 (61%) isolats et aucun n'a été mal identifié.

CONCLUSION : Les kits commerciaux ne permettent pas de résoudre l'identification des espèces NTM difficile à identifier. L'analyse combinée du séquençage reste la méthode de choix. MALDI-TOF MS a émergé comme un outil puissant et accessible avec un potentiel pour l'identification rapide d'NTM d'intérêt clinique dans le laboratoire de diagnostic. Sa précision peut être maximisée par la mise en place d'une base de données personnalisée de NTM.

RESUMEN

MARCO DE REFERENCIA: Existen más de 150 micobacterias no tuberculosas (NTM) que son potencialmente patógenas y presentan un desafío al diagnóstico de laboratorio clínico.

OBJETIVO: Evaluar diferentes enfoques para la identificación de 40 aislamientos clínicamente relevantes de NTM cuyas especies no fueron resueltas mediante nuestra rutina diagnóstica, la que consiste en pruebas fenotípicas y reacción en cadena de la polimerasa–análisis de restricción de *hsp*65.

DISEÑO: Se aplicó 1) análisis de secuenciación de cuatro regiones genéticas conservadas: 16S rDNA, *rpo*B, *hsp*65 y *sod*A; 2) dos pruebas comerciales de hibridación reversa; y 3) análisis de proteínas mediante la identificación de proteínas por espectrometría de masas (MALDI-TOF MS).

RESULTADOS: El análisis combinado de secuencias, pero no el individual, permitió la identificación de especie de 30/40 (75%) aislamientos, incluidas especies no registradas con anterioridad en Argentina. Las pruebas comerciales superaron nuestra rutina diagnóstica en sólo 5/35 aislamientos y clasificaron erróneamente muchos más. MALDI-TOF MS identificó correctamente las especies de 22/36 (61%) aislamientos y no arrojó ninguna clasificación errónea.

CONCLUSIONES: Las pruebas comerciales no resolvieron la asignación de especies de NTM difíciles de identificar. El análisis combinado de secuencias continúa siendo el método de elección. MALDI-TOF MS emerge como una herramienta poderosa y accesible con potencial para la identificación rápida de NTM de interés clínico en el laboratorio diagnóstico. Su precisión puede ser maximizada mediante la construcción de bases de datos personalizadas de espectros de especies de NTM.