

# *rpoB* Gene Sequencing for Identification of Rapidly Growing Mycobacteria

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## Abstract

**Background:** Rapidly growing mycobacteria (RGM) are increasingly recognized as a cause of human infections. Rapid and reliable identification of RGM at species level should be carried out as a means of effective patient managements.

**Methods:** Twenty clinical samples of RGM isolated from suspected tuberculosis (TB) patients were included. Different phenotypic tests and a hsp65-PCR restriction analysis (PRA) method were used to identify the isolated organisms to species level. Sequence analysis of the *rpoB* gene was also used for molecular identification of clinical isolates.

**Results:** Phenotypic evaluation of clinical isolates assigned 19 (95%) isolates of RGM to *M. fortuitum* complex. Using hsp65-PRA, 13 isolates of *M. fortuitum* complex were identified as *M. fortuitum*, 4 isolates as *M. abscessus* and 1 isolates as *M. chelonae*. However, two isolates had identical hsp65-PRA patterns; one was indistinguishable from *M. conceptionense* and *M. senegalense* and another was indistinguishable from *M. peregrinum* and *M. porcinum*. By the *rpoB* gene sequence analysis, all species studied were readily discriminated from each other.

**Conclusions:** *rpoB* gene sequencing has a high discriminatory power, which easily permits the identification of clinical isolates of RGM to the species level. It unambiguously differentiates between closely related species with restricted biochemical and PRA differences. This procedure is suggested as a first-line identification method for RGM.

**Keywords:** Mycobacterium, Sequencing, Iran, *rpoB* Gene

## 1. Background

Rapidly growing mycobacteria (RGM) are increasingly recognized as a cause of human infections (1, 2). They are capable of causing serious illnesses such as pulmonary disease, skin and soft tissue infection and disseminated infection in both immunocompetent and immunocompromised individuals (3-5). This group of mycobacteria is heterogeneous in terms of epidemiology, clinical disease spectrum and drug sensitivity. It is therefore important to identify RGM to the species level (5-7). In most laboratories RGM identification currently relies on phenotypic tests. These phenotypic tests, however, are cumbersome and time-consuming and interpretation of the results is sometimes ambiguous. Phenotypic tests sometimes fail to discriminate between closely related species, such as *Mycobacterium abscessus* and *Mycobacterium chelonae* (8-10). Molecular tools including analyses of the 16S rRNA gene for the identification of mycobacteria have been developed in recent years (11-13). However, there is little variability

within the mycobacterial 16S rRNA gene sequence in RGM, making this target a poor discriminator for closely related species (11, 12, 14). *rpoB* is a single-copy gene encoding the  $\beta$ -subunit of the bacterial RNA polymerase gene, which is present in all mycobacteria. It is more variable than the 16S rRNA gene sequence and is therefore potentially useful for the identification of genetically related species (1, 15). The aim of this work was to evaluate the potential of partial *rpoB* sequencing for the rapid identification of this group of emerging pathogens.

## 2. Methods

### 2.1. Mycobacterial Isolates

In this cross-sectional study, 20 clinical isolates of RGM collected by our clinical microbiology laboratory (Tehran reference laboratory) from January 2014 to December 2015 were included. They were isolated from sputum of suspected tuberculosis (TB) patients. If the patient had multiple longitudinal sampling, only the first set of samples was

included into the study. The ethics committee of Shahid Beheshti University of Medical Sciences approved the study and all the patients have signed informed consent.

## 2.2. Phenotypic Identification

The clinical isolates were identified by conventional methods, i.e. growth rate, macroscopic and microscopic morphological features, growth at different temperatures, tween 80 hydrolysis, nitrate reduction, niacin production, arylsulfatase, urease production, tellurite reduction, salt tolerance and catalase production, according to standard procedures (16).

## 2.3. Restriction Analysis

PCR restriction analysis (PRA) was used to speciate mycobacteria. Genomic DNA was extracted using a QI-Aamp DNA Mini Kit (QIAGEN, USA). According to the kits instruction, for PRA, approximately 441 bp fragment of hsp65 gene was amplified by PCR using two specific primers Tb11 (50-ACCAACGATGGTGTGCCAT-30) and Tb12 (50-CTGTGCGAACCGCATACCT-30) (17). PCR products were digested with 5 U of restriction enzyme Hae III and Bst II for 24 hours at 37°C. The pattern of digested products was analyzed using 8% polyacrylamide gel. *M. fortuitum* (ATCC 49404) and double distilled water were used as positive and negative control in all PCR experiments, respectively. Species identification was performed using algorithm proposed by Chimara et al. (18).

## 2.4. PCR and Sequencing of rpoB Gene

As previously described, a 750 bp fragment of the *rpoB* gene was amplified and sequenced using two specific primers MycoF (50-GGCAAGGTCACCCGAAGGG-30) and MycoR (50-AGCGGCTGCTGGGTGATC-30) (1). The obtained sequences for each isolate from different loci were aligned separately and compared with all existing relevant sequences of mycobacteria retrieved from the GenBank database at the NCBI website via the nucleotide BLAST search (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

## 3. Results

### 3.1. Species Identification by Phenotypic Tests

On the basis of growth characteristics, the isolates studied were classified within the RGM group. According to phenotypic tests, 19 (95%) isolates assigned to the *M. fortuitum* complex and the one remaining isolate was unidentifiable (Table 1).

### 3.2. hsp65-PRA-Based Identification

According to hsp65-PRA results, an identical pattern was detected for the isolated microorganisms from each patient. Using hsp65-PRA, 13 isolates of *M. fortuitum* complex, which had been identified by phenotypic tests, were identified as *M. fortuitum*, 4 isolates as *M. abscessus* and 1 isolate as *M. chelonae* (Table 2). Thus, *M. abscessus*, formerly *Mycobacterium chelonae* subsp. *abscessus*, was easily distinguished from *M. chelonae* and *M. fortuitum*. Two isolates had identical hsp65-PRA patterns: one was indistinguishable from *M. conceptionense* and *M. senegalense* due to the same digestion pattern and another was indistinguishable from *M. peregrinum* and *M. porcinum*.

### 3.3. Identification by rpoB Gene Sequencing

By using *rpoB* gene sequencing, all species studied were readily discriminated from each other. *M. senegalense* and *M. porcinum*, which could not be differentiated from closely related species by phenotypic and genotypic methods, were easily differentiated by *rpoB* sequencing (Table 2).

## 4. Discussion

There are increasing reports of nontuberculous mycobacterial (NTM) infection not only in the immunocompromised but also in immunocompetent. Amongst those species that are currently recognized, rapidly growing species such as *M. fortuitum*, *M. chelonae* and *M. abscessus* are frequently encountered and are clinically important (19). RGM are amongst the most common NTM isolate associated with nosocomial diseases (19). Previous studies demonstrated that tap water, processed tap water used for dialysis, as well as piped water systems in hospital settings are the usual nosocomial sources of NTM infections (19). Compared to *M. tuberculosis*, RGM are even more difficult to eradicate with common decontamination practices and are relatively resistant to standard anti-TB drugs (20, 21). Thus, transmission and spread of such NTM species from nosocomial sources may constitute the major part of the problem in hospital control strategies. As a result, rapid and reliable identification of RGM, at species level, should be carried out as a means of effective patient management and molecular epidemiology. PRA is one of the methods that is used and is simple and convenient (18, 22, 23). In the current study, phenotypic evaluation assigned 19 (95%) isolates to a species or complex. However, by the hsp65-PRA analysis, nearly all RGM isolates were accordingly speciated. Similar to our study, other reports from different parts of the world have shown that hsp65-PRA was significantly more accurate than the phenotypic methods (22-24). Nonetheless, the presence of unknown patterns as a

**Table 1.** Results of RGM Identification by Phenotypic Tests

Numbers of Isolates	Phenotypic Characterization of Clinical Isolates												Result
	Growth at 37°C	Growth at 42°C	Growth on Mac-Conkey Agar	Urease Production	Tween 80 Hydrolysis	Pigment Production	Growth Rate (Days)	Niacin Production	Nitrate Reduction	Heat Stable Catalase	Tellurite Reduction	Arylsulfatase (3 days)	
13	+	+	+	+	-	N	< 7	-	1+	+	+	+	<i>M. fortuitum</i> complex
4	+	-	+	+	+	N	< 7	-	-	+	-	+	<i>M. fortuitum</i> complex
1	+	-	+	-	+	N	< 7	+	+	+	-	+	<i>M. fortuitum</i> complex
1	+	-	+	+	+	N	< 7	-	2+	+	+	+	<i>M. fortuitum</i> complex
1	+	-	+	+	-	N	< 7	-	-	+	+	+	<i>Mycobacterium</i> sp.

**Table 2.** Results of RGM Identification by Genotypic Test and *rpoB* Sequencing

Numbers of Isolates	Phenotypic Tests	Patterns by <i>hsp65</i> -PRA		Identification by PRA	Identification by <i>rpoB</i> Sequencing
		Bst E II	Hae III		
13	<i>M. fortuitum</i> complex	235/120/85	145/120/60/55	<i>M. fortuitum</i>	<i>M. fortuitum</i>
4	<i>M. fortuitum</i> complex	235/210	145/70/60/55	<i>M. abscessus</i>	<i>M. abscessus</i>
1	<i>M. fortuitum</i> complex	235/210	145/140/100/50	<i>M. peregrinum</i> or <i>M. porcinum</i>	<i>M. porcinum</i>
1	<i>M. fortuitum</i> complex	235/120/85	140/125/60/55	<i>M. conceptionense</i> or <i>M. senegalense</i>	<i>M. senegalense</i>
1	<i>Mycobacterium</i> sp.	320/130	200/60/55/50	<i>M. chelonae</i>	<i>M. chelonae</i>

result of gel-to-gel variation due to small restriction fragment sizes as well as identical patterns to that of *M. conceptionense* or *M. senegalense* based on the *hsp65*-PRA method emphasized the need for a more reliable identification method (16). *rpoB* gene sequencing is one of most common technique currently used for *Mycobacterium* species identification (15, 25). In comparison with *hsp65*-PRA, sequence analysis of *rpoB* can markedly improve molecular identification of clinical isolates. In this study, all clinical isolates were easily identified by using *rpoB* gene sequences analyses. The isolates belonging to closely related species such as *M. conceptionense* or *M. senegalense* and *M. peregrinum* or *M. porcinum*, which were poorly discriminated by the *hsp65*-PRA, were clearly delineated as *M. senegalense* and *M. porcinum*, respectively. This indicated that the *hsp65*-PRA was less discriminatory than the *rpoB* gene for RGM identification. This result was consistent with prior reports, which confirmed the high discriminatory power of the *rpoB* gene for species identification of RGM (15, 16).

In conclusion, *rpoB* gene sequencing has a high discriminatory power, which easily permits the identification of clinical isolates of RGM to the species level. It unambiguously differentiates between closely related species with re-

stricted biochemical and PRA differences, such as *M. conceptionense* and *M. senegalense*. On the basis of the data presented here, we consider *rpoB* sequencing an appropriate identification method for RGM isolated from human samples.

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## Footnote

**Conflict of Interests:** None.

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