***Mycobacterium wolinskyi* Infection Confirmed by *rpoB* Gene Sequencing**

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Identification of rapidly growing mycobacteria (RGM) is problematic because there are many taxonomic changes. 16S rRNA gene is commonly used to identify *Mycobacterium* species, but alternative gene targets have been introduced for more accurate identification. We report a rare case of a prosthetic knee infection due to *Mycobacterium wolinskyi*. The isolate was not identified by 16S rRNA gene sequencing

alone and substantially confirmed by *rpoB* gene sequencing. The identification was delayed because our laboratory did not routinely identify RGM to the species level. Simultaneous sequencing of both 16S rRNA and *rpoB* genes will allow rapid and accurate identification of *M. wolinskyi* isolates. J. Clin. Lab. Anal. 26:325–327, 2012. © 2012 Wiley Periodicals, Inc.

Key words: *Mycobacterium wolinskyi*; *rpoB* gene; 16S rRNA gene

INTRODUCTION

The rapidly growing mycobacteria (RGM) are widely distributed in the soil, water, and other environments. During the past two decades, RGM have been increasingly recognized as significant human pathogens.

Conventional laboratory identification of the RGM is based mainly on growth rate, pigmentation, and some biochemical tests. However, these methods are time consuming and proved inadequate for the identification of some species (1, 2). Accurate identification is important because the clinical significance and antibiotic susceptibilities differ among RGM species.

Recently, molecular diagnostic methods such as 16S rRNA or *rpoB* gene sequencing have been introduced for the identification of the RGM species (3, 4). Here, we describe a case of prosthetic knee infection due to *Mycobacterium wolinskyi* identified by *rpoB* gene sequencing.

CASE REPORT

A 65-year-old woman who underwent a total knee replacement arthroplasty for primary osteoarthritis was admitted with knee pain, swelling, and redness. Laboratory tests showed an elevated C-reactive protein level (88.6 mg/l) and erythrocyte sedimentation rate (39 mm/hr). The peripheral blood leukocyte count was nor-

mal (8,000 cells/ μ l) with a slight increase of monocyte (14.3%). Grossly purulent synovial fluid obtained upon aspiration was inoculated into BacT/Alert (bioMérieux Inc., Durham, NC) blood culture bottles. Gram staining of the fluid revealed many leukocytes but no bacteria. The aerobic culture bottle became positive after 3 days of incubation at 35°C. The subculture on 5% sheep blood agar plate revealed smooth, opaque white colonies without pigmentation. Gram stain of the isolate showed irregular Gram-positive bacilli. On the day of admission, empirical antibiotic treatment was initiated with a combination of ciprofloxacin (800 mg/day) and vancomycin (2 g/day). Surgical debridement was performed and the histopathologic finding revealed a granulomatous inflammation with massive necrosis. Same organism was isolated from intraoperative tissue culture on 3% Ogawa agar. The organism was positive for Ziehl-Neelsen staining and tentatively identified as an RGM. Based on a preliminary report, antibiotics were changed

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TABLE 1. Antimicrobial Susceptibility of the *M. wolinskyi* Isolate

Agent	Interpretation	MIC (μ g/ml)
Amikacin	S	16
Cefoxitin	R	≥ 256
Ciprofloxacin	S	1
Clarithromycin	R	64
Doxycycline	S	0.5
Imipenem	R	≥ 64
Moxifloxacin	S	≤ 0.125
Rifampicin	R	≥ 16
Sulfamethoxazole	R	≥ 128
Tobramycin	R	≥ 32

S, susceptible; R, resistant.

to clarithromycin (1 g/day). Despite the antibiotic treatment, the patient's condition was not improved. Secondary surgical debridement was performed. An accurate identification of the isolate was delayed because our laboratory did not routinely identify RGM to the species level. After 2 weeks, partial 16S rRNA gene sequence analysis was carried out with primer sets g2R (5'-GAGAAT TCGTGCT TAACACAT GCAAGTCG-3') and rM582R (5'-ATGGAT CCGTGAGATTT CACGAACAA CGC-3') (2). Using the BLAST (Basic Local Alignment Search Tool) search of the GenBank database (<http://www.ncbi.nih.gov>), sequence identity of 99.2% was observed with *M. wolinskyi* ATCC 700010 (GenBank accession no. AY457083) and *M. jacuzzii* (GenBank accession no. DQ137412), followed by *M. canari-asense* CIP 107998 (GenBank accession no. AY943207) with 99%. The isolate was not classified as a single species by 16S rRNA gene sequencing. Alternate target gene, *rpoB* gene sequence analysis was performed using primers MF (5'-CGACCACTTCGGCAACCG-3') and MR (5'-TCGATCGGGCACATCCGG-3') (5). The isolate demonstrated 99.7% sequence similarity with *M. wolinskyi* ATCC 700010 followed by *M. smegmatis* strain MC2 155 (GenBank accession no. CP000480) with 98.3%. Finally, the isolate was definitively identified as *M. wolinskyi*. Antibiotic susceptibilities were determined by a broth microdilution assay based on CLSI (Clinical and Laboratory Standards Institute) recommendations (Table 1). Since the organism was resistant to clarithromycin, the drug regimen was changed to amikacin (500 mg/day), ciprofloxacin (800 mg/day), and doxycycline (300 mg/day). The patient improved and subsequently discharged.

DISCUSSION

The primary pathogenic RGM are *M. fortuitum* group, *M. chelonae/abscessus* group, and *M. smegmatis* group (1). The *M. smegmatis* group is composed of *M. smeg-*

matis sensu stricto and the newly described *M. goodii* and *M. wolinskyi*. *M. wolinskyi* is usually associated with post-traumatic wound infections and surgical site infections, often with extensive suppuration and, in some cases, tissue necrosis.

M. wolinskyi was first identified in 1999 by Brown et al. (6). Previously, it would be identified as *M. smegmatis* group without further species differentiation. With the advance of molecular identification techniques, *M. wolinskyi* is increasingly recognized in clinical specimens (7–9).

The 16S rRNA gene is most widely used for the identification of *Mycobacterium* species. However, for RGM, the interspecies similarities are 95.7–99.7%, which is too high to discriminate closely related species (10). Alternative molecular targets have been used to identify these organisms reliably. The *rpoB* gene sequence is more divergent than those of 16S rRNA gene (11, 12). This suggests that *rpoB* gene provides better discrimination for the identification of RGM species. In our case, the isolate was not classified as a single species by 16S rRNA gene sequencing alone and substantially confirmed by *rpoB* gene sequencing. Therefore, identification of some isolates will be more accurate when both 16S rRNA and *rpoB* genes are examined.

The antimicrobial susceptibility pattern often differs among RGM species (1, 9). These differences are important not only for therapeutic purposes but also for the identification of the organism. The *M. smegmatis* group is susceptible to ethambutol but not clarithromycin. This feature is helpful in distinguishing it from other RGM groups. *M. wolinskyi* is resistant to tobramycin, which is distinctive from other *M. smegmatis* group members.

Delayed identification of RGM species may lead to inappropriate antimicrobial therapy (10, 12). In our case, we first identified the pathogen simply as an RGM, and it took several weeks for identifying as *M. wolinskyi*. The patient was treated with clarithromycin, which was considered to be the drug of choice for RGM. However, the patient did not improve despite the antibiotic treatment because the isolate was not susceptible to clarithromycin. Therefore, the rapid and accurate identification of RGM to the species level is important for the adequate treatment of patient.

In summary, we report a rare case of a prosthetic knee infection due to *M. wolinskyi* confirmed by *rpoB* gene sequence analysis. To avoid any delay in diagnosis and treatment, it is important to identify pathogenic RGM to the species level by molecular tools in clinical laboratory.

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