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Identification and pathogenicity analysis of a novel non-tuberculous mycobacterium clinical isolate with nine-antibiotic resistance

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Abstract

With mycobacteriosis increasing, the study of non-tuberculous mycobacteria is imperative for clinical therapy and management. Non-tuberculous mycobacteria are naturally resistant to most anti-tuberculosis drugs. Accordingly, it is important to decipher the biology of the novel non-tuberculous mycobacteria through complete genomic analysis of novel pathogenic mycobacteria. We describe *Mycobacterium sinense* JDM601, a novel, slow-growing mycobacterium of the *Mycobacterium terrae* complex resistant to nine antibiotics, by clinical presentation, cultural and biochemical characteristics, minimal inhibitory concentrations, and genome-sequencing analysis. JDM601 is closest to *Mycobacterium nonchromogenicum* according to mycolic acid composition, but closest to *Mycobacterium algericum* sp. nov according to 16S rDNA. JDM601 is resistant to isoniazid, streptomycin, rifampin, euteropas, protionamide, capromycin, ciprofloxacin, amikacin and levofloxacin but not ethambutol. The clinical information, mycolic acid composition, and virulence genes indicate that JDM601 is an opportunistic pathogen.

Keywords: Antibiotic resistance, complete genome, *Mycobacterium sinense*, *Mycobacterium terrae* complex, non-tuberculous mycobacterium

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Introduction

Infections caused by non-tuberculous mycobacteria (NTM) have risen steadily over the last several decades in China and around the world [1,2]. The NTM are naturally resistant to most anti-tuberculosis drugs. Traditional therapy to cure complicated infections caused by novel NTM, especially tuberculosis-like disease, has been limited. A definite identification of a given clinical isolate may lead to a better estimation of the pathogenicity and epidemiology of this organism.

Mycobacterium terrae complex (MTEC) includes *M. terrae*, *M. triviale*, *M. nonchromogenicum* and *M. hiberniae*. Despite the common opinion that MTEC isolates are non-pathogenic, these organisms are occasionally identified in the clinical disease setting [3,4]. *Mycobacterium senuense* and *M. paraterrae* are two novel MTEC species recently isolated from pulmonary infection patients [5,6]. Because of the presumed non-pathogenic nature of MTEC, there has been little effort to distinguish the species of this complex in the clinical setting in the past century. However, MTEC infection can cause debilitating disease that is relatively resistant to antibiotic therapy. We identified a novel pathogenic mycobacterium resistant to nine antibiotics that was isolated from a patient

with tuberculosis-like disease, and determined the whole genome sequence for further analysis (accession number CP002329) [7].

Materials and Methods

Patient

A 34-year-old woman presented to her primary physician with chest pain in March 2006. Left-lung infiltrates were found using chest X-ray. The detection of acid-fast bacilli initially led to treatment with anti-tuberculosis drugs. As a consequence of the progression of the pulmonary infiltrates, she was transferred to another hospital 6 months later. There, computerized tomography of her chest suggested left pneumonitis. The acid-fast bacillus staining gave a strongly positive result, graded as 4-plus smear, so she was primarily diagnosed with pulmonary tuberculosis and was treated empirically with isoniazid, rifampin, pyrazinamide and ethambutol for 3 months. On the basis of mycobacterial identification and *in vitro* drug-susceptibility test results, second-line antibiotics were added, and specific antibiotics were administered. Finally, the infection cleared after prolonged multidrug antibiotic therapy (ending in October 2008).

Strain identification

Strain JDM601 was isolated from the sputa of the patient with a symptomatic pulmonary infection. Successive isolations of the same strain as that isolated from the sputa of this patient had been observed three times without isolation of other mycobacteria.

Detailed conventional biochemical testing procedures and quality control measures were performed [8]. The phenotypic characteristics of strain JDM601 and reference strain *M. tuberculosis* $H_{37}R_v$ were analysed and compared. The mycolic acids of JDM601 were analysed using the MIDI Sherlock® Microbial Identification System. We performed taxonomy analysis for JDM601 based on the 16S rDNA and *hsp*65 sequences of 19 mycobacteria from different species. Reference sequences were obtained from GenBank.

Antibiotic resistance and MIC measurement

The use of *in vitro* susceptibility testing for JDM601 was consistent with the Clinical and Laboratory Standards Institute (CLSI) recommendations. Minimal inhibitory concentrations (MICs) for ten drugs, including isoniazid, streptomycin, rifampin, euteropas, protionamide, capromycin, ciprofloxacin, amikacin, levofloxacin and ethambutol, were determined [9].

Results

Species identification

JDM601 was a slow-growing mycobacterium (SGM); 3 weeks or more were required to form mature colonies on Lowenstein–Jensen medium. The biochemical characteristics of JDM601 are shown in Table 1. JDM601 was distinguished from the other species.

TABLE 1. Biochemical characteristics of *Mycobacterium sinense* JDM601 compared with other mycobacteria

Characteristic	1	2	3	4	5	6	7	8	9
Growth at:									
28°C	+	–	+	+	–	+	–	+	+
37°C	+	+	+	+	+	+	+	+	+
Growth speed	S	S	S	R(S)	S	S(R)	S	S	R
Morphology	R'	R'	S'(R')	S'	S'(R')	S'(R')	R'(S')	S'	R'
Pigmentation	N	N	N	N	N	P	N	N	N
Nitrate reductase	–	+	+	+	V	–	–	–	+
Arylsulphatase (9 days)	–	–	+	–	+	+	V	–	–
Tellurite reductase	+	+	+	+	+	U	+	+	U
Tween-80 hydrolysis									
<5 days	–	–	+	V	+	+	–	–	+
>10 days	+	+	+	+	+	+	V	–	+
Urease	+	+	–	–	–	+	+	–	+
Growth on/in:									
TCH	+	+	+	+	+	+	–	+	+
Para-nitrobenzoic acid	–	–	+	–	V	V	–	+	+
5% NaCl	+	–	–	–	–	–	–	–	+
MacConkey agar	–	–	U	–	–	U	U	U	U
Picric acid	–	–	U	–	–	U	U	U	+
Catalase	–	–	+	U	+	V	–	V	+
Nicotinic acid	–	+	–	U	U	V	–	–	–
Phosphatase	–	–	+	U	+	+	–	–	–
Iron absorption (7 days)	+	–	+	U	U	–	–	–	+

1, *M. sinense* JDM601; 2, *M. tuberculosis* $H_{37}R_v$; 3, *M. terrae*; 4, *M. senuense* [5]; 5, *M. nonchromogenicum*; 6, *M. marinum*; 7, *M. bovis*; 8, *M. avium*; 9, *M. smegmatis*;1 ~ 3 and 5 ~ 9 [23]. R, rapid; S, slow; V, partly positive; +, positive; –, negative; U, unknown. R', rough; S', smooth; N, non-photochromogenic; P, photochromogenic.
TCH, thiophene-2-carboxylic acid hydrazide.