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Mycobacterium branderi Isolated from Pus of a Right Pulmonary Cavitory Lesion

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A 22-year-old male truck driver who presented for a regular physical checkup was found to have an abnormal shadow in the right lung and was therefore referred to Tokushima Red Cross Hospital in September, 2004. Chest CT revealed a cavitory lesion in the right upper lung field. He was hospitalized at Higashi-Tokushima National Hospital for probable pulmonary tuberculosis, and at that time there were no symptoms. Chest CT showed an encapsulated cavitory lesion about 3 cm in diameter in the right S2 area adjacent to the pleura, and no dispersing abnormal shadows. Results of various tests included: tuberculin test 11 × 11 mm, sputum negative for *Mycobacterium tuberculosis*, PCR negative for *M. tuberculosis*, ESR 3 mm/h, CRP 0.0 mg/dl, serum *Aspergillus* antigen >5 positive, *Cryptococcus neoformans* antigen negative, β-D-glucan <5 negative. Partial thoracoscopic resection of the right lower lobe was performed. The pus from the cavitory lesion showed Gaffky 5, acid-fast bacteria that could not be identified by PCR and DNA-DNA hybridization (Kyokuto Pharmaceutical Industry Co., Tokyo, Japan). The Research Institute of Tuberculosis (RIT) was therefore asked to identify the bacteria. Histopathological examination of the surgical specimen showed caseating pulmonary granulomas with Ziehl-Neelsen-stained bacteria. No fungi were evident.

As shown in Table 1, we carried out routine biochemical tests on the clinical isolate in comparison with standard strains of *Mycobacterium branderi* (ATCC51789) and *Mycobacterium celatum* (ATCC51131). The growth characteristics and biochemical test results for this *M. branderi* were similar to those of standard strains. Only tellurite reduction showed a difference between *M. branderi* and *M. celatum*. Although it has been reported that the *M. celatum* type I standard strain is positive for tellurite reduction whereas clinical isolates are negative, our isolate was positive for tellurite reduction whereas the *M. celatum* standard strains in our laboratory were negative (1). We carried out an *rpo B* assay (2), but this was unable to distinguish *M. branderi* from *M. celatum*. We then performed 16S rRNA sequencing of this isolate, the *M. branderi* standard strain, and *M. celatum*. At present, 16S rRNA gene sequencing remains the sole definitive means of distinguishing between them (3). Results of a similarity search using a partial 16S ribosomal DNA database showed that our isolate had 99.7% identity with *M.*

branderi and 93.3% identity with *M. celatum*. Our isolate was therefore judged to be *M. branderi*.

We then carried out susceptibility tests (Table 2). Previous studies have provided interpretations of MICs determined for *Mycobacterium avium* isolates (4-6), and these guidelines were the only basis available for a tentative interpretation of the susceptibility patterns of the *M. branderi* isolates in this study. The susceptibility patterns observed for the clinical isolate and the *M. branderi* standard strain included resistance to amikacin and rifampin and susceptibility to clarithromycin, ciprofloxacin, ethambutol, ethionamide and streptomycin (7). These patterns were similar to that of *M. celatum*.

Our isolate is similar to the Helsinki strain and *M. branderi* in the second of two cases reported by Wolfe et al. (1). Finally, we carried out animal infection experiments using Hartley female guinea pigs. When 1 × 10⁷ CFU of the isolate was administered subcutaneously and necropsy was performed 7 weeks later, several small granulomas were observed in the lungs and the liver microscopically (Fig. 1). Such granulomas were not recognized in the spleen or kidneys of the guinea pig.

From the medical history of this patient, the cavitory lesion in the right lung appeared to have been induced

Table 1. Growth characteristics and biochemical test results for the clinical isolate in comparison with standard strains¹⁾

Biochemical test	Clinical isolate	<i>M. branderi</i>	<i>M. celatum</i>
Growth temperature			
25°C	+	+	+
28°C	+	+	+
31°C	+	+	+
42°C	+	+	+
Pigment production	-	-	-
Niacin	-	-	-
Nitrate reductase	-	-	-
Catalase	-	-	-
Arylsulfatase	+	+	+
Tween 80 hydrolysis	-	-	-
Urease	-	-	-
Tellurite reduction	+	+	-
Pyrazinamidase	+	+	+
Acid production from			
Mannitol	-	-	-
Sorbitol	-	-	-

¹⁾: +, positive; -, negative.

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Table 2. Antimicrobial sensitivity results for the clinical isolate, *M. branderi* and *M. celatum*

Antibiotic	MIC ($\mu\text{g/ml}$)		
	Clinical isolate	<i>M. branderi</i>	<i>M. celatum</i>
Streptomycin	0.5 (S)	0.5	0.25
Ethambutol	2 (S)	2	2
Kanamycin	8	4	0.5
Isoniazid	4	8	4
Rifampin	16 (R)	32	32
Clarithromycin	0.03 (S)	0.03	0.03
Ethionamide	8 (S)	8	8
Amikacin	8 (R)	8	8
Levofloxacin	0.125	0.125	0.125
Sparfloxacin	0.03	0.03	0.06
Ciprofloxacin	0.125 (S)	0.125	0.125

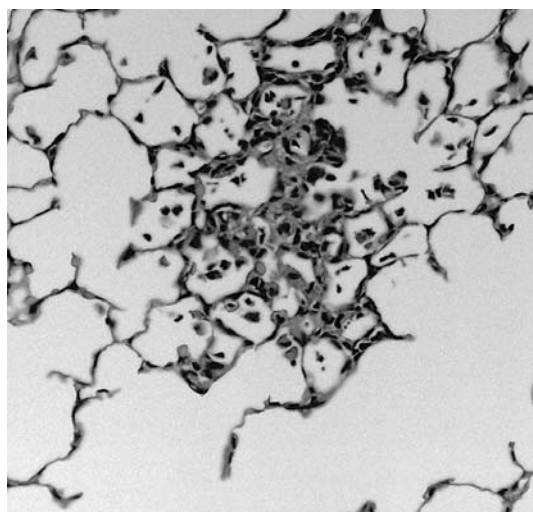


Fig. 1. Histopathology of the guinea pig lung infected with the clinical isolate. Small granuloma consisting of macrophages, neutrophils and lymphocytes is recognized in the lung. $\times 200$. Hematoxylin & eosin stain.

by *Aspergillus fumigatus*, because repeated cultures were negative for *M. tuberculosis*. The cause of the *Aspergillus* granuloma remains unknown because there was no evidence of immunological abnormality. *M. branderi* infection was secondary to the initial infection. However, this isolate induced granulomas in the lungs and liver of guinea pigs and was slightly pathogenic. Due to its low pathogenicity, it seems reasonable to conclude that the cavitary pulmonary granuloma was not induced by *M. branderi*. The condition

of our patient is currently improved, but he will need to be followed up carefully. Additional studies will be required to further characterize *M. branderi* and clarify the role of this species as a human pathogen (8).

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