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Isolation of *Mycobacterium shimoidei* from a Patient with Cavitary Pulmonary Disease

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Mycobacterium shimoidei **was isolated from the sputum of a man hospitalized for cavitary pulmonary disease. This is the fourth isolation of *M. shimoidei* to be reported; the organism has also been isolated in Japan, Australia, and Germany.**

Mycobacterium shimoidei, first isolated by the Japanese microbiologist H. Shimoide (6), was described and named by M. Tsukamura (4).

Subsequent isolations of this mycobacterium have been extremely rare. Only two other cases, one from Australia (reported by Z. Blacklock and D. Dawson; cited in reference 7) and one from Germany (2), have been reported. (The third report of the International Working Group of Mycobacterial Taxonomy [8] reports a further strain of unknown clinical significance, isolated in Mississippi, that on the basis of numerical taxonomy could be attributed to the same cluster as *M. shimoidei*).

M. shimoidei is an acid-fast rod, 0.5 by 3 to 5 μm, that does not branch and does not form cords; cross-barring has been described. On egg or synthetic media for mycobacteria this species grows by forming rough colonies after 2 to 4 weeks of incubation at 37°C.

TABLE 1. Comparison of three of four known cases in which *M. shimoidei* was isolated

Characteristic	Value of results in case described in indicated reference		
	6	2	This study
Clinical features of patient			
Age (yr) at the first isolation	56	77	68
Sex	Male	Male	Male
Disease type[a]	TB-like	TB-like	TB-like
PPD status	Unknown	Unknown	Positive
Concomitant disease(s)		Silicosis	Addison's disease, cor pulmonale
Radiographic picture	Cavities	Cavities	Cavities
Therapeutic regimen[b]	Unknown	INI, PRT, RIF; later STR, ISO	INI, STR, EMB, RIF, KAN, PAS
Outeome of therapy	Unknown	Improvement	Improvement
Microbiological features			
Isolation site	Sputum	Sputum	Sputum
Direct examination	Unknown	Positive	Negative
No. of positive cultures	7	14	1
Digestant	Unknown	Nekal BX	NaOH
Medium or media	Salicylate, *p*-nitrobenzoic acid	L-J, Stonebrink, Gottsacker	L-J
M. tuberculosis isolation	None	None	None
Susceptibility to:			
STR	Resistant	Intermediate	Susceptible
INI	Resistant	Resistant	Resistant
PAS	Resistant		Susceptible
Ethionamide	Resistant		Susceptible
RIF	Resistant	Resistant	Resistant
EMB	Susceptible	Susceptible	Susceptible
KAN	Susceptible		
Capreomycin	Susceptible		
Ciprofloxacin			Resistant

[a] TB, tuberculosis.
[b] EMB, ethambutol; INI, isoniazid; ISO, isoprodian; KAN, kanamycin; PAS, *p*-aminosalicylic acid; PRT, protionamide; RIF, rifampin; STR, streptomycin.

Case report. One strain of *M. shimoidei* was isolated in 1989 from sputum of a 68-year-old male hospitalized at Cortona Hospital, Cortona, Italy, with a diagnosis of relapse of pulmonary tuberculosis.

Tubercular disease was first diagnosed in 1946, but the patient recovered from the attack and there is no further record

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TABLE 2. Results of differential tests performed for the characterization of *M. shimoidei* strains

Test	Result from indicated reference		
	10[a]	2	This study
Niacin	-		-
Nitrate reduction	-	-	-
Catalase	+	+	+
68°C catalase	+	+	+
Semiquantitative catalase (foam > 45 mm)	-		-
Photochromogenicity	-	-	-
Scotochromogenicity	-	-	-
Growth rate	3 wk	Slow	3 wk
Growth at 25°C	-		-
Growth at 45°C	+	+	+
Colony type	Rough	Rough	Rough
Tween 80 hydrolysis, 5 days	+		+
Tween 80 hydrolysis, 10 days	+	+	+
Tellurite reduction	-		-
Arylsulfatase, 3 days	-	-	-
Urease	-		-
β-Galactosidase	-	-	-
Acid phosphatase	+	+	+
MacConkey agar	-		-
Growth in presence of:			
NaCl (5%)	-		-
p-Nitrobenzoic acid (500 μg/m1)	+		+
Thiophene-2-carboxylic acid hydrazide (1 μg/ml)	+		+
Thiacetazone (10 μg/m1)	+		+
Hydroxylamine HCl (125 μg /ml)	+		+
Hydroxylamine HCl (500 μg/ml)	-		-
Isoniazid (1 μg/ml)	+	+	+
Isoniazid (10 μg/ml)	-		+
Oleic acid (250 μg/m1)	-		-
Picrate (0.2%)	-		-
Ethambutol (5 μg /ml)	-	-	-

[a] data from *Bergey's Manual* (10) are based on strains described by Tsukamura et al. (6).

of the illness until a short hospitalization in 1975 in Arezzo, Italy, when inactive tuberculosis was diagnosed; the only report about the purified protein derivative (PPD) reactivity of the patient dates from this period, when a Mantoux test was strongly positive. The man was admitted to the same hospital again in 1979 and remained there for several months; this time, on the basis of radiographic data accompanied by typical symptoms, the diagnosis was cavitary pulmonary tuberculosis and Addison's disease. All of the acid-fast stains and cultures of sputum performed during the hospitalization were negative. The pulmonary lesions responded to antitubercular therapy, and the patient was released from the hospital some months later.

In August 1989, the patient was hospitalized again in Cortona, and radiographic examination revealed a relapse of pulmonary tuberculosis. Some colonies were recovered from only one of the various cultures of sputum whose direct examination was negative and were shown to be acid-fast bacilli by Ziehl-Neelsen staining.

A Löwenstein-Jensen slant was sent to the Bacteriological and Virological Laboratory of Careggi Hospital in Florence, Italy, for identification and susceptibility testing. Meanwhile, the patient, who had been moved to the Hospital of Arezzo, was placed on therapy with isoniazid, streptomycin, ethambutol, kanamycin, rifampin, and *p*-aminosalicylic acid, and 4 months later he was discharged. Some months after his release from the hospital, the patient, who was also suffering from cor pulmonale, died. The death was imputed to cardio-respiratory conditions.

The clinical features of this case and two previously described cases of *M. shimoidei* isolation in pulmonary infections (no published description of the Australian case, reported in the first report of the International Working Group on Mycobacterial Taxonomy [7], could be found) show several similarities (Table 1). The most significant are probably the presence of tuberculosis-like lesions and the lack of isolation of *Mycobacterium tuberculosis.*

Microbiological tests. On the Löwenstein-Jensen slant sent to our laboratory, about 20 colonies with an *M. tuberculosis*-like morphology were grown. As hybridization with DNA probes specific for *M. tuberculosis* complex and for *Mycobacterium avium* complex (Gen-Probe, San Diego, Calif.) were negative *(M. tuberculosis* complex hybridization, 1.9%; *M. avium* hybridization, 3.3%; *Mycobacterium intracellulare* hybridization, 0.9%), conventional biochemical and culture tests (3), whose results are reported in Table 2, were performed.

On the basis of data reported in *Bergey's Manual of Systematic Bacteriology (10),* the only species matching the pattern of results appeared to be *M. shimoidei.* Considering the extreme rarity of this species, the strain was sent to the Institut Pasteur, Paris, France, for confirmation.

Drug susceptibility was determined by plating the strain onto control and drug-impregnated 7H10 agar plates (3). By using the 1% endpoint after incubation for 3 weeks at 36°C with 5% CO_2, the strain was found to be susceptible lo streptomycin (2 μg/ml), ethambutol (2 μg/ml), ethionamide (5 μg/m1), and *p*-aminosalicylic acid (2 μg/m1) and resistant to isoniazid (0.2 μg/m1), rifampin (1 μg/m1), and ciprofloxacin (1 μg/ml).

The analysis of the mycolic acid pattern (1), which revealed the presence of types i (α-mycolates), ii (α'-mycolates), iv (ketomycolates), and vi (dicarboxylic mycolates) and the absence of types iii (methoxymycolates) and v

TABLE 3. Comparison of *M. shimoidei* with other nonchromogenic slowly growing mycobacteria and with *M. xenopi*.

Species or complex	Characteristic[a]							
	Colony morphology	Growth at:		Catalase (foam > 45 mm)	Tween 80 hydrolysis (10 days)	Resistance to:		
		25°C	45°C			$MH_2OH·HCl$ (500 μg/ml)	Ethambutol (5 μg/ml)	*p*-Nitrobenzoic acid (500 μg/ml)
M. shimoidei	R	-	+	-	+	-	-	+
M. avium complex	S	V	V	-	-		+	+
M. terrae complex	R/S[b]	+	-	+	+	+	-	+
M. gastri	S	+	-	-	+	-	-	-
M. malmoense	S	+	-	-	+		+	+
M. haemophilum	S	+	-	-	-	-	-	-
M. ulcerans	S	+	-	-	-	-	-	+
M. xenopi	S	-	+	-	-	-	+	V

[a] R, rough colonies; S, smooth colonies; + ≥81% of strains are positive; ±, 61 to 80% of strains are positive; V, 41 to 60% of strains are positive; -, ≤20% of strains are positive. Data are derived from references 4, 6, 9 and 10.

[b] Colony morphology was variable; some strains were R while other were S.

(epoxymycolates), and the repetition of conventional tests, performed at the Institut Pasteur, confirmed the identification of the strain as *M. shimoidei.*

Discussion. Various studies (4, 5, 7, 8), on the basis of numerical taxonomy, have shown that *M. shimoidei* is distinct from all other species of slowly growing mycobacteria; in the third report of the International Working Group on Mycobacterial Taxonomy (8), the third cluster (slowly growing mycobacteria are grouped into 14 clusters) consists solely of the species *M. shimoidei.* There are various features that distinguish this species from other nonchromogenic mycobacteria (the only ones that can be confused with *M. shimoidei).* With regard lo the key tests, growth al 45°C and Tween 80 hydrolysis (10 days) are positive, and growth at 25°C, catalase (45-mm foam), and resistance to inhibition by hydroxylamine HCl (500 μg/ml) and by ethambutol (5 μg/m1) are negative (Table 3). Mycolic acid analysis is necessary for correct identification (1).

The fact that *M. shimoidei* has never been isolated from environmental cultures and the presence of tuberculosis-like pulmonary cavities in the patients from whose sputa *M. shimoidei* has been isolated seem to suggest a pathogenic role for this microorganism. In the present case, the finding of only one isolate, exclusively during the last worsening of the pulmonary disease, does not entitle us to assert that any of the previous episodes were due to *M. shimoidei*; moreover, the PPD status of the patient could indicate concomitant or previous tubercular infection. Nothing is known about the reaction to PPD of other patients from whose sputa *M. shimoidei* has been previously isolated, and nothing, as far as we know, is known about the possible cross-reactivity of the antigens of this species and PPD.

No hypothesis about the possible route of infection can be made; we know only that the patient was a farmer who had never gone abroad and consequently could not have been in any of the countries in which *M. shimoidei* had previously been isolated.

The number of strains tested is too small to draw certain conclusions about the drug susceptibility of *M. shimoidei*; however results of previous tests (2, 5) an those obtained in the present study suggest that this species is susceptible to ethambutol but resistant lo isoniazid and rifampin (Table 1).

Quite inexplicable is the extreme rarity of isolation of this microorganism, above all if we consider that *M. shimoidei* does not have unusual growth requirements. The fact that almost all microbiological handbooks do not mention this mycobacterial species may be only in part responsible for this phenomenon, since in many countries the identification of mycobacteria other than *M. tuberculosis is* performed in reference (third-level) laboratories that certainly are aware of this novel species.

Only the isolation of more strains will. shed further light on M. shimoidei, which, even though uncommon, deserves to be studied intensively because of its probable pathogenicity.

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