

Mycobacterium thermoresistibile: a New Pathogen for Humans

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The first evidence of the potential pathogenicity of *Mycobacterium thermoresistibile* is presented. This mycobacterium, initially identified as *Mycobacterium gordonae*, was isolated repeatedly from sputum, a bronchoscopy specimen, and later, an open lung biopsy. The distinctive characteristics are described, including the unique ability of the organism to grow at 52°C.

Sputum cultures and a culture of fluid from a bronchoscopy specimen obtained from a patient hospitalized with a respiratory tract infection yielded moderate amounts of yellowish-pigmented colonies identified in the hospital laboratory as *Mycobacterium gordonae*. Smears from all clinical specimens were negative for acid-fast bacilli. One of the authors (M.C.), suspecting that these isolates could be the etiological agent, requested the Mycobacteriology Unit of the Bureau of Laboratories, New York City Department of Health, to verify the identification.

Case report. A middle-aged Caucasian woman was admitted to the hospital for fevers of up to 38.2°C. The patient had had weight loss (20 lbs [9,071.84 g]), cough, and fever for approximately 3 weeks. She first became ill in Hawaii, where she complained of an upper respiratory tract infection. On admission to the hospital, she was noted to be chronically ill and febrile to 37.8°C. She had minimal physical findings except for a nonproductive cough, fever, and rales at both bases. There was no evidence of immunoincompetency. A chest X ray, however, revealed several small cavities interpreted as abscesses. Her purified protein derivative skin tests were negative to 5 TU and 250 TU. She was treated with penicillin for a presumed aspiration pneumonia and showed no response. Various drugs and combinations of drugs were tried, and again she showed no response. The patient continued to run a low-grade fever and experience gradual weight loss over several months. A sputum culture obtained early on admission grew an atypical mycobacterium initially thought to be *M. gordonae*. A fiberoptic bronchoscopy was

performed which showed greyish bronchi thought to reflect chronic disease. A culture of fluid obtained at this time revealed the same mycobacteria as the sputum culture. Because the patient continued to run low-grade fevers, an open lung biopsy was performed. Tissue obtained at this time revealed numerous microabscesses and granulomata with giant cells of the Langhans type. No bacteria, mycobacteria, or fungi were seen in the tissue sections or on acid-fast smears. A culture of this tissue also grew the same mycobacteria as those which the two previous cultures had grown. The patient was placed on rifampin, ethambutol, and streptomycin, and over a period of 1 week, she became afebrile, felt subjectively improved, and gained weight. Her chest X ray also showed improvement.

The laboratory received one culture from sputum followed by cultures from another sputum specimen and a bronchoscopy specimen. These were streaked on Middlebrook 7H11 medium and incubated at 35°C in an atmosphere of 5% CO₂.

Three yellowish colony types were obtained from each of these specimens: one was domed and smooth, one was rough and flat, and one was smooth with an apron. All three colony types and a culture later obtained from a lung biopsy showed the same properties in the routine tests performed. These properties were as follows: bacilli were acid fast with prominent beading; colonies showed moderately rapid growth (7 days) and were yellowish-orange when young, becoming brown with age; growth occurred at 25°C and was better at 37 and 45°C; niacin negative; nitrate negative (test repeated

five times); tellurite reduction negative; iron uptake negative; arylsulfatase negative after 3 days and after 2 weeks; Tween 80 hydrolysis positive (rapid); catalase 68°C positive and catalase semiquantitative, >45 mm; tolerance to 5% NaCl; urease positive; sensitive to streptomycin (10 µg/ml), ethambutol (5.0 and 10 µg/ml), and rifampin (1 µg/ml); and resistant to streptomycin (2 µg/ml), isoniazid (0.5, 1, and 5 µg/ml), *p*-aminosalicylic acid (2 µg/ml), and ethionimide (20 and 30 µg/ml [Lowenstein Jensen medium]).

Since our isolates did not fit the characteristics of any of the slowly or rapidly growing mycobacteria described in appropriate texts (1-3), we checked our reprint file until we came across a paper by Tsukamura on a study of Adansonian classification of mycobacteria (4). With the exception of the nitrate test, our isolates appeared to match the properties of *M. thermoresistibile* listed in Table 10 of reference 4. *M. thermoresistibile* shares with *M. phlei* the unique property of being thermoresistant and growing well at 52°C. When we performed this test, we found that our isolates were not only capable of growth at that temperature but also were able to survive exposure to 60°C for 4 h. Our negative controls, *M. flavescens*, *M. gordonae*, and *M. scrofulaceum*, did not survive these tests.

The isolate from the lung was sent for confirmation to Michio Tsukamura, who originally described the characteristics of *M. thermoresistibile* (4), and to others in the United States and abroad. Tsukamura concluded after the performance of 120 tests that the culture from the lung was indeed *M. thermoresistibile* and fell within the range of variation of that species, as described in a study of 44 isolates (5). In that study, for example, 41 of 44 strains reduced nitrate, but three did not.

P. A. Jenkins (Cardiff, United Kingdom) reported our culture to be faintly scotochromic but unclassified, since it did not fit into their system of classification and had no specific lipid pattern. He also found this isolate to be sensitive to streptomycin, rifampin, ethambutol, and cycloserine but resistant to isoniazid, *p*-aminosalicylic acid, ethionimide, capreomycin, and thiacetazone. One laboratory reported the culture to be "probably *M. thermoresistibile*," and one laboratory reported, "Unable to classify, moderately rapid growing AF, *M. thermoresistibile*?" Jean Hawkins at the West Haven, Conn. Veterans Administration Hospital obtained results in agreement with ours in routine tests except

for sensitivity to streptomycin at 2.0 µg/ml, and she is submitting this isolate to the International Working Group on Mycobacterial Taxonomy for studies. She also found our isolate to be sensitive to 250 µg of hydroxylamine per ml and resistant to 1.0 µg of thiophene-2-carboxylic acid hydrazide (TCH) per ml. R. C. Good and Vella A. Silcox at the Centers for Disease Control, Atlanta, Ga., obtained growth even on 10 µg of TCH per ml. Their results were, however, negative for 5% NaCl. We found that optimal growth was at 45°C, at which point growth occurred in 7 days or less. This is in general agreement with the results reported by other laboratories. *M. thermoresistibile* has been described by Tsukamura as both a rapid (4) and slow grower (5). He and his colleagues recently reported that *M. thermoresistibile* forms a unique taxonomic cluster with *M. flavescens*, intermediate between the slowly growing and rapidly growing mycobacteria (6). We also concluded that growth was intermediate if the organism was incubated at 37°C.

M. thermoresistibile is listed in *Bergey's Manual of Determinative Bacteriology*, 8th ed., as "species incertae sedis" (1) but is included on the approved list of mycobacterial species as reported by Kubica (2).

This report provides the first evidence of the potential pathogenicity of this species (personal communication from Tsukamura). Tsukamura reported that 39 of the 44 isolates he studied were obtained from the soil in Japan and form a homogeneous group (4). The sources of the other isolates were not revealed. It is of interest that our patient visited a "hot springs" in Hawaii shortly before developing her respiratory tract ailment. One can conjecture as to whether *M. thermoresistibile* is limited to the Far East, has been erroneously reported elsewhere as *M. gordonae*, or has simply been dismissed without identification.

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