

Clinical Relevance of *Mycobacterium szulgai* in The Netherlands

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Background. The clinical relevance of *Mycobacterium szulgai* isolates is unknown, and available literature focuses on case reports of *M. szulgai* disease. We assessed the clinical relevance of *M. szulgai* isolated from patients in The Netherlands.

Methods. We reviewed medical files for all 21 patients in The Netherlands from whom *M. szulgai* was isolated during 1999–2006, applying the diagnostic criteria of the American Thoracic Society for nontuberculous mycobacterial infection. Random amplified polymorphic DNA genotyping was performed using IS986, OPA-2, and OPA-18 as primers.

Results. Of the 21 patients, 16 (76%) met the American Thoracic Society diagnostic criteria and were thus likely to have *M. szulgai* disease. Pulmonary *M. szulgai* disease was the most common presentation, with extrapulmonary disease restricted to patients with an impaired systemic immunity. Although treatment regimens varied in content and duration, the outcomes were mostly favorable. Both overtreatment and undertreatment were noticed. Random amplified polymorphic DNA genotyping revealed a higher degree of interpatient variability, with limited intrapatient variability, suggesting persisting monoclonal infection and good reproducibility. No genotype was associated with clinical relevance.

Conclusions. Clinical isolation of *M. szulgai* generally represents true disease and demands careful follow-up. Extrapulmonary disease occurs in patients with impaired immunity. Adherence to diagnostic guidelines can be improved.

Mycobacterium szulgai is a slow-growing nontuberculous *Mycobacterium* (NTM) that was first described in 1972 by Marks et al. [1] and that was named after Dr. T. Szulga, who developed the lipid analysis method that identified this NTM as a new species. NTMs are opportunistic pathogens, with local events (e.g., chronic obstructive pulmonary disease [COPD] or healed tuberculosis) or systemic impaired immunity (e.g., receipt of immunosuppressive medication, HIV infection, or hematological malignancy) as the predisposing condition [2]. Pulmonary *M. szulgai* disease, which resembles tuberculosis, is the most common presentation, al-

though extrapulmonary and disseminated disease has been recorded [1–6].

M. szulgai has been recovered from environmental sources, including a snail, aquarium water, swimming pool water, and tropical fish [3–6]. The environment is the suspected source of human NTM infection [4, 7]. Therefore, *M. szulgai* disease has to be distinguished from pseudoinfection because of the occasional presence of *M. szulgai* in clinical samples or contamination of samples in the laboratory [2, 7].

The American Thoracic Society (ATS) published guidelines for the diagnosis and treatment of NTM infection [2]. The guidelines consist of clinical, radiologic, and bacteriologic criteria and are summarized in figure 1.

We reviewed the medical files of all patients in The Netherlands from whom *M. szulgai* was isolated during the period from January 1999 through January 2006. We assessed the frequency and clinical relevance of *M. szulgai* isolation using the current ATS diagnostic criteria [2], and we evaluated drug susceptibility, treat-

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<p>American Thoracic Society Diagnostic Criteria of Nontuberculous Mycobacterial Lung Disease</p> <p>Clinical criteria</p> <ol style="list-style-type: none"> 1. Pulmonary symptoms, nodular or cavitary opacities on chest radiograph, or an HRCT scan that shows multifocal bronchiectasis with multiple small nodules. <p>and</p> <ol style="list-style-type: none"> 2. Appropriate exclusion of other diagnoses. <p>Microbiological criteria</p> <ol style="list-style-type: none"> 1. Positive culture results from at least two separate expectorated sputum samples. (If the results from the initial sputum samples are nondiagnostic, consider repeat sputum AFB smears and cultures.) <p>or</p> <ol style="list-style-type: none"> 2. Positive culture results from at least one bronchial wash or lavage. <p>or</p> <ol style="list-style-type: none"> 3. Transbronchial or other lung biopsy with mycobacterial histopathologic features (granulomatous inflammation or AFB) and positive culture for NTM or biopsy showing mycobacterial histopathologic features (granulomatous inflammation or AFB) and one or more sputum or bronchial washings that are culture positive for NTM.

Figure 1. Summary of the 2007 American Thoracic Society diagnostic criteria

ment regimens, and outcome. In addition, we analyzed the genotypes of all clinical isolates that were evaluated, to study the molecular epidemiology of *M. szulgai* in The Netherlands.

METHODS

To determine clinical relevance, we examined the medical records of all patients in The Netherlands from whom *M. szulgai* was isolated between January 1999 and January 2006. We recorded sex, age, predisposing factors, symptoms, chest imaging results, treatment and outcome, drug susceptibility, and status in accordance with the diagnostic criteria of the ATS [2]. We defined cure as clinical and radiographic improvement during treatment and absence of positive culture results after completion of treatment.

In all patients, the isolates were subjected to laboratory diagnosis by the Dutch National Institute for Public Health and the Environment (RIVM; Bilthoven, The Netherlands) or a self-sufficient hospital laboratory. All self-sufficient hospitals used 16S rDNA gene sequence analysis for identification and granted access to their databases, to ensure full national coverage. The RIVM is the national reference laboratory that performs identification, drug susceptibility testing, and epidemiological typing of mycobacterial isolates for all hospitals in The Netherlands. At the RIVM, we used 16S rDNA gene sequence analysis to identify mycobacteria, after ruling out the *M. tuberculosis* complex with a Hain GenoType MTBC strip (Hain Lifescience) or the more common species of NTM with an INNO-LiPA Mycobacteria v2 (Innogenetics) reverse line-blot. *M. szulgai* is not incorporated in this line-blot.

We used the agar dilution method for drug susceptibility testing. Drugs tested were isoniazid, rifampicin, ethambutol, streptomycin, cycloserine, prothionamide, amikacin, ciprofloxacin, clofazimine, clarithromycin, and rifabutin [8].

Genotypes were determined using random amplified polymorphic DNA (RAPD) fingerprinting [9]. We selected OPA2, OPA18, and IS986 as primers. The study was approved by the regional ethics committee.

RESULTS

Files were reviewed for 21 patients. The patients' clinical characteristics are detailed in table 1. Sixteen patients (76%) met the current ATS diagnostic criteria and therefore we considered them to have *M. szulgai* disease. Eighteen patients (86%) were male, and 20 (95%) were of Dutch origin; the mean age was 56 years. Most primary *M. szulgai* isolates were obtained from pulmonary samples (sputum samples, 7 isolates [33%]; bronchoalveolar lavage fluid samples, 8 isolates [38%]). Remaining primary isolates were obtained from skin specimens (2 isolates [10%]) and from feces, joint aspirate, lymph node biopsy, and pleural fluid samples (1 isolate each [5%]). Primary samples tested positive for acid-fast bacilli by direct microscopy for just 4 patients (19%). There were no relapses of prior *M. szulgai* disease. Remarkably, 3 patients had a history of Billroth II gastrojejunostomy; all 3 had *M. szulgai* disease. Impaired systemic immunity was associated with extrapulmonary disease (OR, 4.0; 95% CI, 1.71–9.35; $P = .006$) but not with true disease overall ($P = .647$).

Of the 15 patients with pulmonary isolates (patients 7–21), 11 (73%) had true disease (table 1). Thirteen patients (87%) were male, and all were of Dutch origin; the mean age was 60 years. Twelve patients (80%) had preexisting pulmonary disease, mostly COPD (10 patients [67%]), and 11 patients (73%) were current or past smokers. Impaired immunity was noted in 2 patients (diabetes for one patient and use of TNF- α -neutralizing agents for the other).

Reported symptoms included productive cough (14 patients [93%]), hemoptysis (4 patients [27%]), dyspnea (8 patients [53%]), weight loss (8 patients [53%]), and fever and malaise (6 patients for each [40%]). All patients underwent chest radiography, and abnormalities were noted in 14 patients. Infiltrates (7 patients [47%]), cavities (6 patients [40%]), bronchiectasis (3 patients [20%]), nodular opacities (1 patient [7%]), pleural thickening (4 patients [27%]), and scars due to previous lung disease (7 patients [47%]) were observed. In 10

Table 1. Clinical characteristics of all 21 patients from whom *Mycobacterium szulgai* isolates were recovered.

Patient	Sex/age, years	No. of positive culture results/no. of cultures (smeared result)	Sample cultured	Risk factor	Symptom(s)	Chest radiograph finding(s)	ATS criteria	Treatment (duration, months)	Outcome
1	M/62	5/7 (-)	Skin	Steroid treatment ^a	Nodular skin lesions	No abnormalities	Met	Eth, Cla, Cip (12)	Cured
2	M/19	1/1 (-)	Lymph node	IFN- γ RD	Painless swollen neck	No abnormalities	Met	Eth, Cla, Cip (9)	Cured
3	F/90	1/1 (-)	Pleural fluid	None	PC, dyspnea, malaise/fatigue	Infiltrate, pleural fluid collection	Not met	None	NA
4	M/37	1/4 (-)	Feces	HIV infection (CD4 cell count, 20 cells/ μ L)	PC, dyspnea, WL, malaise/fatigue	Nodules	Met	Eth, Rb, Cla (26)	Cured
5	M/41	4/4 (+)	Skin	HIV infection (CD4 cell count, 40 cells/ μ L), B2G	Skin ulcers	No abnormalities	Met	Eth, Rb, Cla, Ofi (6)	Death
6	M/17	1/1 (+)	Joint	IFN- γ RD	Ankle joint swelling, fever, NS, WL, malaise/fatigue	Nodules, coarse linear scarring	Met	Mer, Ofi, Dox (2); Eth, Rif, Cla, Ofi (18)	Cured
7	M/71	3/7 (-)	BAL	COPD, TB	PC	Infiltrate, coarse linear scarring	Met	Izd, Eth, Rif, Cip (12)	Death
8	M/55	1/3 (-)	Sputum	COPD	PC, dyspnea, fever	Infiltrate, bulla, air-fluid level, emphysema	Not met	Rif, Eth, Cla (12)	NA
9	M/87	2/5 (-)	BAL	B2G	PC, WL, malaise/fatigue	Infiltrate, coarse linear scarring	Met	None	Death
10	F/54	1/1 (-)	BAL	COPD, TB	Hemoptysis	Emphysema	Not met	None	NA
11	M/44	6/9 (-)	Sputum	Diabetes	PC, fever, WL	Cavities, infiltrate	Met	Rif, Eth, Cla (12)	Cured
12	M/53	6/12 (-)	BAL	Anti-TNF, COPD	PC, dyspnea, fever	Infiltrate, cavities, emphysema	Met	Rif, Eth, Cla (12)	Cured
13	M/49	1/7 (-)	BAL	COPD	PC, dyspnea	Pulmonary mass	Not met	None	NA
14	M/67	2/4 (+)	Sputum	COPD, BR	Hemoptysis, WL, malaise/fatigue	Cavities, pleural thickening, BR, emphysema, coarse linear scarring	Met	Izd, Rif, Pyr, Eth (1); Rif, Eth, Cla (12)	Cured
15	M/70	6/6 (-)	BAL	COPD, TB	PC, dyspnea	Fibrosis, coarse linear scarring	Met	Refused	Deteriorated
16	M/44	4/4 (+)	Sputum	Metabolizing fluid exposure, smoking	PC, dyspnea, WL	Cavities	Met	Izd, Rif, Pyr, Eth (5); Rif, Eth, Cla (7)	Cured
17	M/68	2/3 (-)	BAL	COPD, BR, B2G	PC, dyspnea, fever, WL, malaise/fatigue	Infiltrate, emphysema, BR, nodules, pleural thickening, coarse linear scarring	Met	Izd, Rif, Pyr, Eth (1); Rif, Eth, Cla (6)	Cured
18	M/77	1/2 (-)	BAL	COPD	PC, NS, WL, malaise/fatigue	Infiltrate, cavities, pleural thickening, coarse linear scarring	Met	None	Death
19	M/38	1/3 (-)	Sputum	HIV (CD4 cell count, 280 cells/ μ L)	PC, dyspnea, fever, WL, malaise/fatigue	No abnormalities	Not met	None	NA
20	M/59	2/4 (-)	Sputum	Asthma, recurrent RTIs	Hemoptysis	Emphysema, BR, coarse linear scarring	Met	None	Cured
21	F/70	4/6 (-)	Sputum	COPD, TB	Hemoptysis, dyspnea, fever, WL, malaise/fatigue	Cavities, emphysema, pleural thickening, coarse linear scarring	Met	None	Death

NOTE. Anti-TNF, TNF- α neutralizing treatment; BAL, bronchoalveolar lavage; BR, bronchiectasis; B2G, Billroth II gastrojejunostomy; Cip, ciprofloxacin; Cla, clarithromycin; COPD, chronic obstructive pulmonary disease; Dox, doxycycline; Eth, ethambutol; Izd, isoniazid; Mer, meropenem; NA, not applicable; NS, night sweats; Ofi, ofloxacin; PC, productive cough; Pyr, pyrazinamide; Rb, rifabutin; RD, receptor deficiency; Rif, rifampicin; RTI, respiratory tract infection; TB, prior tuberculosis; WL, weight loss.

^a Receipt of >15 mg of prednisone per day for >3 months.

patients, additional CTs of the thorax were performed, revealing 1 additional case of cavitory disease and 1 additional case of nodular bronchiectatic disease (in patients 7 and 20, respectively) (table 1). All other CT findings were concomitant with the findings on the chest radiograph.

Six patients (patients 1–6) had *M. szulgai* isolates recovered from extrapulmonary sites (table 1). Five (83%) of the 6 patients met the ATS diagnostic criteria, having skin (2 patients) and joint, intestinal, and lymph node infections (1 patient each). The skin, joint, and intestinal diseases represented disseminated disease. Five patients (83%) were male, and 5 (83%) were of Dutch origin; the mean age was 44 years. Predisposing conditions were HIV infection (2 patients [33%]), IFN- γ receptor deficiency (2 patients [33%]), and receipt of high-dose steroid treatment (1 patient [17%]). Aside from local signs and symptoms, which are detailed in table 1, patients reported productive cough (3 patients [50%]), dyspnea (4 patients [67%]), malaise (3 patients [50%]), and weight loss (2 patients [33%]). Three patients had abnormalities noted by chest radiography that consisted of nodular opacities in 2 patients (patients 4 and 6) and an infiltrate and pleural fluid collection in 1 patient (patient 3). Similar findings were noted in additional CTs of the thorax.

Reported symptoms and radiographic abnormalities were not significantly associated with fulfillment of the ATS diagnostic criteria, neither overall nor specifically among patients infected with pulmonary or extrapulmonary isolates.

A total of 12 patients received antimycobacterial treatment, of whom 11 met the former ATS diagnostic criteria, which were available during the studied period [10]. On average, treatment lasted for 12 months (range, 6–26 months) and consisted of rifampicin or rifabutin with ethambutol and clarithromycin and/or a quinolone antibiotic. Two patients (patients 2 and 6) received concurrent treatment with IFN- γ because of a proven underlying IFN- γ -receptor deficiency. Follow-up sputum microscopy or culture during treatment was not performed at regular intervals; 5 patients with pulmonary *M. szulgai* disease were known to have negative culture results after 6 months of treatment. Treatment outcomes are detailed in table 1; we recorded no failures or relapses. Two patients died: one died during treatment, and the other died just after therapy (due to HIV-related cryptococcal meningitis in one and lung cancer in the other). The average duration of follow-up after treatment was 42 months (range, 9–84 months). Three patients met the former ATS diagnostic criteria but were not treated; 1 died, 1 refused treatment, and 1 experienced conversion of culture results to negative. No signs of active NTM disease were noted during the follow-up for patients who did not meet the ATS criteria. Isolation measures were taken for 4 patients until PCR results for *M. tuberculosis* complex were proven to be negative.

In vitro intermediate susceptibility to isoniazid (MIC, 0.5–

1 $\mu\text{g}/\text{mL}$) and susceptibility to all other compounds in the test panel was noted for all isolates. The MICs for rifampicin, ethambutol, and clarithromycin were 0.5–1, 2–5, and <2 $\mu\text{g}/\text{mL}$, respectively.

Genotyping of the isolates from our study period revealed little inpatient and good interpatient variability (figure 2). Four major groups of strains are apparent. No associations between genotype and predisposing factors, patient origin, or clinical relevance were observed. Clustering that potentially indicates (pseudo-)epidemics or laboratory cross-contamination was not recorded. Two patients (patients 14 and 15, represented by lanes M and N in figure 2) had similar genotypes of *M. szulgai*, which was cultured in different laboratories and during different periods. They were not epidemiologically linked. In 1 patient (patient 5, represented by lanes W/X and Y in figure 2), 2 different *M. szulgai* strains were isolated; one sample (shown in lane Y) was cultured from feces, whereas the others (shown in lanes W and X) were from skin lesions.

DISCUSSION

M. szulgai isolation was found to be clinically relevant in 16 (76%) of the 21 patients included in this study. O'Brien et al. [11] also reported a high rate of true *M. szulgai* infection (8 [57%] of 14 patients) in the first national survey from the United States. The current ATS criteria state that “cultures yielding *M. szulgai* almost always have a pathological significance” [2, p. 401]; our findings are in accordance with this. No specific determinants significantly associated with *M. szulgai* disease were observed; the limited number of patients and the observed comorbidities do not allow greater statistical certainty. Previous literature on *M. szulgai* disease focused mainly on case reports [1, 3–6, 12, 13]. To our knowledge, we report the largest group of isolates examined thus far for their clinical relevance. Still, we may have missed *M. szulgai* isolates, because 16S rDNA gene sequencing is not a free service at the national reference laboratory, whereas identification by the INNO-LiPA Mycobacteria v2 reverse-line blot method—which does not recognize *M. szulgai*—is. However, few strains that are unidentifiable to the species level by this method are not sequenced, on the basis of an appraisal of the costs by the referring hospital. If cases of *M. szulgai* isolation have been missed, they will thus be very few. In addition, strains submitted for drug susceptibility testing are always identified to the species level, with 16S rDNA sequencing applied as appropriate.

The rate of extrapulmonary disease (31% of all cases of *M. szulgai* disease) was similar to that reported in prior publications by Marks et al. [1] (43%), Benator et al. [3] (29%), and Maloney et al. [12] (33%). This may reflect the degree of impairment of immunity seen in this patient category, although it might also be a species-specific phenomenon. We considered 1 positive pleural fluid culture for a patient with *Streptococcus*

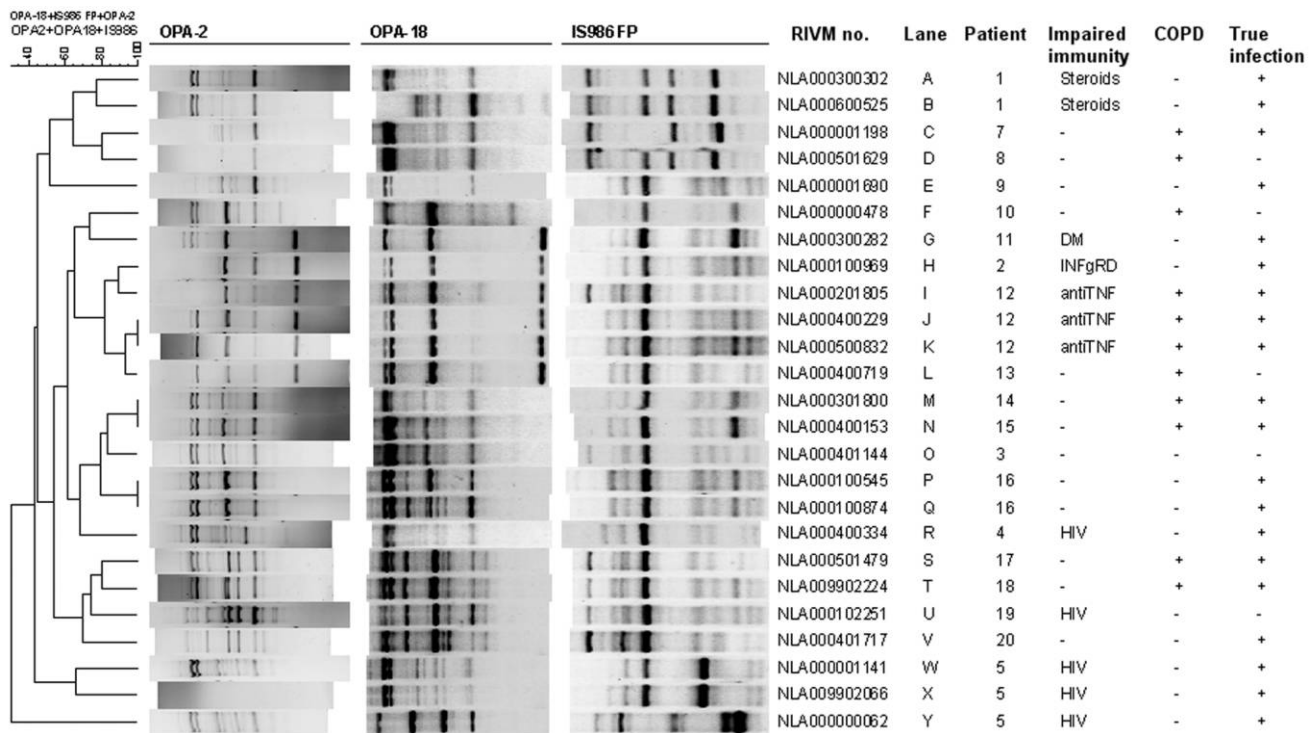


Figure 2. Random amplified polymorphic DNA (RAPD) genotyping results of 25 strains isolated from 19 patients. Four major strain types are represented: lanes A–E, type 1; lanes F–R, type 2; lanes S–V, type 3; and lanes W–Y, type 4. No association with geographical region, clinical relevance, or specific predisposing conditions exists. Limited inpatient variability is noted for patients with >2-year intervals between follow-up cultures (lanes A and B and lanes I and J). Patient 5 has 2 distinct types from different body sites (lanes W/X and Y). Anti-TNF, TNF- α -neutralizing treatment; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus type 1; INFgRD, IFN- γ receptor deficiency; RIVM, Dutch National Institute for Public Health and the Environment.

pneumoniae pneumonia with pleural effusion to have been contaminated on the basis of the patient's clinical and radiographic improvement after receipt of treatment with penicillin, with complete resolution of the pleural fluid collection. The stool sample from 1 patient with HIV/AIDS was considered to be representative of disseminated infection, as determined on the basis of a low CD4 count, symptoms, and associated chest radiography and CT abnormalities, which improved—as did the patient's symptoms—after the initiation of antimycobacterial therapy; both interpretations remain debatable, because the pathway to diagnosis was not optimal.

The clinical and demographic features of our study group are comparable to those in previous reports [1–5, 12, 13]. The number of patients with previous Billroth II gastrectomy was an unexpected observation; gastric surgery has been recognized as a risk factor for the development of tuberculosis [14]. Maloney et al. [12] reported 1 case of *M. szulgai* disease with partial gastrectomy, smoking, and alcohol abuse as predisposing factors. The 3 patients in our study also had multiple predisposing factors: they were smokers, with AIDS, COPD, and old age as individual risk factors. It remains controversial whether gastric surgery is a predisposing condition or a result

of other predisposing conditions (e.g., heavy smoking and alcohol abuse) that cause gastric ulcers that require surgery. Contrary to reports of *Mycobacterium avium* complex or *Mycobacterium abscessus* pulmonary disease in the United States [2], we noted pulmonary *M. szulgai* disease only in patients with established predisposing conditions. This finding may reflect a difference in the pathophysiology of infections with different NTMs.

In our cohort, we observed a favorable treatment outcome for all but 2 patients, and none of the cases in our study constituted relapses of prior *M. szulgai* disease. Previous studies recorded similar results, with the few relapses attributable to noncompliance with drug treatment or receipt of an inadequate drug regimen [1, 3, 12, 13]. Our findings imply that the observed average treatment regimen—12 months of rifampicin, ethambutol, and clarithromycin—leads to favorable outcomes without bacteriological relapse. In vitro drug susceptibility testing results supported this assumption. Randomized, controlled trials are needed to investigate the optimal drug treatment and its duration. We realize, however, that these studies are difficult to perform, because they require a large, multiple-center approach to collect a large enough sample size. In our cohort,

the treatment regimens were based on data from the available literature or expert consultation [10]; the susceptibility of the isolates to the most frequently used drugs did not necessitate changes in therapy regimens.

We observed possible instances of both undertreatment (i.e., patients who met the diagnostic criteria were not treated) and overtreatment (i.e., treatment of persons who did not meet diagnostic criteria). This, as well as the lack of bacteriological follow-up during treatment and handling of the uncertain cases of extrapulmonary disease discussed above, could reflect a lack of knowledge of and experience with the diagnosis and management of NTM disease in physicians or clinical circumstances that were not captured in our file review. Both over- and undertreatment can be harmful for patients.

The mostly unique genotypes imply acquisition of *M. szulgai* from the local environment rather than human-to-human transmission or laboratory contamination, as was found in previous studies [4, 7, 9]. The uncertain pathogenesis and environmental reservoirs, as well as the low frequency of isolation, are important drawbacks to these molecular epidemiological studies and to the interpretation of their results. The absence of an association between RAPD genotype and fulfillment of the ATS diagnostic criteria may reflect the importance of patient—rather than mycobacterial—factors in the etiology of *M. szulgai* disease. The limited inpatient variability suggests good reproducibility and persisting monoclonal infections. In 1 patient (patient 5), 2 infecting strains were found in specimens from separate body sites (as denoted in lanes W/X and Y in figure 2). The minor changes observed in serial isolates may hint at either limited reproducibility of RAPD genotyping or genetic drift of the infecting strain during chronic infection.

In conclusion, the vast majority (76%) of 21 patients with *M. szulgai* isolates in our study experienced true *M. szulgai* disease. Therefore, clinical *M. szulgai* isolation demands careful follow-up. Extrapulmonary disease occurs in patients with impaired immunity. Stricter adherence to diagnostic guidelines seems desirable. Treatment with rifampicin, ethambutol, and a macrolide antibiotic leads to favorable outcomes; its optimal duration, as well as the efficacy of alternative regimens, requires

additional study. *M. szulgai* is most likely contracted from the environment, although this subject also requires further study.

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Potential conflicts of interest. All authors: no conflicts.

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