PDF hosted at the Radboud Repository of the Radboud University Nijmegen

The following full text is a publisher's version.

For additional information about this publication click this link.
http://hdl.handle.net/2066/69075

Please be advised that this information was generated on 2019-11-15 and may be subject to change.
Clinical Relevance of *Mycobacterium szulgai* in The Netherlands

Jakko van Ingen, Martin J. Boeree, Wiel C. M. de Lange, Petra E. W. de Haas, P. N. Richard Dekhuijzen, and Dick van Soolingen

1Department of Pulmonary Diseases, Radboud University Nijmegen Medical Center, Nijmegen, and 2National Mycobacteria Reference Laboratory, National Institute for Public Health and the Environment, Bilthoven, The Netherlands

**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, although 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-
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 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

![Figure 1. Summary of the 2007 American Thoracic Society diagnostic criteria](http://cid.oxfordjournals.org/figure1.jpg)
<table>
<thead>
<tr>
<th>No. of positive culture results/No. of cultures (smear result)</th>
<th>Sample cultured</th>
<th>Risk factor</th>
<th>Symptom(s)</th>
<th>Chest radiograph finding(s)</th>
<th>ATS criteria</th>
<th>Treatment (duration, months)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 M/62 5/7 (+)</td>
<td>Skin</td>
<td>Steroid treatment&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Nodular skin lesions</td>
<td>No abnormalities</td>
<td>Met</td>
<td>Eth, Cla, Cip (12)</td>
<td>Cured</td>
</tr>
<tr>
<td>2 M/19 1/1 (+)</td>
<td>Lymph node</td>
<td>IFN-γ RD</td>
<td>Painless swollen neck</td>
<td>No abnormalities</td>
<td>Met</td>
<td>Eth, Cla, Cip (9)</td>
<td>Cured</td>
</tr>
<tr>
<td>3 F/90 1/1 (+)</td>
<td>Pleural fluid</td>
<td>None</td>
<td>PC, dyspnea, malaise/fatigue</td>
<td>Infiltrate, plural fluid collection</td>
<td>Not met</td>
<td>None</td>
<td>NA</td>
</tr>
<tr>
<td>4 M/37 1/4 (+)</td>
<td>Feces</td>
<td>HIV infection (CD4 cell count, 20 cells/mL)</td>
<td>PC, dyspnea, WL, malaise/fatigue</td>
<td>Nodules</td>
<td>Met</td>
<td>Eth, Rb, Cla (26)</td>
<td>Cured</td>
</tr>
<tr>
<td>5 M/41 4/4 (+)</td>
<td>Skin</td>
<td>HIV infection (CD4 cell count, 40 cells/mL), B2G</td>
<td>Skin ulcers</td>
<td>No abnormalities</td>
<td>Met</td>
<td>Eth, Rb, Cla, Ofl (6)</td>
<td>Death</td>
</tr>
<tr>
<td>6 M/17 1/1 (+)</td>
<td>Joint</td>
<td>IFN-γ RD</td>
<td>Ankle joint swelling, fever, NS, WL, malaise/fatigue</td>
<td>Nodules, coarse linear scarring</td>
<td>Met</td>
<td>Mer, Ofl, Dox (2); Eth, Rb, Cla, Ofl (18)</td>
<td>Cured</td>
</tr>
<tr>
<td>7 M/71 3/7 (+)</td>
<td>BAL</td>
<td>COPD, TB</td>
<td>PC</td>
<td>Infiltrate, coarse linear scarring</td>
<td>Met</td>
<td>Izd, Eth, Rb, Cip (12)</td>
<td>Death</td>
</tr>
<tr>
<td>8 M/95 1/3 (+)</td>
<td>Sputum</td>
<td>COPD</td>
<td>PC, dyspnea, fever</td>
<td>Infiltrate, bulla, air-fluid level, emphysema</td>
<td>Not met</td>
<td>Rb, Eth, Cla (12)</td>
<td>NA</td>
</tr>
<tr>
<td>9 M/97 2/5 (+)</td>
<td>BAL</td>
<td>B2G</td>
<td>PC, WL, malaise/fatigue</td>
<td>Infiltrate, coarse linear scarring</td>
<td>Met</td>
<td>None</td>
<td>Death</td>
</tr>
<tr>
<td>10 F/64 1/1 (+)</td>
<td>BAL</td>
<td>COPD, TB</td>
<td>Hemoptysis</td>
<td>Emphysema</td>
<td>Not met</td>
<td>None</td>
<td>NA</td>
</tr>
<tr>
<td>11 M/44 5/9 (+)</td>
<td>Sputum</td>
<td>Diabetes</td>
<td>PC, fever, WL</td>
<td>Cavities, infiltrate</td>
<td>Met</td>
<td>RF, Eth, Cla (12)</td>
<td>Cured</td>
</tr>
<tr>
<td>12 M/51 6/12 (+)</td>
<td>BAL</td>
<td>Anti-TNF, COPD</td>
<td>PC, dyspnea, fever</td>
<td>Infiltrate, cavities, emphysema</td>
<td>Met</td>
<td>RF, Eth, Cla (12)</td>
<td>Cured</td>
</tr>
<tr>
<td>13 M/49 1/7 (+)</td>
<td>BAL</td>
<td>COPD</td>
<td>PC, dyspnea</td>
<td>Pulmonary mass</td>
<td>Not met</td>
<td>None</td>
<td>NA</td>
</tr>
<tr>
<td>14 M/67 2/4 (+)</td>
<td>Sputum</td>
<td>COPD, BR</td>
<td>Hemoptysis, WL, malaise/fatigue</td>
<td>Cavities, plural thickening, BR, emphysema, coarse linear scarring</td>
<td>Met</td>
<td>Izd, Rb, Pyr, Eth (1); Rb, Eth, Cla (12)</td>
<td>Cured</td>
</tr>
<tr>
<td>15 M/70 6/6 (+)</td>
<td>BAL</td>
<td>COPD, TB</td>
<td>PC, dyspnea</td>
<td>Fibrosis, coarse linear scarring</td>
<td>Met</td>
<td>Refused</td>
<td>Deteriorated</td>
</tr>
<tr>
<td>16 M/44 4/4 (+)</td>
<td>Sputum</td>
<td>Metalworking fluid exposure, smoking</td>
<td>PC, dyspnea, WL</td>
<td>Cavities</td>
<td>Met</td>
<td>Izd, Rb, Pyr, Eth (9); Rb, Eth, Cla (7)</td>
<td>Cured</td>
</tr>
<tr>
<td>17 M/68 2/3 (+)</td>
<td>BAL</td>
<td>COPD, BR, B2G</td>
<td>PC, dyspnea, fever, WL, malaise/fatigue</td>
<td>Infiltrate, emphysema, BR, nodules, pleural thickening, coarse linear scarring</td>
<td>Met</td>
<td>Izd, Rb, Pyr, Eth (1); Rb, Eth, Cla (6)</td>
<td>Death</td>
</tr>
<tr>
<td>18 M/77 1/2 (+)</td>
<td>BAL</td>
<td>COPD</td>
<td>PC, NS, WL, malaise/fatigue</td>
<td>Infiltrate, cavities, plural thickening, coarse linear scarring</td>
<td>Met</td>
<td>None</td>
<td>Death</td>
</tr>
<tr>
<td>19 M/38 1/3 (+)</td>
<td>Sputum</td>
<td>HIV (CD4 cell count, 280 cells/mL)</td>
<td>PC, dyspnea, fever, WL, malaise/fatigue</td>
<td>No abnormalities</td>
<td>Not met</td>
<td>None</td>
<td>NA</td>
</tr>
<tr>
<td>20 M/59 2/4 (+)</td>
<td>Sputum</td>
<td>Asthma, recurrent RTIs</td>
<td>Hemoptysis</td>
<td>Emphysema, BR, coarse linear scarring</td>
<td>Met</td>
<td>None</td>
<td>Cured</td>
</tr>
<tr>
<td>21 F/70 4/6 (+)</td>
<td>Sputum</td>
<td>COPD, TB</td>
<td>Hemoptysis, dyspnea, fever, WL, malaise/fatigue</td>
<td>Cavities, emphysema, plural thickening, coarse linear scarring</td>
<td>Met</td>
<td>None</td>
<td>Death</td>
</tr>
</tbody>
</table>

**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; Ofl, ofloxacin; PC, productive cough; Pyr, pyrazinamide; Rb, rifabutin; RD, receptor deficiency; Rif, rifampicin; RTI, respiratory tract infection; TB, prior tuberculosis; WL, weight loss.

<sup>a</sup> Receipt of >15 mg of prednisone per day for >3 months.
patients, additional CTs of the thorax were performed, revealing 1 additional case of cavitary 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.

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 pneumoniae, 2 skin lesions, 1 possible pleural fluid collection, and 1 possible case of meningitis, as well as 1 positive blood culture, 1 possible case of meningitis, and 1 positive urine culture, as species-specific phenomena.

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 extrapolunmonary 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 pneumoniae, 2 skin lesions, 1 possible pleural fluid collection, and 1 possible case of meningitis, as well as 1 positive blood culture, 1 possible case of meningitis, and 1 positive urine culture, as species-specific phenomena.

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.

**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 extrapolunmonary 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 pneumoniae, 2 skin lesions, 1 possible pleural fluid collection, and 1 possible case of meningitis, as well as 1 positive blood culture, 1 possible case of meningitis, and 1 positive urine culture, as species-specific phenomena.

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 extrapolunmonary 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 pneumoniae, 2 skin lesions, 1 possible pleural fluid collection, and 1 possible case of meningitis, as well as 1 positive blood culture, 1 possible case of meningitis, and 1 positive urine culture, as species-specific phenomena.

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 extrapolunmonary 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 pneumoniae, 2 skin lesions, 1 possible pleural fluid collection, and 1 possible case of meningitis, as well as 1 positive blood culture, 1 possible case of meningitis, and 1 positive urine culture, as species-specific phenomena.

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 extrapolunmonary 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 pneumoniae, 2 skin lesions, 1 possible pleural fluid collection, and 1 possible case of meningitis, as well as 1 positive blood culture, 1 possible case of meningitis, and 1 positive urine culture, as species-specific phenomena.
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 gastrojejunostomy 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 intrapatient 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.

**Acknowledgments**

**Potential conflicts of interest.** All authors: no conflicts.

**References**


