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Clinical Mycobacterium conspicuum Isolation from Two Immunocompetent Patients in The Netherlands

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CASE REPORT

The first patient, a 63-year-old Dutch male living in the south of The Netherlands, presented at a regional hospital with dyspnea and a productive cough. No fever, malaise, hemoptysis, night sweats, or weight loss was reported. His medical history included chronic obstructive pulmonary disease. Since a chest X ray revealed a single opacity in the right upper lobe, computed tomography scanning (CT) was performed, demonstrating calcification in a mediastinal lymph node and a solid mass with spicule in the right upper lobe, suggestive of malignancy. In the diagnostic workup, a bronchoalveolar lavage was performed. No malignant cells were found in the cytology sample of the lavage fluid. Although no acid-fast bacilli were seen on direct microscopy, the routine mycobacterial culture of the lavage fluid yielded Mycobacterium conspicuum. Serological examination lowered the suspicion of an infectious process, with an erythrocyte sedimentation rate of 5 mm/h and a white blood cell count of 7.8 per nl. Although no malignancy was cytologically proven, a mediastinoscopy was performed for staging of the suspected malignancy. No evidence of either malignancy or mycobacterial infection was found in the lymph node samples. Based on the radiographic image and the mediastinoscopy result, the patient underwent a lobectomy of the right upper lobe and a squamous cell carcinoma was diagnosed histologically. No histological evidence of mycobacterial infection was found. Based on the single positive culture, nonsuspect radiographic image, and alternative diagnosis, the M. conspicuum isolate was considered a contaminant; hence, no antymycobacterial treatment was started.

The second patient was a 65-year-old Dutch male from the eastern part of The Netherlands whose medical history consisted of radiographic stage IV sarcoidosis. His sarcoidosis was not treated with immunosuppressive agents. The patient presented at another regional hospital with dyspnea and a productive cough, without a fever, night sweats, weight loss, or malaise. A chest X ray and CT revealed further progression of preexistent interstitial nodular opacities, thick septa, bronchiectasis, fibrotic scarring, bullae, emphysema, and a ground-glass aspect. During the next 2 years, 13 mycobacterial sputum cultures were performed, 10 of which yielded M. conspicuum; 3 cultures remained negative, although two samples were initially PCR positive for a nontuberculous mycobacterium (NTM) identified as M. conspicuum by sequencing of the PCR product. All acid-fast bacillus smears were negative. The serological parameters of infection were slightly raised; the erythrocyte sedimentation rate ranged between 21 and 32 mm/h, the C-reactive protein concentration ranged between 13 and 44 mg/liter, and the white blood cell count was 12.2 per nl. The disease progressed in these 2 years, with increasingly frequent Pseudomonas aeruginosa infections and chronic hypoxia, making the patient oxygen dependent. It was decided that the patient was infected with the M. conspicuum infection, we would have been treated, and the patient underwent bilateral lung transplantation 2 years and 8 months after the first M. conspicuum culture. The last sputum culture yielding M. conspicuum was performed 8 weeks prior to transplantation. The patient died of respiratory failure 17 days after transplantation; the donor lungs were colonized by P. aeruginosa and an Acinetobacter species. Autopsy revealed an acute necrotizing pneumonia in both lungs, most likely caused by P. aeruginosa. The native lungs were not examined.

Based on the progression of symptoms and radiographic abnormalities as well as multiple positive cultures, this patient met the American Thoracic Society (ATS) diagnostic criteria (1) and thus was likely to have M. conspicuum pulmonary disease. However, fulfillment of these criteria does not necessitate treatment per se; this is a decision based on potential risks and benefits of therapy for the individual patient (1). In addition, the progression of symptoms and radiographic abnormalities may have been due to sarcoidosis alone. For these reasons, no antymycobacterial treatment was initiated. Histological examination of the native lungs could have provided additional proof of M. conspicuum infection. Previously, the condition of persistent culture positivity with little or no clinical or radiographic deterioration has been referred to as colonization. However, airway colonization without infection is an unproven condition for NTM and recent studies with high-resolution CT, summarized in the ATS statement, have re-

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vealed progressive nodular bronchiectatic disease, as in our 
patient, considered to be due to NTM (1).

Since its isolation and subsequent description as a novel 
*Mycobacterium* species in 1995 by Springer et al., no reports on 
clinical *M. conspicuum* isolation have been recorded in the 
literature (2). *Mycobacterium conspicuum* is a rare clinical iso-
phylogenetically most closely related to *Mycobacterium malmoense* 
and *Mycobacterium szulgai* on the basis of 16S 
rRNA gene sequences (2).

In our country, over an 8-year period, bacteria of this species 
have been isolated only from the pulmonary samples of the two 
patients presented. The mycobacteria were cultured in the 
MB/BacT system (Biomerieux, Boxtel, The Netherlands) as 
well as on Middlebrook 7H10, Ogawa, and Coletos-T (Bio-
merieux) solid media at 35°C. Growth was observed after 13 
days in the MB/BacT liquid medium and after 28 to 35 days on 
the solid media. No growth was observed after 35 days on 
Löwenstein-Jensen media with or without pyruvate incubated 
at 35°C. On Middlebrook 7H10 media, colonies were small, 
smooth, and white.

Identification was performed at the Dutch national myco-
bacteria reference laboratory (RIVM) by sequencing of the 
151-bp hypervariable region in the 16S rRNA gene after ruling 
out the *M. tuberculosis* complex by using the Hain GenoType 
*M. tuberculosis* complex (Hain Lifesciences GmbH, Nehren, 
Germany) line probe assay and the more common species of 
NTM by using the INNO-LiPA MYCOBACTERIA v2 (Inno-
genetics, Ghent, Belgium) reverse line blot assay. The obtained 
sequences were compared to those in the Ribosomal Differ-
entiation of Medical Microorganisms database (http://rdna.
ridom.de). All isolates from patient 2 were also identified as 
*M. conspicuum* in the regional hospital laboratory by 16S gene 
sequence analysis.

We performed drug susceptibility testing for four *M. con-
spicuum* isolates (one from patient 1 and three from patient 2), 
using a previously published Middlebrook 7H10 agar dilution 
method (3). The drugs included in the panel, with their break-
point concentrations in parentheses, are isoniazid (1 μg/ml), 
rifampin (1 μg/ml), ethambutol (5 μg/ml), streptomycin (5 
μg/ml), cycloserine (50 μg/ml), prothionamide (5 μg/ml), ami-
kacin (5 μg/ml), ciprofloxacin (2 μg/ml), clofazimine (2 μg/ml), 
clarithromycin (16 μg/ml), and rifabutin (2 μg/ml). Growth at 
the breakpoint concentration is reported as susceptible, and 
growth at higher concentrations of the drug is considered re-
sistant. The results are detailed in Table 1.

The original species description featured two immunocom-
promised patients suffering from disseminated infections (1, 
2). The patients presented here were not known to suffer from 
impairment of systemic immunity, but their preexisting pulmonary 
diseases predisposed them to NTM infection. For patient 1, 
follow-up cultures in the 3 years since *M. conspicuum* isolation 
have yielded other NTM (one *Mycobacterium avium* and one 
*Mycobacterium kansasii*) isolate, indicative of increased suscept-
ibility to mycobacterial infections in general. The demonstra-

tics, with the patients being male, white, and older than the 
average tuberculosis patient, are common for NTM infections 
and probably reflect the characteristics of the predisposing 
lung disease.

To assess the clinical relevance of *M. conspicuum* isolation 
from these patients, we used the diagnostic criteria set in the 
recent ATS statement (1). Although these criteria fit best with 
the *M. avium* complex, *M. kansasii*, and *Mycobacterium absces-
sus*, the authors state that “it is assumed, but not proven, that 
the concepts outlined in these guidelines are pertinent for 
other less common NTM respiratory pathogens” (1).

Our findings indicate that *M. conspicuum* is not only a caus-
itive agent of disseminated infections in immunocompromised 
patients. This species may occasionally cause pulmonary infec-
tions in immunocompetent persons with preexisting pulmonary 
diseases or be a mere contaminant. This is in line with what has 
been found for most other NTM species (1).

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### TABLE 1. In vitro drug susceptibility results for four 
*Mycobacterium conspicuum* strains

<table>
<thead>
<tr>
<th>Drug</th>
<th>MIC (μg/ml) for indicated isolate</th>
<th>Overall status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>No. 2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Rifampin</td>
<td>&gt;5</td>
<td>5</td>
</tr>
<tr>
<td>Rifabutin</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>5</td>
<td>≤1</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Amikacin</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>≤2</td>
<td>≤2</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>≤1</td>
<td>≤1</td>
</tr>
<tr>
<td>Cycloserine</td>
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<td>10</td>
</tr>
<tr>
<td>Prothionamide</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Clofazimine</td>
<td>2</td>
<td>0.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> Isolate from patient 1.

<sup>b</sup> Isolate from patient 2.