

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/2066/51520>

Please be advised that this information was generated on 2018-04-19 and may be subject to change.

**Clinical *Mycobacterium conspicuum*
Isolation from Two Immunocompetent
Patients in The Netherlands**

J. van Ingen, M. J. Boeree, F. S. Stals, C. C. M. Pitz, J. J. C.
M. Rooijmans-Rietjens, A. G. M. van der Zanden, P. N. R.
Dekhuijzen and D. van Soolingen
J. Clin. Microbiol. 2007, 45(12):4075. DOI:
10.1128/JCM.00867-07.
Published Ahead of Print 17 October 2007.

Updated information and services can be found at:
<http://jcm.asm.org/content/45/12/4075>

	<i>These include:</i>
REFERENCES	This article cites 3 articles, 3 of which can be accessed free at: http://jcm.asm.org/content/45/12/4075#ref-list-1
CONTENT ALERTS	Receive: RSS Feeds, eTOCs, free email alerts (when new articles cite this article), more»

Information about commercial reprint orders: <http://journals.asm.org/site/misc/reprints.xhtml>
To subscribe to to another ASM Journal go to: <http://journals.asm.org/site/subscriptions/>

Clinical *Mycobacterium conspicuum* Isolation from Two Immunocompetent Patients in The Netherlands[∇]

J. van Ingen,^{1,2*} M. J. Boeree,¹ F. S. Stals,³ C. C. M. Pitz,⁴ J. J. C. M. Rooijmans-Rietjens,⁵
A. G. M. van der Zanden,⁶ P. N. R. Dekhuijzen,¹ and D. van Soolingen²

Dept. of Pulmonary Diseases, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands¹; National Mycobacteria Reference Laboratory, National Institute for Public Health and the Environment, Bilthoven, The Netherlands²; Dept. of Clinical Microbiology, Laurentius Hospital, Roermond, The Netherlands³; Dept. of Pulmonary Diseases, Laurentius Hospital, Roermond, The Netherlands⁴; Dept. of Pulmonary Diseases, Gelre Hospital, Apeldoorn, The Netherlands⁵; and Dept. of Clinical Microbiology, Gelre Hospital, Apeldoorn, The Netherlands⁶

Received 25 April 2007/Returned for modification 1 June 2007/Accepted 21 September 2007

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 spiculae 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 antimycobacterial 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 sarcoidosis, rather than the *M. conspicuum* infection, would be 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 antimycobacterial 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-

* Corresponding author. Mailing address: RIVM; LIS, National Mycobacteria Reference Laboratory, Antonie van Leeuwenhoeklaan 9, P.O. Box 1, 3720 BA Bilthoven, The Netherlands. Phone: 31-30-2742886. Fax: 31-30-2744418. E-mail: jakko.van.ingen@rivm.nl.

[∇] Published ahead of print on 17 October 2007.

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 isolate phylogenetically most closely related to *Mycobacterium malmhoense* 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 (Biomérieux, Boxtel, The Netherlands) as well as on Middlebrook 7H10, Ogawa, and Coletsos-T (Biomérieux) 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 mycobacteria 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 (Innogenetics, Ghent, Belgium) reverse line blot assay. The obtained sequences were compared to those in the Ribosomal Differentiation 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. conspicuum* 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 breakpoint 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), amikacin (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 resistant. The results are detailed in Table 1.

The original species description featured two immunocompromised patients suffering from disseminated infections (1, 2). The patients presented here were not known to suffer from impaired systemic immunity, but their preexisting pulmonary diseases predisposed them to NTM infection. For patient 1,

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

Drug	MIC (mg/liter) for indicated isolate				Overall status
	No. 1 ^a	No. 2 ^b	No. 3 ^b	No. 4 ^b	
Isoniazid	10	2	5	5	Resistant
Rifampin	>5	5	>5	5	Resistant
Rifabutin	0.5	0.5	0.5	0.5	Susceptible
Ethambutol	5	≤1	2	2	Susceptible
Streptomycin	10	5	5–10	5	Variable
Amikacin	10	10	20	10	Resistant
Clarithromycin	≤2	≤2	≤2	≤2	Susceptible
Ciprofloxacin	≤1	≤1	≤1	≤1	Susceptible
Cycloserine	10	10	10	10	Susceptible
Prothionamide	5	5	5	2	Susceptible
Clofazimine	2	≤0.5	1	1	Susceptible

^a Isolate from patient 1.

^b Isolate from patient 2.

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 susceptibility to mycobacterial infections in general. The demographics, 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 abscessus*, 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 causative agent of disseminated infections in immunocompromised patients. This species may occasionally cause pulmonary infections 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).

REFERENCES

- Griffith, D. E., T. Aksamit, B. A. Brown-Elliott, A. Catanzaro, C. Daley, F. Gordin, S. M. Holland, R. Horsburgh, G. Huitt, M. F. Iademarco, M. Iseman, K. Olivier, S. Ruoss, C. Fordham von Reyn, R. J. Wallace, Jr., and K. Winthrop. 2007. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am. J. Respir. Crit. Care Med.* **175**:367–416.
- Springer, B., E. Tortoli, I. Richter, R. Grünwald, S. Rüscher-Gerdes, K. Uschmann, F. Suter, M. D. Collins, R. M. Kroppenstedt, and E. C. Bottger. 1995. *Mycobacterium conspicuum* sp. nov., a new species isolated from patients with disseminated infections. *J. Clin. Microbiol.* **33**:2805–2811.
- van Klingeren, B., M. Dessens-Kroon, T. van der Laan, K. Kremer, and D. van Soolingen. 2007. Drug susceptibility testing of *Mycobacterium tuberculosis* complex using a high throughput, reproducible, absolute concentration method. *J. Clin. Microbiol.* **45**:2662–2668.