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Clinical relevance of *Mycobacterium chelonae–abscessus* group isolation in 95 patients

Jakko van Ingen [a,b,*], Rina de Zwaan [b], Richard P.N. Dekhuijzen [a], Martin J. Boeree [a], Dick van Soolingen [b]

[a] *Department of Pulmonary Diseases, Radboud University Nijmegen Medical Center, PO Box 9101, 6500HB Nijmegen, The Netherlands*
[b] *National Mycobacteria Reference Laboratory, Laboratories for Infectious Diseases and Perinatal Screening, National Institute for Public Health and the Environment (RIVM), PO Box 1, 3720 BA, Bilthoven, The Netherlands*

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Opportunist mycobacteria

Summary *Objectives:* To determine the clinical relevance of *Mycobacterium chelonae–abscessus* group isolation from clinical samples.
Methods: We retrospectively reviewed medical files of all patients from whom these mycobacteria were isolated between January 1999 and January 2005 and re-identified the isolates by *rpoB* sequencing. We applied the American Thoracic Society (ATS) diagnostic criteria to establish clinical relevance.
Results: Ninety-five patients were traced (56 *M. chelonae*, 25 *Mycobacterium abscessus*, 8 *Mycobacterium massiliense*, 6 *Mycobacterium bolletii*). Most isolates were cultured from pulmonary samples in patients with pre-existing pulmonary disease. Among patients with pulmonary isolates, 27% (20/74) meets ATS criteria; *M. abscessus* is most relevant (50%; 9/18), followed by *M. massiliense* (29%; 2/7), *M. bolletii* (20%; 1/5) and *M. chelonae* (18%; 8/44). Extrapulmonary disease presented as disseminated skin disease, eye disease specific for *M. chelonae* and otomastoiditis for *M. abscessus*. Treatment, especially for pulmonary *M. abscessus* disease, yielded limited results.
Conclusions: One-fourth of the patients with pulmonary *M. chelonae–abscessus* group isolates met the ATS criteria; this percentage differs by species. Species distribution and clinical relevance differ from other regions. *M. abscessus* isolation in cystic fibrosis patients warrants special attention. Current ATS criteria might be too lenient to diagnose *M. chelonae–abscessus* group disease.

Introduction

For a long time *Mycobacterium chelonae* and *Mycobacterium abscessus* have been thought to represent the subgroups of a single species, due to overlap in biochemical

* Corresponding author at: National Institute for Public Health and the Environment (RIVM; LIS), Mycobacteria Reference Laboratory, PO Box 1, 3720 BA Bilthoven, The Netherlands. Tel.: +31 30 2742886; fax: +31 30 2744418.
E-mail address: jakko.van.ingen@rivm.nl (J. van Ingen).

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and genetic properties; only in 1992 *M. abscessus* was granted a separate species status, supported by DNA-DNA hybridization of less than 70%.[1] This taxonomical status was recently changed, when subsets of isolates formerly identified as *M. abscessus* were elevated to separate species status, as *Mycobacterium bolletii* and *Mycobacterium massiliense*, based on <97% *rpoB* gene sequence homology.[2] In the Netherlands, the *M. chelonae–abscessus* group bacteria are the most frequently encountered rapidly growing nontuberculous mycobacteria (NTM), making up 55% of all referred rapid growers. (Source: National Mycobacteria Reference Laboratory).

In general, NTM are opportunistic pathogens and pulmonary infections mostly affect patients with pre-existing pulmonary disease. Extrapulmonary disease generally occurs after trauma or in patients with systemic impaired immunity, i.e., immunosuppressive medication, HIV infection or hematological malignancy.[3] Improvements in laboratory facilities for culture and species identification, increasing notification, and growing awareness of their pathogenic potential have led to increased interest in the NTM in general.[3] *M. abscessus* infections in cystic fibrosis (CF) patients, and the problematic resistance of these bacteria to antimycobacterial drugs, have received special attention.[1,3–6]

The environment is the suspected source of infections by NTM, as person-to-person transmission has not been proven.[3,4] Bacteria of these species have been recovered from water and soil.[1,3] Their presence in water and resistance to common disinfectants can result in pseudo-outbreaks due to contamination of laboratory materials[7] or medical equipment such as bronchoscopes.[8] Hence, clinical *M. chelonae*, *M. abscessus*, *M. massiliense* or *M. bolletii* isolation, especially from the respiratory tract, does not represent disease *per se*. To aid in the differentiation between NTM disease and pseudo-infection or contamination, the American Thoracic Society (ATS) provides diagnostic criteria, summarized in Box 1, with a specific emphasis on *M. abscessus*.[3]

In the current study we establish the clinical relevance of *M. chelonae*, *M. abscessus*, *M. massiliense* and *M. bolletii* isolation by studying the clinical and demographical data of patients from whom these species were isolated and determining the percentage of patients that meets the ATS criteria.

Methods

We retrieved the medical records of all patients in the Netherlands from whom *M. chelonae* or *M. abscessus* was isolated between January 1999 and January 2005. We recorded demographical, clinical and microbiological data and status according to the diagnostic criteria by the ATS.[3]

All patient isolates had been subjected to laboratory diagnosis at the National Institute for Public Health and the Environment (RIVM, Bilthoven, the Netherlands), the national reference laboratory. All isolates had been identified as *M. chelonae* or *M. abscessus* using the INNO-LiPA MYCOBACTERIA v2 (Innogenetics, Ghent, Belgium) assay or, prior to 2004, 16S rRNA gene sequence analysis and the picric acid test.[9]

For this study, we re-subjected all isolates to identification using *rpoB* gene sequence analysis.[2] The DNA sequence results are compared to the GenBank (National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov) sequence database.

For all NTM, susceptibility to isoniazid, rifampicin, ethambutol, streptomycin, cycloserine, prothionamide, amikacin, ciprofloxacin, clofazimine, clarithromycin and rifabutin is tested using an agar dilution method,[10] but only upon request of the referring physician.

Pearson χ^2, Fisher exact and *t*-tests were used for statistical correlations. The regional ethics committee approved the study.

Box 1. Summary of the 2007 American Thoracic Society diagnostic criteria.[3]

American Thoracic Society Diagnostic Criteria of Nontuberculous Mycobacterial Lung Disease

Clinical criteria

1. Pulmonary symptoms, nodular or cavitary opacities on chest radiograph, or an HRCT scan that shows multifocal bronchiectasis with multiple small nodules.
   and
2. Appropriate exclusion of other diagnoses.

Microbiological criteria

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.)
   or
2. Positive culture results from at least one bronchial wash or lavage.
   or
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.

HRCT, high resolution computed tomography; AFB, acid-fast bacilli; NTM, nontuberculous mycobacteria.
Note: Three or more pulmonary samples should be analyzed to apply these criteria.