Microbiological Diagnosis of Nontuberculous Mycobacterial Pulmonary Disease

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KEYWORDS

• Nontuberculous mycobacteria • Pulmonary disease • Microbiology • Diagnosis

KEY POINTS

- Despite its central role in the diagnosis of nontuberculous mycobacterial pulmonary disease, few studies have been performed to specifically address the optimization of microbiological diagnosis.
- Given their widespread environmental presence, isolation of nontuberculous mycobacteria (NTM) from specimens of nonsterile body sites such as the respiratory tract does not indicate disease per se.
- Diagnosis of NTM lung disease starts with procuring a good-quality respiratory sample.
- Both liquid and solid media should be incubated to increase sensitivity of culture.
- Clinical relevance differs by species; molecular identification of NTM isolates can aid in the distinction between occasional presence of NTM and true NTM lung disease.

BACKGROUND

Nontuberculous mycobacteria (NTM) are increasingly recognized as causative agents of mostly opportunistic infections of humans. Of all NTM diseases, pulmonary disease is by far the most frequent.[1] Other relatively common NTM diseases are skin infections after inoculation, cervical lymphadenitis in children, and disseminated disease in the severely immunocompromised.[1] Three distinct pulmonary disease manifestations are known: fibrocavitary disease, nodular bronchiectatic disease, and hypersensitivity pneumonitis. All 3 affect distinct patient categories.[2]

Given their widespread environmental presence, isolation of NTM from specimens of nonsterile body sites such as the respiratory tract does not indicate disease per se. Current diagnostic criteria presented in a Statement by the American Thoracic Society and Infectious Disease Society of America (ATS/IDSA) account for this discrepancy between isolation and disease. In short, to diagnose NTM pulmonary disease (NTM-PD), patients should have symptoms and radiologic signs suggestive of NTM-PD, and cultures of multiple respiratory tract samples must grow the same NTM species.[2]

Thus, clinicians and microbiologists face the task of acquiring optimal samples from the respiratory tract and making sure that NTM present in the sample are detected and identified. But what are the optimal methods to acquire and process the samples? And what could be the role of serology in diagnosing NTM-PD? This review summarizes currently available data on techniques involved in the microbiological diagnosis of NTM-PD, and aims to provide a framework for such optimal microbiological diagnosis.

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LITERATURE REVIEW

The literature search was performed using the PubMed database (US National Library of Medicine; National Center for Biotechnology Information: http://www.ncbi.nlm.nih.gov/pubmed). The following Medical Subject Heading (MeSH) terms were used in the search, both alone and in combinations: "Mycobacterium/isolation and purification," "Mycobacterium/microbiology," "Mycobacterium avium-intracellulare Infection/diagnosis," "Mycobacterium Infections, Nontuberculous/diagnosis," and "Mycobacterium Infections, Nontuberculous/microbiology." Only English-language studies involving humans and published after 1990 were included. Case reports, case series, editorials, and literature reviews were excluded from analysis; the review focused on laboratory diagnostic studies. Reference lists of selected articles were searched for further articles for review.

SPECIMEN SUBMISSION

Given the central role of culture in the diagnosis of NTM lung disease, good diagnostics start with procuring a good-quality respiratory sample. Instruction of patients has been shown to increase the yield of acid-fast bacilli (AFB) smears for tuberculosis (TB) diagnostics; in a randomized study, well-instructed patients produced sputum samples of which 39% proved AFB-positive on direct microscopy, versus only 27% in the group that was not instructed.[3] Good instruction is therefore likely to be of benefit to NTM patients as well. The high prevalence of underlying chronic lung diseases in these patients[1,2] makes it likely that some patients have already been instructed on sputum expectoration. Hence, the added benefit may be smaller in NTM than in TB patients.

Visual inspection of the sputum sample is a helpful first assessment of its suitability. Saliva cultures are not useful to diagnose NTM-PD because NTM are occasionally present in the human oral cavity, where they may even be part of the normal commensal flora.[4] Thus, if a sample does not appear mucoid or purulent, it is best to request a new sample. Moreover, the purulence of a sputum sample may serve as a marker for disease severity, as it does in patients with bronchiectasis.[5]

The effect of delay in processing of sputum samples on the microscopy and culture results has not been investigated for NTM. *Mycobacterium tuberculosis* viability decreases if sputum samples are stored at room temperature. In one study, 163 sputum specimens of TB patients were split for immediate processing and storage for 3, 5, or 7 days. Smear microscopy results were not affected by storage duration, but although 92% of samples were culture-positive before storage, this rate diminished to 83% after 3 days of storage at room temperature.[6] In another study in 43 TB patients in Malawi, sputum storage at room temperature and at 4°C were compared; viability of cultures was best preserved by refrigeration, and significant losses in viability only appeared after more than 2 weeks of refrigeration.[7] Similar studies with sputum samples containing *Pseudomonas aeruginosa* showed that bacterial loads remained stable during storage at 4°C, decreased at −20°C, and increased during storage at 25°C for 48 hours.[8] Hence, samples are preferably incubated on relevant media on the same day.[2] Mailing samples to the laboratory is possible without significant losses in sputum yield if the time in the mail system is short (ie, <72 hours) and the sample arrives during normal laboratory opening hours.[9] If the latter fails, refrigeration on arrival is warranted.

HOW MANY SAMPLES AND AT WHICH INTERVALS?

For pulmonary TB diagnosis, the World Health Organization has long used the spot-morning-spot algorithm to obtain 3 consecutive sputum samples within a 24-hour period. After systematic reviews revealed that the increase in sensitivity brought about by the third sample was only 2%, only 2 sputum samples are now requested.[10] For the diagnosis of NTM-PD, this may not be very helpful. Temporary presence of an NTM species in the airways after environmental exposure may lead to consecutive positive samples, yet have no clinical significance. For this reason, the current ATS Statement on NTM disease states that "to establish the diagnosis of NTM lung disease, the collection of three early-morning specimens on different days is preferred."[2] Given the slow course of the disease, an interval of a week ensures that repeat positive cultures are unlikely to reflect a transient contamination of the airways after a single environmental exposure.

The rationale for the multiple sputum specimens and the prerequisite of having at least 2 positive cultures with the same species come largely from a study in Japan, which showed that radiologic evidence of disease (infiltrates or cavitary lesions) and progression was found in 98% of the patients who had 2 or more positive sputum cultures for *Mycobacterium avium* complex (MAC), versus just 2% in those with a single positive culture during 12 months of observation.[11] For 97% of patients, the first 2 positive cultures grew from the initial 3 sputum specimens. This approach may be less applicable to the