Public Health Ontario: Best Practices for Pulmonary Nontuberculous Mycobacteria

Introduction
Nontuberculous mycobacteria (NTM) comprise over 170 species that are widespread environmental organisms found in soil and water, including treated drinking water distribution systems, and may be concentrated in showerheads and faucets (1). Human NTM infections are most often pulmonary and typically cause slowly progressive respiratory and systemic symptoms. Pulmonary NTM infections are increasingly common (2) and difficult to treat. Less common nonpulmonary NTM infections may include: localized infections of skin and soft tissue, lymph nodes, joints or other sites and disseminated disease (3). Disseminated disease occurs almost exclusively in the presence of systemic immune deficiency.

In Ontario, we have observed increased pulmonary NTM isolation and disease over the past two decades while more recently identifying that rates of nonpulmonary NTM isolation are stable or decreasing (4). As health care providers are encountering more individuals who may have NTM disease, we identified a need for clinical guidance on NTM disease and management in Ontario and released a “primer” on NTM with the following content:

- Sample collection and microbiology “disease” versus “colonization”
- Diagnostic and treatment criteria for NTM pulmonary disease
- Most common clinical presentations
- Public health implications
- NTM infections in animals
- NTM and TB co-isolation
- Treatment
- NTM pulmonary disease (NTM-PD) transmission between people
- Environmental sources and avoidance of exposure

As the incidence of NTM infection is increasing in many jurisdictions, some areas of particular interest include public health implications, surveillance and reportability of NTM pulmonary and/or nonpulmonary infections (see below), and developing a knowledge base to support recommendations on prevention/exposure avoidance.

Below are excerpts from “Best Practices for Pulmonary Nontuberculous Mycobacteria”:
Public health implications

NTM-PD is not reportable in Ontario. Although NTM-PD rates have increased over the past decade, it is not clear that there is an effective and timely public health response to these infections, given the widespread exposure to NTM from the environment and the likelihood that individual host susceptibility is the determining factor for developing the disease. In Queensland, Australia, an active microbiological surveillance system, including the routine analysis of case numbers and geographic distribution, has been felt to be helpful in understanding environmental sources of pulmonary NTM (5). Respiratory outbreaks in institutions are reportable, regardless of the causative organism. Additionally, health care providers with concerns about potential community outbreaks should contact public health.

Nonpulmonary NTM infections continue to occur with a low and stable frequency in Ontario. Skin and soft tissue NTM infections usually occur after direct inoculation, although the recent attention focused on risks of exposure to *M. chimaera* from heater-cooler units involved in cardiac surgery indicate that other routes of exposure are possible (6). Direct inoculation may occur during medical procedures, esthetic procedures such as pedicures after soaking in foot baths containing high NTM concentrations, tattooing with contaminated inks, or from working with contaminated fish or aquaria. Although NTM infections are not reportable, identifying the source of nonpulmonary NTM infections may enable prevention of additional cases from contaminated sources. There is very limited literature on the public health response to clusters of nonpulmonary NTM infections given that few jurisdictions have designated these infections reportable, with the notable exception of recently published experience from Oregon (7).

Environmental sources and avoidance of exposure

Although no data supports specific exposure avoidance measures, for individuals with prior NTM-PD or cystic fibrosis (CF) who may be susceptible to NTM-PD, it is likely appropriate to minimize potential exposures. The inhalation of water aerosols carrying NTM organisms could cause lung infection. Hot tubs, showers and humidifiers all generate aerosols containing NTM that may be inhaled. Use of indoor swimming pools (possibly due to the lack of ventilation compared with outdoor pools) has been associated with NTM-PD among individuals with CF (8). Although drinking water is likely associated with lower risk than these activities, aspiration (with or without reflux) may lead to lung infection with organisms in drinking water. Among people at risk of aspiration, avoidance of dispenser systems that could promote reproduction of NTM (e.g., refrigerated filter pitchers, ice dispensers, water dispensers through refrigerators) might reduce exposure. Minimizing inhalation of soil aerosols may reduce the risk of infection with species like *M. intracellulare*, thought to be primarily contracted from soils. Avoiding “turning over” soil and working with dry soil (which may be associated with higher aerosol generation) may reduce inhalation of soil aerosols. Suggested measures have been offered by NTM Info & Research, Inc.

As described above, although NTM infections are not reportable, identifying the source of nonpulmonary infections may enable prevention of additional cases from contaminated sources, including but not limited to such sources as nail salon foot baths, tattoo inks and surgical equipment (9, 10). Due to the pervasiveness of...
potential exposures for pulmonary infections (inhalation of drinking water or soil aerosols), the belief that host susceptibility is the major determinant of disease development, and the uncertainties as to the effectiveness of the public health outcomes in response to cases or clusters, reportability for pulmonary disease would appear to be less relevant. Additional work is required to understand in which circumstances public health investigations and interventions may be useful when cases of NTM-PD are identified.

Helpful resources:

- American Thoracic Society / Infectious Diseases Society of America Guidelines for the Diagnosis and Management of NTM Disease
- Canadian Thoracic Society / Public Health Agency of Canada Nontuberculous Mycobacteria Guidelines
- NTM Info and Research – a non-profit organization providing patient support, medical education, and research funding for NTM
- US Cystic Fibrosis Foundation and European Cystic Fibrosis Society consensus recommendations for managing NTM in individuals with CF

References
The Importance of Macrolides and *erm* Gene Sequencing in Rapidly Growing Mycobacteria Antimicrobial Susceptibility Testing

Introduction
For over two decades, macrolides have been the cornerstone for treatment of many nontuberculous mycobacteria (NTM), including the *Mycobacterium abscessus* complex (composed of three subspecies: *Mycobacterium abscessus* subsp. *abscessus*, subspecies *massiliense* and subspecies *bolletii*) and collectively referred to as the *M. abscessus* complex (1-3). Among these three subspecies, *M. abscessus* subsp. *abscessus*, and *M. abscessus* subsp. *massiliense* are the two most frequent causes of pulmonary infections associated with rapidly growing mycobacteria (RGM), including bronchopulmonary infections in patients with cystic fibrosis and chronic pulmonary diseases (3, 4), and skin and soft tissue infections (3).

In general, the most often prescribed macrolides for treatment of infections caused by NTM include clarithromycin and azithromycin (3). Azithromycin is technically considered an azalide due to the presence of a nitrogen in the chemical structure, but it has the same mechanism of action as does clarithromycin and thus is included in the discussion of macrolides (5). In the laboratory, testing of azithromycin is technically difficult and not recommended due to the problem of preparing solutions with sufficiently high concentrations of the antimicrobial. Thus, the Clinical and Laboratory Standards Institute (CLSI), a global organization which promotes the development and use of consensus standards and guidelines worldwide, has not developed or recommended breakpoints for this antimicrobial (6). Clarithromycin is considered the class drug for which resistance and susceptibility have been defined (6).

Macrolide Resistance and the Description of the *erm* Gene
The mechanism by which the macrolides act is the binding of the peptide exit tunnel of the ribosome (a small part of the cytoplasm of living cells that functions as a protein synthesis “factory”) to prevent the growing
peptide chain from leaving the peptidyl-transferase center of the ribosome. This action is believed to “gum-up” the ribosome and prevent further lengthening of the peptide chain \(^7\).

The primary mechanism for acquired resistance (sometimes seen during or after macrolide treatment) in NTM is detected by sequencing of the 23S rRNA (\(rrl\)) gene for single point mutations in the adenine at position 2058G or 2059 \(^8\). This is also known as an “\(rrl\)” mutation where “\(rrl\)” is the gene encoding a 23S peptidyl transferase in the ribosome \(2, 8\).

Within the past decade, another mechanism of macrolide resistance involving an erythromycin ribosomal resistance methylase gene (\(erm\)), which induces macrolide resistance in the presence of the macrolide, has been described in RGM \(2, 9-13\). These \(erm\) genes encode an rRNA methylase which confers macrolide resistance by methylating an adenine base (2058 or 2059) in the peptidyl-transferase region of the 23S rRNA gene, resulting in reduced binding of macrolide antibiotics to the ribosome. The RGM species without functional \(erm\) genes include: \(M. chelonae\), \(M. immunogenum\), and the \(M. mucogenicum\) group \(10-13\). Additionally, \(M. abscessus\) subsp. \(massiliense\) has a truncated \(erm\) gene sequence which results in a defective, nonfunctional gene \(12, 14, 15\). Untreated (wild) isolates of these aforementioned species would be expected to be susceptible to macrolide antibiotics unless they have acquired macrolide resistance. This finding is of vital importance in evaluating the effectiveness of macrolide treatment regimens for RGM.

Inducible macrolide resistance has been elucidated and described in at least 10 species of RGM \(9-13\). These include \(erm(38)\) of \(M. goodii\) and \(M. smegmatis\); \(erm(39)\) of \(M. boenickei\), \(M. fortuitum\), \(M. houstonense\), \(M. neworleansense\), and \(M. porcinum\); \(erm(40)\) of \(M. mageritense\) and \(M. wolinskyi\); and most recently a functional \(erm(41)\) present in the majority of the strains of \(M. abscessus\) subspecies \(abscessus\) and \(M. abscessus\) subsp. \(bolletii\) \(9, 10\).

**Laboratory Detection of the \(erm\) Gene**

Previous studies describing the \(erm\) gene functionality in the known RGM species help to explain why some patients tend to respond poorly to macrolide-based treatment regimens. A 2011 study by Koh and colleagues showed that patients with isolates of \(M. abscessus\) subsp. \(massiliense\) (i.e., nonfunctional \(erm\) gene) had an approximately 75% successful treatment outcome compared to only a 25% favorable treatment outcome for patients with isolates of \(M. abscessus\) with functional \(erm\) genes \(4\).

In the laboratory, detection of inducible macrolide resistance is accomplished in two ways. The most time-consuming and least efficient method involves extended incubation of the isolates in the presence of the macrolide. Using this phenotypic method, minimum inhibitory concentrations (MICs) should be read so that the final MIC reading of clarithromycin is at 14 days unless resistance (defined as \(>16 \mu g/mL\)) is recognized at an earlier time \(6, 14\).

The second method of detection of inducible macrolide resistance is performed by sequencing of the \(erm\) gene. In RGM species that do not harbor functional \(erm\) genes (see Table 1), an \(rpoB\) sequence identification of that species would be sufficient to indicate the absence of the functional \(erm\) gene \(6, 9, 10, 12\). A proposal to the
CLSI has been made, but not yet accepted, to enable laboratories to substitute sequencing of the *erm* gene rather than requiring extended incubation to detect the inducible *erm* gene in isolates of some RGM, including the *M. abscessus* complex (14).

In a 2014 study of 349 isolates of *M. abscessus* subsp. *abscessus* (excluding those with acquired resistance detected by 23S rRNA gene mutations) from a large U.S. reference laboratory, approximately 80% of the isolates exhibited inducible macrolide resistance (defined as clarithromycin MICs ≥16 μg/mL) after the initial MIC reading at 3-5 days. In the same study, sequence of the *erm* genes revealed 10 sequevars. The type strain of *M. abscessus*, ATCC 19977 was designated as sequevar Type 1 and most of the macrolide-resistant isolates were of this sequevar. Six other sequevars contained isolates with clarithromycin MICs >16 μg/mL. A T to C change at position 28 (T28C) substitution in the *erm*(41) that has been associated with macrolide susceptibility. This change is thought to be due to altered conformation of the *erm*(41) gene and inability to bind to the ribosomal binding region, also known as Domain V. This substitution (position 28 T to C) was seen in less than 20% of the isolates studied (14-16). Since this study, six other sequevars associated with macrolide resistance have been found (unpublished data, Brown-Elliott BA and Wallace RJ). Thus, sequencing of the *erm*(41) gene appears to be predictive of the inducible macrolide susceptibility/resistance of these isolates (14).

For the *M. fortuitum* group, except those listed without functional *erm* genes (see Table 1), extended incubation is needed until sufficient sequencing data is available to obviate the need for the continued phenotypic testing (6).

**Summary**

Recent studies suggest that macrolides may no longer be as useful as initially thought in the treatment of infections caused by the majority of isolates of *M. abscessus* subsp. *abscessus* and some other species of RGM. Moreover, molecular detection and characterization of the presence or absence *erm* gene generally correlates well with the phenotypic detection of macrolide resistance although some isolates of *M. abscessus* that have a full-length *erm*(41) gene that is nonfunctional and some that have truncated (nonfunctional) *erm* genes have been described (14-16).

<table>
<thead>
<tr>
<th>Table 1. Rapidly growing mycobacteria species with <em>erm</em> gene designation</th>
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<tbody>
<tr>
<td><strong>Species/subspecies</strong></td>
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<tr>
<td><em>M. goodii</em>, <em>M. smegmatis</em></td>
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<tr>
<td><em>M. fortuitum</em>, <em>M. porcinum</em>, <em>M. neworleansense</em>, <em>M. houstonense</em></td>
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<tr>
<td><em>M. mageritense</em>, <em>M. wolinskyi</em></td>
</tr>
<tr>
<td><em>M. abscessus</em> subsp. <em>abscessus</em>, <em>M. abscessus</em> subsp. <em>bolletii</em></td>
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<tr>
<td><em>M. chelonae</em>, <em>M. immunogenenum</em>, <em>M. mucogenicum group</em> (<em>M. mucogenicum</em>, <em>M. phocaicum</em>, <em>M. aubagnense</em>), <em>M. senegalense</em>, <em>M. peregrinum</em>, <em>M. abscessus</em> subsp. <em>massiliense</em> (has truncated <em>erm</em> gene), <em>M. abscessus</em> subsp. <em>abscessus</em> [erm gene sequevar 2 (MAB30**)]</td>
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Co-editors: Charles Daley, MD and Max Salfinger, MD, FIDSA, FAAM
*Approximately 80% of isolates

**Approximately 20% of isolates

References


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Recent Staff Publications (PubMed)


Meetings/Conferences/Lectures

22nd Annual TB Conference, he Union-North America Region, February 28-March 3, 2018, Westin Michigan Avenue Hotel, Chicago, Illinois. For more information and to register, please visit: https://bc.lung.ca/support-services/union-north-america

55th Annual Denver TB Course, April 4-7, 2018, Molly Blank Conference Center, National Jewish Health, Denver, CO

The Denver TB Course provides a broad overview of active and latent TB, including its epidemiology, transmission, pathogenesis, diagnosis, treatment and management. The purpose of this course is to present this body of knowledge to health care providers who will be responsible for the management and care of patients with tuberculosis. CME/CNE available. For more information and to register, please visit: http://www.njhealth.org/TBCourse2018

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