Isolation prevalence of pulmonary non-tuberculous mycobacteria in Ontario 1997-2003

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ABSTRACT

Rationale: The prevalence of pulmonary non-tuberculous mycobacterial (NTM) infection is reportedly increasing.

Objectives: To study the “isolation prevalence” of pulmonary NTM in Ontario, Canada, between 1997 and 2003, and the frequency of pulmonary NTM “disease.”

Methods: In a retrospective cohort, we studied “isolation prevalence” by reviewing all positive NTM culture results from the Tuberculosis and Mycobacteriology Laboratory, Ministry of Health and Long-Term Care, in Toronto, from 1997 to 2003. This laboratory identifies at least 90% of NTM isolates in Ontario, Canada. Prevalence was compared between years using a negative binomial model. To study the frequency of “disease” (defined by American Thoracic Society criteria), we reviewed records of patients who had an isolate in 2003 and had been assessed at our hospital.

Measurements and Main Results: There were 22,247 pulmonary isolates from 10,231 patients. The “isolation prevalence” of all species (excluding Mycobacterium gordonae) was 9.1/100,000 in 1997 and rose to 14.1/100,000 by 2003 (p<0.0001), with an average annual increase of 8.4%. Similar increases were observed for individual species. Two hundred patients assessed at our institution were studied using American Thoracic Society criteria for “disease.” Microbiologic criteria were fulfilled by 37%. Of patients with adequate data, 74% fulfilled clinical criteria, 77% fulfilled radiologic criteria and 33% fulfilled all criteria.

Conclusions: The “isolation prevalence” of pulmonary NTM has significantly and rapidly increased in Ontario, a sizeable proportion of patients are likely to have “disease.”
INTRODUCTION

Pulmonary non-tuberculous mycobacteria (NTM) infection can cause severe progressive illness, that may be preceded by a period of colonisation(1;2). Treatment of pulmonary NTM is complicated, requiring multiple anti-mycobacterial drugs for greater than 12 months(1;2). Treatment is usually initiated in the setting of “disease,” defined by the American Thoracic Society as the presence of symptoms, radiographic abnormalities and multiple positive cultures(1). Colonisation on the other hand, may be defined by the absence of one or more diagnostic criteria(3). In the setting of colonisation, treatment is often withheld due to its potential toxicities and the uncertain rate of progression. Ideally, therapy should be initiated before irreversible lung damage occurs or progresses.

Studies of the epidemiology of pulmonary NTM infections are generally limited in several ways. First since NTM are often isolated from the environment, including potable water, specimen contamination is a significant concern.(1;2) Second, the mere presence of NTM in the lungs does not necessarily imply significant or progressive pulmonary disease.(1;2) This has resulted in the confusing terminology, whose definition is critical to the interpretation of studies. Third, because the isolation of NTM does not mandate in forming a “reportable disease” office in many jurisdictions, surveys of NTM epidemiology often comprise incomplete samples. Although all epidemiologic studies of pulmonary NTM, including the ones referenced herein, have major limitations, it seems clear that the prevalence of pulmonary NTM varies by geographic region and is increasing significantly. All well-defined population-based studies of temporal trends of pulmonary NTM consistently report increases in frequency of 1.5 to 6 fold, over 5 to 30 years.(4-9) The universally observed increases in frequency of pulmonary NTM infection makes this emerging infectious disease relevant to all pulmonary and infectious disease practitioners. The precise magnitude of the problem is not well understood for several reasons. In addition to the limitations outlined above, increasing prevalence and geographic variability make studying this infection challenging. Changing prevalence requires frequent study and geographic variability limits generalisability of results of studies across regions. In addition, many previous studies have been limited by representing either small or inadequately defined areas and populations, or incomplete sampling of large and varying areas and populations.(10;11) There is a lack of recent and comprehensive data regarding the frequency of NTM infection in Canada and Ontario. Furthermore, the proportion of patients with colonisation versus disease remains even less well studied. In a systematic review, median annual rates of colonisation in previous studies were 4.4 per 100,000 population in North America, 7 per 100,000 in Europe and 5.4 per 100,000 in Australia.(10) The corresponding median rates of disease were 1.8 per 100,000 in North America, 1.3 per 100,000 in Europe and 1.8 per 100,000 in Australia.(10) We studied the “isolation prevalence” of pulmonary NTM in Ontario, and whether the prevalence changed between 1997 and 2003. In a secondary analysis, we studied a subset of patients to determine the proportion therein with “disease.”

METHODS

To study the isolation prevalence of pulmonary NTM in Ontario, data were obtained retrospectively, from the TB and Mycobacteriology Laboratory of the Ministry of Health and Long-Term Care, in Toronto (MOH TB laboratory). We determined the number of people with a positive pulmonary NTM culture between 1997 and 2003. Due to data constraints, we were unable to study the total number of isolates per person for all years. The study period began in January 1997, when high performance liquid chromatography (HPLC) was introduced in the mycobacteriology laboratory, providing an accurate species identification method for the most common NTM species. The study period ended in December 2003, the last year for which data were complete when we initiated this study. The Toronto area MOH TB laboratory processes the majority of specimens for mycobacterial culture in Ontario, and also acts as a reference laboratory for species identification for cultures from most other laboratories in the province. We communicated with laboratory directors and senior technologists from other Ontario laboratories that
identify NTM organisms to estimate the fraction of provincial NTM isolates that are identified in the Toronto MOH TB laboratory. During the last five years of the study (1999-2003), there were approximately 200 isolates per year in other labs. Based on our results, we therefore estimate that at least 90% of NTM isolates in Ontario are identified in the Toronto MOH TB laboratory. We therefore decided that excluding isolates from other laboratories would not significantly alter frequency estimates and included data only from the Toronto area MOH TB laboratory.

To explore the frequency of disease among individuals with pulmonary NTM, we studied a subgroup of patients with pulmonary NTM isolates in 2003. Among patients with pulmonary NTM isolates, records of patients who had been assessed at our hospital network, the University Health Network (an academic institution providing ambulatory and inpatient care) within 6 months of sample collection were reviewed.

Specimen culture was performed using the Bactec 460 TB system until May 2000, and thereafter with the BACTEC MGIT 960 system, shown to be slightly more sensitive for NTM,(12) (Becton Dickinson Microbiology Systems, Sparks, Maryland, USA). Specimens were processed and analyzed as described by Hanna et al.(12) NTM cultures were speciated by DNA probes (AccuProbe, Gen-Probe, San Diego, California, USA) for \textit{M. avium} complex (MAC) and \textit{M. gordonae}, and by HPLC for other species.(13)

The isolation prevalence of pulmonary NTM was calculated for each of the study years as follows: A prevalent case was defined as a patient with an isolate during a given calendar year (isolation prevalence = total number of patients with an isolate during a given year / contemporary provincial population). To study whether isolation prevalence changed over time, isolation prevalence was compared between years by visually inspecting graphs and statistical significance was assessed using generalized linear models with a negative binomial distribution using prevalence as the outcome variable and year as the predictor variable. This was performed using the SAS Proc Genmod with a negative binomial distribution and a logarithmic link function. Regression was also performed with the SAS Proc Autoreg to verify a lack of autocorrelation.

The above calculations were repeated for the four most commonly isolated species or species-groups in each year, exclusive of \textit{M. gordonae}. Although a common isolate, \textit{M. gordonae} was excluded as it may be a contaminant from water ingested before collecting sputum and is infrequently associated with clinically significant disease.(14) NTM prevalence was compared to the contemporary Ontario tuberculosis case rates.

The proportion of patients with disease was determined only for members of the subset who had been patients at our hospital within six months of NTM isolation. These patients' records were reviewed to determine whether they fulfilled American Thoracic Society clinical and radiographic criteria for disease(1) (Table 1). Clinical criteria were assessed with clinic and inpatient records. Radiologic criteria were assessed with radiologists' reports.
Table 1: American Thoracic Society Diagnostic Criteria for pulmonary NTM Disease

<table>
<thead>
<tr>
<th>Category</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical</td>
<td>Compatible signs/symptoms (cough, fatigue, fever weight loss, hemoptysis, dyspnea), documented deterioration in clinical status and reasonable exclusion of other disease</td>
</tr>
<tr>
<td>Radiographic</td>
<td>On chest radiograph, any of:</td>
</tr>
<tr>
<td></td>
<td>- infiltrates (progressive or persistent &gt;2 months)</td>
</tr>
<tr>
<td></td>
<td>- cavitation</td>
</tr>
<tr>
<td></td>
<td>- multiple nodules</td>
</tr>
<tr>
<td></td>
<td>On high resolution computed tomography, any of:</td>
</tr>
<tr>
<td></td>
<td>- multiple small nodules</td>
</tr>
<tr>
<td></td>
<td>- multifocal bronchiectasis</td>
</tr>
<tr>
<td></td>
<td>(if baseline films &gt;1 year old, there should be evidence of progression)</td>
</tr>
<tr>
<td>Microbiologic</td>
<td>With at least 3 available sputum / bronchial wash samples in 1 year, any of:</td>
</tr>
<tr>
<td></td>
<td>- 3 positive cultures</td>
</tr>
<tr>
<td></td>
<td>- 2 positive cultures with any acid fast bacilli seen on at least 1 smear</td>
</tr>
<tr>
<td></td>
<td>With only 1 available bronchial wash and inability to obtain sputum, any of:</td>
</tr>
<tr>
<td></td>
<td>- positive culture with 2+, 3+ or 4+ growth</td>
</tr>
<tr>
<td></td>
<td>- positive culture with more than rare acid fast bacilli seen on smear assessment</td>
</tr>
<tr>
<td></td>
<td>With a tissue biopsy</td>
</tr>
<tr>
<td></td>
<td>- any growth on bronchopulmonary tissue biopsy</td>
</tr>
<tr>
<td></td>
<td>- granuloma and/or acid fast bacilli on lung biopsy with at least 1+ growth from sputum / bronchial wash</td>
</tr>
</tbody>
</table>

* A patient must meet clinical, radiographic and microbiologic criteria to be classified as having pulmonary NTM disease according to American Thoracic Society guidelines.

Data were entered directly into an electronic database in Microsoft® Access 2000 (Redmond, WA, USA). All statistical analyses were performed using SAS® version 8.02 (SAS Institute, Cary, North Carolina, USA) and Epi Info 2000 (Centers for Disease Control and Prevention, Atlanta, Georgia, USA). Ethics approval was granted by the appropriate institutional review board.

RESULTS

Between 1997 and 2003, there were 23,823 NTM isolates from 11,186 patients, including all body sites. Limiting specimens to pulmonary sites yielded 22,247 isolates from 10,231 patients. Ninety-three percent of all NTM isolates were from a pulmonary source and 91% of all patients with NTM isolates had specimens from a pulmonary source. Table 2 presents the number of isolates and individual people by anatomic site per year.

Table 2: Number of NTM isolates and patients with isolates per year by anatomic site

<table>
<thead>
<tr>
<th>Year</th>
<th>Pulmonary / patients</th>
<th>Blood / patients</th>
<th>Urine / patients</th>
<th>Bone marrow / patients</th>
<th>Unknown / patients</th>
<th>Total* / patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>1997</td>
<td>1658 / 1172</td>
<td>55 / 36</td>
<td>25 / 24</td>
<td>0 / 0</td>
<td>232 / 194</td>
<td>1970 / 1386</td>
</tr>
<tr>
<td>1998</td>
<td>1890 / 1214</td>
<td>32 / 23</td>
<td>4 / 4</td>
<td>1 / 1</td>
<td>285 / 249</td>
<td>2212 / 1433</td>
</tr>
<tr>
<td>1999</td>
<td>2407 / 1537</td>
<td>32 / 20</td>
<td>0 / 0</td>
<td>0 / 0</td>
<td>155 / 143</td>
<td>2594 / 1659</td>
</tr>
</tbody>
</table>
### Table 1

<table>
<thead>
<tr>
<th>Year</th>
<th>Total Isolates</th>
<th>Pulmonary Isolates</th>
<th>Non-pulmonary</th>
<th>Non-pulmonary Origin</th>
<th>MAC</th>
<th>M. xenopi</th>
<th>Rapidly Growing</th>
<th>M. kansasii</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>22247 / 10231</td>
<td>590 / 112</td>
<td>60 / 35</td>
<td>2 / 1</td>
<td>924 / 807</td>
<td>23823 / 11186</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2001</td>
<td>2349 / 2126</td>
<td>137 / 114</td>
<td>10 / 8</td>
<td>0 / 0</td>
<td>105 / 92</td>
<td>3636 / 2410</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2002</td>
<td>3471 / 2319</td>
<td>23 / 14</td>
<td>5 / 4</td>
<td>0 / 0</td>
<td>137 / 114</td>
<td>3349 / 2126</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2003</td>
<td>3200 / 2090</td>
<td>28 / 14</td>
<td>10 / 10</td>
<td>0 / 0</td>
<td>122 / 103</td>
<td>3360 / 2186</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total*</td>
<td>22247 / 10231</td>
<td>590 / 112</td>
<td>60 / 35</td>
<td>2 / 1</td>
<td>924 / 807</td>
<td>23823 / 11186</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Total patients does not equal sum of columns or rows due to patients with isolates from different body sites, multiple species or repeatedly positive cultures over years.

Of the 7% of isolates that were non-pulmonary, 59% (924/1,576) were of unknown origin, while the remainder was from blood (37%, 590/1,576), urine (4%, 60/1,576), and bone marrow (0.1%, 2/1,576). Of the 9% of patients who had non-pulmonary isolates, 85% (807/955) of isolates were of unknown origin, while the remainder was from blood (12%, 112/955), urine (4%, 35/955), and bone marrow (0.1%, 1/955). The number of patients with pulmonary isolates increased each year, with the exception of the final study year. Conversely, the number of patients with isolates not known to be pulmonary tended to decrease during the study period, including the number of patients with isolates from blood, urine and unknown anatomic sources. MAC was isolated in 59% of cases, *M. xenopi* was isolated in 26% of cases, rapidly growing mycobacteria (*M. abscessus, chelonae and fortuitum*) were isolated in 13% and *M. kansasii* was isolated in 2% of cases.

Isolation prevalence and temporal trends of pulmonary NTM, along with the contemporary provincial rates of tuberculosis, are presented in Table 3. The isolation prevalence for the four most common NTM species combined was 9.1/100,000 in 1997 and rose to 14.1/100,000 by 2003, a statistically significant increase (p<0.0001), with an average annual increase of 8.4%. Similar increases in prevalence, all statistically significant, were observed for each individual NTM species (Table 3).

To investigate whether the increase in isolation prevalence was due to a surveillance bias, we analysed the frequency of microscopy smear-positive cases in each year. We reasoned that if there was no change in the fraction of cases that was smear-positive, surveillance bias was probably not important. The frequency of smear-positive specimens ranged from a low of 10.5% in 1997, to a high of 17.4% in 2000, and was 11.1% in 2003. There was no obvious temporal trend in the fraction of smear-positive cases. If smear-positive status is a reasonable surrogate for NTM disease, then the relatively stable fraction of cases with smear-positive isolates over time, suggests that the frequency of NTM disease probably increased during the study period.
Table 3: Annual isolation prevalence of pulmonary NTM and reported tuberculosis cases in Ontario 1997-2003

<table>
<thead>
<tr>
<th>Year</th>
<th>Ontario population* (thousands)</th>
<th>Prevalence** (total cases)</th>
<th>M. avium complex</th>
<th>M. xenopi</th>
<th>Rapidly growing mycobacteria†</th>
<th>M. kansasii</th>
<th>All species‡</th>
<th>Tuberculosis§</th>
</tr>
</thead>
<tbody>
<tr>
<td>1997</td>
<td>11,168.4</td>
<td></td>
<td>5.6 (629)</td>
<td>2.6 (293)</td>
<td>1.2 (134)</td>
<td>0.09 (10)</td>
<td>9.1 (1,020)</td>
<td>6.9 (776)</td>
</tr>
<tr>
<td>1998</td>
<td>11,336.0</td>
<td></td>
<td>6.1 (688)</td>
<td>2.1 (241)</td>
<td>1.4 (156)</td>
<td>0.17 (19)</td>
<td>9.4 (1,064)</td>
<td>6.5 (741)</td>
</tr>
<tr>
<td>1999</td>
<td>11,506.4</td>
<td></td>
<td>7.2 (824)</td>
<td>3.4 (388)</td>
<td>1.4 (165)</td>
<td>0.17 (20)</td>
<td>11.8 (1,360)</td>
<td>6.0 (696)</td>
</tr>
<tr>
<td>2000</td>
<td>11,685.4</td>
<td></td>
<td>7.6 (892)</td>
<td>3.3 (389)</td>
<td>1.7 (194)</td>
<td>0.22 (26)</td>
<td>12.5 (1,465)</td>
<td>6.0 (696)</td>
</tr>
<tr>
<td>2001</td>
<td>11,897.6</td>
<td></td>
<td>9.4 (1,115)</td>
<td>4.0 (474)</td>
<td>2.0 (240)</td>
<td>0.32 (38)</td>
<td>15.0 (1,780)</td>
<td>5.8 (688)</td>
</tr>
<tr>
<td>2002</td>
<td>12,096.6</td>
<td></td>
<td>9.9 (1,196)</td>
<td>4.7 (571)</td>
<td>2.3 (282)</td>
<td>0.35 (42)</td>
<td>16.5 (1,991)</td>
<td>5.6 (677)</td>
</tr>
<tr>
<td>2003</td>
<td>12,238.3</td>
<td></td>
<td>8.4 (1,027)</td>
<td>4.2 (513)</td>
<td>2.0 (240)</td>
<td>0.23 (28)</td>
<td>14.1 (1,734)</td>
<td>5.4 (657)</td>
</tr>
<tr>
<td>Average annual change</td>
<td>N/A</td>
<td></td>
<td>7.7%</td>
<td>11.3%</td>
<td>9.6%</td>
<td>23.1%</td>
<td>8.4%</td>
<td>-4.0%</td>
</tr>
<tr>
<td>P value§§</td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Prevalence calculated as the number of cases per 100,000 Ontario population in a given year
N/A Not applicable
** Prevalence for NTM species defined as the isolation prevalence and the prevalence of tuberculosis was defined according to reported cases of tuberculosis
† M. abscessus, chelonae and fortuitum
‡ Data for M. avium complex, M. xenopi, M. fortuitum, M. chelonae, M. abscessus and M. kansasii combined
§ Total number of tuberculosis cases from Public Health and Epidemiology Reports Ontario, (15;16) rate calculated according to population from Statistics Canada
§§ P value for comparing prevalence over time using generalized linear models with a negative binomial distribution
Isolation prevalence of pulmonary NTM increased while the incidence of tuberculosis in Ontario slowly and progressively decreased. The isolation prevalence of individual NTM species, all NTM species combined, and the contemporary rates of tuberculosis are presented graphically in the figure. The isolation prevalence of pulmonary NTM was greater than the rate of tuberculosis throughout the study period, and the ratio of NTM isolation prevalence to tuberculosis case prevalence increased during the study period.

Of 2,090 patients with pulmonary NTM isolates (other than M. gordonae) in 2003, 200 (10%) were assessed utilizing American Thoracic Society diagnostic criteria for disease, based on at least one of a clinical or radiographic assessment at our hospital. Isolate sources were sputum in 171 patients (85.5%) and by bronchoscopy in 29 patients (14.5%). NTM species included MAC in 59%, M. xenopi in 33.5%, rapidly growing mycobacteria in 6.5% and M. kansasii in 1%. American Thoracic Society microbiologic criteria were fulfilled by 73/200 (37%) of patients. Adequate clinical information was available for 139 patients and 103/139 (74%) fulfilled clinical criteria. Adequate radiologic information was available for 180 patients and 139/180 (77%) fulfilled radiologic criteria. Adequate information for all American Thoracic Society criteria was available for 119 patients and 39/119 (33%) fulfilled all criteria.

DISCUSSION

We observed a high and increasing isolation prevalence of pulmonary NTM in Ontario, Canada, between 1997 and 2003. Based on a systematic review(10) and more recent work,(17) the median rates of pulmonary NTM isolation have been approximately 6.2/100,000 in North America, 8.3/100,000 in Europe, 15/100,000 in Asia and 7.2/100,000 in Australia. Our results represent a remarkably higher prevalence than generally reported in North American studies and studies from most other areas. Throughout our study period, the isolation prevalence of pulmonary NTM was approximately twice as great as the rates of tuberculosis cases (mean isolation prevalence of pulmonary NTM 12.6, versus 6.0 for tuberculosis case rates).(15;16) In addition to the high isolation prevalence, the increase over time is an important observation that is consistent with previous studies. All well-defined population based studies that presented data on the frequency of pulmonary NTM isolation or disease over time have shown increases.(4-9) The numerous reports of increasing frequency are consistent with our results and suggest that pulmonary NTM infection is a growing problem. Increases in the frequency pulmonary NTM throughout the world have not been adequately explained, but there are several possible contributing factors. The increase could be either spurious or smaller than perceived, if increased awareness has led to more investigations, or improved laboratory methods have led to isolation and identification of previously unrecognized organisms. Studies have been published that support(18) and refute(19) this argument. We believe such factors cannot completely explain the observed increases. Part of the increase we observed could be explained by the greater sensitivity of the Bactec MGIT 960 system. However, this could only explain a portion of the increase for at least two reasons. First, the increase in prevalence began before and continued well after the culture system was changed in June, 2000. Second the greater sensitivity of the new system is expected to increase the total number of positive cultures by less than 7%,12 while the number of prevalent cases we observed increased by more than 30% between the year before and the year after introducing the new system. In addition, since the same microbiologic systems are used to detect M. tuberculosis complex and NTM organisms, the concurrent decrease in rates of TB in Ontario,(15) may argue against an apparent increase in NTM isolation simply due to more sensitive microbiologic techniques. It may have been possible that changing referral patterns from other laboratories in Ontario contributed to the increase in NTM prevalence that we observed. If some laboratories had previously performed NTM identification, but switched to referring NTM isolates to the Toronto MOH TB laboratory during the study period, then our increase in prevalence may be an overestimate. However, based on a review of referral patterns and the number of isolates from relevant laboratories, changing referral patterns could potentially have accounted for only a 3-9% increase over the entire study period. Since we observed
an increase of over 50%, we believe that changing referral patterns had no important effect on our observation of increased prevalence.

True changes in the frequency of pulmonary NTM isolation could be due to host changes, pathogen changes or an interaction between host and pathogen. Host changes leading to increased susceptibility could play an important role. There may be more immunocompromised patients due to HIV infection, malignancy or simply advanced age.(19) As proposed elsewhere, changes in host immunity may be relevant. If infections with \textit{M. tuberculosis} complex species(20;21) and BCG,(22;23) provide cross-immunity or protection against pulmonary NTM, and if infections with these "protective" mycobacteria decrease, then pulmonary NTM may secondarily increase. Decreasing rates of tuberculosis concomitant with increases in pulmonary NTM have generally been observed.(20;21) Our observations in Ontario have been similar, with a small progressive concomitant decline in tuberculosis during our study period,(15;16) as presented in Table 3 and the Figure. In addition, studies from Sweden(23) and the Czech Republic(22) reported that children who were not vaccinated with BCG had a far higher rate of extra-pulmonary NTM infection, also consistent with the notion that cross-immunity offers protection from infections across mycobacterial species. Pathogen changes, such as enhanced virulence, should be considered as a possible contributor to increased rates of NTM infection. NTM isolates from patients have been shown to vary widely in virulence.(24) Examples of genetically identified virulence factors include a conserved coding sequence of a macrophage-induced gene(25) and the presence of numerous or large plasmids.(26) Finally, an interaction between host and pathogen could involve an increase in host exposure to the pathogen. This may have occurred as a result of the increase in popularity of showering as a form of bathing,(27) a habit that may greatly increase respiratory exposure to water-borne contaminants through aerosolization. Regardless of the explanation, increasing frequencies of pulmonary NTM are consistently observed, suggesting that the importance of pulmonary NTM infection continues to grow.

\textit{Mycobacterium xenopi} comprised 25-30% of prevalent cases in our study, a relatively high rate compared with systematic surveys in other parts of North America.(4;6;20;21;28-30) This finding is consistent however with investigators who have presented large series of pulmonary \textit{M. xenopi} infection in southern Ontario(31;32) and northwestern New York State, USA.(33) We hypothesize that this is related to our proximity to large bodies of fresh water.

In the subset of patients who had adequate clinical information available for review, 33% met American Thoracic Society diagnostic criteria for clinically important disease. This is slightly higher than the expected rate of 20-25% based on the findings of a systematic review of rates of advanced infection among patients with MAC or \textit{M. xenopi} recovered from respiratory specimens.(10) Although the difference may not be significant, a possible explanation for a slightly higher rate could reflect a biased selection of records for review. It is likely that NTM-infected patients assessed at the UHN have relatively high rates of disease, given that our institution provides tertiary care. We believe that the results of our subgroup study are applicable to patients seen in a teaching institution that provides primary to tertiary care, where NTM isolates may more often signify disease. Further, we note that approximately three-quarters of patients fulfilled clinical and radiologic criteria for disease, while only one-third fulfilled the criteria we use to define disease. We wonder whether our estimate of the frequency of disease in this sample is a significant underestimate, possibly due to inadequate microbiologic sampling. Unfortunately, data regarding the total number of specimens collected were not available to us, so this remains speculative.

Limitations of our study relate to challenges in studying population level epidemiology of the isolation prevalence of pulmonary NTM, and challenges in studying the presence of pulmonary NTM disease in a group of patients with pulmonary NTM isolates. For the population-based study of the isolation prevalence of pulmonary NTM, we chose not to study incidence because of the presumed chronic nature of pulmonary NTM. To confidently identify a new or incident case, a long "look-back" period would be required, to ensure that a patient had not previously had a pulmonary NTM isolate. The uncertain duration
of look-back period required, and the lack of reliable data for an adequate duration prior to our study period dissuaded us from studying incidence, and we instead focused on prevalence. Looking at individual patients over time, we found that few patients had repeat isolates over years. This observation could be due to either resolution of the “infection” or infrequent follow-up specimen collection after the initial isolate. Assuming that pulmonary NTM is usually chronic, we suspect that a large proportion of patients did not have regular follow-up specimens collected, and for this reason, the isolation prevalence we observed may be an underestimate. Another limitation in the population-based study is the lack of data from some Ontario laboratories that identify NTM species. We included only isolates from the Toronto MOH TB laboratory. Isolates in other laboratories were not included unless they were referred to the study laboratory, so the isolation prevalence of pulmonary NTM in Ontario that we measured is an underestimate. The estimate that our study captured more than 90% of NTM isolates in Ontario is based on extensive communications with personnel from other laboratories. Although we are confident that this is a fairly accurate estimate, and that there was no temporal variation in isolation prevalence in other laboratories that could alter our results, our data remain limited in this area. A third limitation in the population-based study involves specimens that were from an unknown source. Approximately 7% of specimens were not identified as to the anatomic site of origin. We suspect that some of these specimens were pulmonary and therefore our results may represent a further underestimate of the isolation prevalence of pulmonary NTM. Overall, we believe that the limitations of the population-based study suggest that our measurements underestimate the isolation prevalence of pulmonary NTM infection, and that the true prevalence is higher than we measured.

Our assessment of the frequency of disease among patients with pulmonary NTM isolates may be limited by incomplete data and biased sampling. Incomplete data regarding clinical and radiographic criteria for advanced infection, would lead to an underestimate of the frequency of disease. On the other hand, selecting patients assessed at an academic institution probably assembled a cohort of patients with pulmonary NTM isolates who have above average rates of disease. Given that these two biases have opposite effects on the estimated frequency of disease, it is difficult to speculate on the generalisability of our estimate to other populations. We believe that our estimate is valid for patients assessed in a tertiary-care centre and that clinicians should strongly consider the possibility that disease is present when pulmonary NTM are isolated in the tertiary-care setting.

Our population-based study of the isolation prevalence of pulmonary NTM in Ontario, Canada revealed prevalence higher than reported in previous studies. The high and consistently increasing prevalence we observed suggest that the burden of pulmonary NTM infection is significant and will continue to grow in importance. Further work is needed to understand the frequency of disease among all patients with pulmonary NTM. In addition, research efforts should be directed toward areas that will lead to strategies for prevention, predicting progression from the colonized state to disease, and improved treatment of disease.

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Figure legend
Prevalence of pulmonary NTM infections and tuberculosis case rates in Ontario 1997-2003: All NTM prevalence rates statistically significantly increased and tuberculosis rates statistically significantly decreased (p< 0.001 for all); Tuberculosis rates calculated from total number of tuberculosis cases,(15;16) and population data from Statistics Canada (for 1999-2003 from http://www.statcan.ca/english/Pgdb/demo02.htm, accessed 2004/10/04) and estimated by back-extrapolation (for 1997 and 1998).
Box: Definitions of terms regarding pulmonary non-tuberculous mycobacteria (NTM)

Isolation Prevalence:
- In a population, the prevalence of the isolation of NTM from pulmonary specimens, regardless of clinical status or chest imaging findings of the individual population members

Colonization:
- Recovery of NTM from pulmonary specimens, and
- Absence of clinical and/or radiographic evidence of infection with important effects

Disease (synonymous with advanced infection, see Table 1):
- Repeated recovery of NTM from pulmonary specimens, and
- Progressive clinical signs or symptoms compatible with NTM infection, not explained by another illness, and