National atypical mycobacteria survey, 2000

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from information supplied by the Australian Mycobacterium Reference Laboratory Network and the Special Interest Group in Mycobacteria within the Australian Society for Microbiology

Abstract

Infections with atypical mycobacteria in Australia during 2000 occurred at a rate of 1.8 cases per 100,000 population. The main sites of disease were the respiratory tract, soft tissue, and the lymphatics. The *Mycobacterium avium* complex was the most common group of mycobacteria isolated from respiratory, lymphatic sites, and blood.The rapidly growing mycobacteria, predominantly the *M. fortuitum–M. abscessus–M. chelonae* group were the most common soft tissue infections. Atypical mycobacteria were isolated from significant numbers of sputum 'smear positive' patients, requiring further tests to exclude *M. tuberculosis*. Geographical differences were observed for some *Mycobacterium* species, notably the isolation of *M. haemophilum* from Western Australia, and *M. ulcerans* from Victoria and Queensland. Newer molecular techniques, while improving precision and accuracy of identification, raise additional questions about the ecology of the atypical mycobacteria and their role in disease. *Commun Dis Intell* 2003;27:180–189.

Keywords: atypical mycobacteria, nontuberculous mycobacteria, atypical mycobacteriosis, laboratory diagnosis, Mycobacterium

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Introduction

Mycobacteria other than the *Mycobacterium tuberculosis* complex (commonly referred to as 'atypical', 'nontuberculous', 'environmental' mycobacteria, etc) have been implicated in a variety of clinical conditions including tuberculosis-like pulmonary disease, lymphadenitis, superficial and soft tissue infections, and severe disseminated disease. In addition to humans, a variety of animals, birds, reptiles and fish are susceptible to infection with atypical mycobacteria (AM). Modern taxonomic tools have identified many new species of AM, the majority of which are considered potential pathogens, given appropriate host conditions. Unlike tuberculosis, atypical mycobacteriosis is rarely a disease of public health importance, although nosocomial and iatrogenic outbreaks have been recorded. On the other hand, clinical management of atypical mycobacteriosis can be difficult, depending on the species involved, the site of disease, and co-existing host factors. Many AM infections are refractory to treatment with standard anti-tuberculosis agents.

In Australia, as in Europe and the United States of America, increased reporting of atypical mycobacterial disease can be attributed to a growing pool of immunosuppressed patients (particularly HIV+), and greater awareness on the part of clinical and laboratory personnel. A substantial proportion of laboratory resources in mycobacterial reference laboratories in industrialised countries is expended in the isolation, identification and susceptibility testing of AM. Many of these isolates are not associated with disease, but represent colonisation or environmental contamination of the patient.

Although the AM are environmental organisms, much remains to be elucidated with regard to their ecology and epidemiology. While disease due to *M. avium* complex (MAC) is apparently common throughout the developed world, species such as *M. xenopi* and *M. malmoense* are encountered more commonly in Europe than in the United States of America or Australia. Data for atypical mycobacteriosis in Australia are limited, primarily because cases are not notifiable in every state or territory, cases are not included in the National Mycobacterial Surveillance Scheme, and not all recovered AM are fully identified.

The results of a collaborative study undertaken by the Australian Mycobacterium Reference Laboratory Network (AMRLN) on patients whose specimens were culture-positive for AM in 2000 are reported here.

Methods

The data in this report are based on clinical specimens that were culture-positive for AM during the calendar year 2000. Almost 80 laboratories performed culture for mycobacteria in 2000 (Royal College of Pathologists of Australasia Quality Assurance Program) and mycobacterial isolates from Australian patients were forwarded to one of the five laboratories that comprise the AMRLN. The reference laboratories identify isolates to species level using standard procedures such as evaluation of phenotypic characteristics, commercial probes, 16S rDNA sequencing, and high performance liquid chromatography.

Reference laboratories provide specific identification of isolates from patients who are likely to have mycobacterial disease, e.g. smear-positive specimens from the respiratory or urinary tract, tissues and biopsies, usually sterile sites, and wound swabs. Single isolates of AM from the respiratory or urinary tract were less likely to be identified to species level as their clinical significance was often doubtful, and the laboratory techniques were expensive and time-consuming. Indeed, these isolates may never be referred to a reference laboratory. For these reasons, the total figure for AM isolations in 2000 (n=1,447) must be regarded as conservative.

For each patient, the nature of the first clinical specimen that yielded an isolate was used to record the nominal site of disease. Patients who had been culture positive for AM in previous years were excluded from the study. For each patient, the following data were collected:

- a unique identifier (usually a laboratory accession number);
- age and sex;
- specimen source of isolate;
- result of acid-fast microscopy; and
- species (or species complex) of isolate.

The reference laboratories liaised with the responsible medical practitioner in an attempt to ascribe clinical significance to each isolate. In cases where HIV-sero-positivity was known, such information was also recorded.

Results were categorised as:

- clinically significant (defined as 'associated with disease');
- not clinically significant (defined as 'colonising' or 'environmental contaminant'); or
- undetermined (including unknown and uncertain).

Data were forwarded to the study co-ordinators in a standard Excel spreadsheet.

Results

Isolates from 1,447 patients were included in the study. The AM isolates identified in 2000 by the patient's state or territory of residence and the specimen type are shown in Table 1. The majority of isolates (78.7%) were from pulmonary sources. When all AM isolates were considered, the incidence was 7.5 cases per 100,000 population. There were wide variations between the jurisdictions, from a low of 5.5 cases (New South Wales) to 71.3 cases (Northern Territory) per 100,000 population. The isolate was considered associated with disease, for 341 of 1,447 (23.6%) patients, an incidence of 1.8 cases per 100,000 of population (Table 1). Incidence rates for disease in jurisdictions ranged from 0.78 cases (New South Wales) to 4.1 cases (Northern Territory) cases per 100,000 population.

Although almost all isolates from lymph node (98.4%) and soft tissue (91.0%) were clinically significant, only 108 (9.5%) of 1,139 pulmonary isolates were associated with disease. Incidence rates for each of the four categories of site of disease were less than one isolate per 100,000 population. These data suggest that soft tissue infections are more prevalent in Queensland, and lymph node infections are more common in Western Australia and Victoria (Table 2), but these data need to be interpreted with caution.

Table 1. Specimen source of new isolates of atypical mycobacteria by state or territory, Australia, 2000

	Specimen source of isolate				Total	Cases per 100,000 population
	Pulmonary	Lymphatic	Soft tissue	Other		
NSW	290 (10)	7 (7)	10 (9)	53 (25)	**360 (51)**	**5.5 (0.78)**
NT	135 (4)	1 (1)	3 (2)	1 (1)	**140 (8)**	**71.3 (4.1)**
Qld	181 (43)	10 (9)	88 (78)	8 (6)	**287 (136)**	**8.0 (3.8)**
SA	73 (12)	3 (3)	4 (4)	4 (2)	**84 (21)**	**5.6 (1.4)**
Vic.	259 (20)	20 (20)	21 (20)	22 (4)	**322 (64)**	**6.7 (1.3)**
WA	201 (19)	20 (20)	18 (18)	15 (4)	**254 (61)**	**13.4 (3.2)**
Total	**1,139 (108)**	**61 (60)**	**144 (131)**	**103 (42)**	**1,447 (341)**	**7.5 (1.77)**
Cases per 100,000 population	**5.91 (0.56)**	**0.32 (0.31)**	**0.75 (0.68)**	**0.53 (0.22)**	**7.5 (1.77)**	

Numbers in parenthesis refer to cases in which the mycobacterium isolated was judged to be a pathogen.

'Pulmonary' refers to any tissue derived from the lungs (sputum, bronchoscopic collections, biopsies, etc) but not pleurae.

'Lymphatic' refers to any tissue, pus or aspirate clearly associated with a lymph node.

'Soft tissue' refers to skin-associated tissue or pus; bone and joint.

'Other' includes pleural fluid, blood, urine and any isolate from an undefined site.

Since there were few isolates from the Australian Capital Territory, isolations were included with those for New South Wales, and Tasmania was included into that for the testing state (mostly Western Australia).

Table 2. Predominant species identified among disease-associated isolates of atypical mycobacteria

	Specimen source of isolate			
	Pulmonary	**Lymphatic**	**Soft tissue**	**Other**
NSW	*n=10* *M. kansasii* (6) *M. avium* complex (4)	*n=7* *M. avium* complex (6) *M. xenopi* (1)	*n=9* *M. fortuitum* comp (4) *M. chelonae* (2) *M. marinum* (2) *M. abscessus* (1)	*n=25* *M. avium* (18) *M. intracellulare* (6)
NT	*n=4* *M. avium* complex (4)	*n=1* *M. avium* complex (1)	*n=2* *M. fortuitum* (2)	*n=1* *M. avium* complex (1)
Qld	*n=43* *M. avium* complex (24) *M. kansasii* (9) *M. abscessus* (7)	*n=9* *M. avium* complex (8) *M.* species (1)	*n=78* *M. fortuitum* comp (28) *M. marinum* (14) *M. chelonae* (10) *M. abscessus* (8) *M. ulcerans* (7)	*n=6* *M. avium* (4) *M. abscessus* (2)
Vic	*n=20* *M. avium* complex (17) *M. kansasii* (1)	*n=20* *M. avium* complex (18) *M. scrofulaceum* (2)	*n=20* *M. chelonae* (5) *M. haemophilum* (3) *M. ulcerans* (2) *M. abscessus* (2) *M. marinum* (2)	*n=4* *M. avium* (2)
SA	*n=12* *M. avium* complex (12)	*n=3* *M. avium* complex (3)	*n=4* *M. marinum* (2) *M. abscessus* (1) *M. fortuitum* (1)	*n=2* *M. avium* complex (2)
WA	*n=19* *M. avium* complex (12) *M. kansasii* (5)	*n=20* *M. avium* complex (12) *M. haemophilum* (6) *M. malmoense* (1) *M.* species (1)	*n=18* *M. haemophilum* (6) *M. marinum* (4) *M. abscessus* (2) *M. fortuitum* (2)	*n=4* *M. avium* (2) *M. abscessus* (2)

Atypical mycobacteria and site of disease

The *M. avium* complex was the predominant disease-associated atypical mycobacteriosis in pulmonary (73/108, 67.6%) and lymphatic (48/60, 80%) sites. *M. kansasii* was found in 21 of 108, (19.4%) pulmonary samples. The *M. fortuitum* complex, the *M. chelonae* – *M. abscessus* group and *M. marinum* were common isolates from soft tissue (37/131, 28.2%, 31/131, 23.7% and 24/131, 18.3% respectively). *M. ulcerans* was only recovered from soft tissue in Queensland (n=7) and Victoria (n=2) (Table 2).

Age and site of disease for clinically relevant atypical mycobacteria

The age distribution of mycobacterial disease for the categories of pulmonary, lymphatic and soft tissues are presented in Table 3.

Lymphatic disease occurred primarily in children, (50/60, 83%), although 7 of 60 (11.7%) cases were diagnosed in adults greater than 50 years of age. In contrast, no pulmonary disease was found in persons less than 10 years of age. Disease of soft tissue occurred less commonly (9%) in the under 10 years age group.

Isolation of atypical mycobacteria from the respiratory tract

There were 1,139 isolates cultured from the respiratory tract representing 78.7 per cent of all isolates (Table 1). Culture-positive specimens collected at bronchoscopy or by gastric lavage were considered to be of pulmonary origin. Only 108 of 1,139 (9.5%) isolates were considered to be disease associated, implying that over 90 per cent of AM cultured from respiratory specimens were not clinically significant. Members of the *Mycobacterium avium* complex (MAC) were the most common organism isolated accounting for 586 of 1,139 (51.4%) pulmonary isolates (Table 4). Seventy-two of 108 (67.3%) clinically relevant isolates were MAC. There were a small number (n=4) of MAC identified as MAC–X's, a group closely related to, but genetically distinct from *M. avium* and *M. intracellulare*[1] (Table 5).

The Queensland, New South Wales and Western Australian reference laboratories use techniques to identify MAC to species level (*M. avium, M. intracellulare* or MAC–X). The other MRL's use a commercial probe to report the three entities as *Mycobacterium avium* complex and these identifications are described as 'MAC' in the tables. *Mycobacterium intracellulare* was recovered almost twice as often as *M. avium* (ratio of 1.8:1) from pulmonary specimens (data not shown). For clinically relevant isolates identified to species level the ratio was slightly lower (1.5:1) (Table 5). Of the other organisms that were fully identified from pulmonary samples, the 'rapidly growing' mycobacteria and *M. gordonae* appear to be the next most commonly isolated organisms. Only 2 of 164 (1.2%) mycobacteria described as unidentified slowly (or rapidly) growing mycobacteria were disease associated (Table 4).

Smear-positivity and association with pulmonary disease

Smear-positivity was not a reliable indicator of disease in pulmonary samples. Approximately 10 per cent of samples in which disease was not established were smear positive for acid fast bacilli. In contrast, some 50 per cent of cases where the recovered AM was deemed to be associated with disease were smear positive (data not shown).

Table 3. Age distribution for new patients from whom a 'pathogenic'atypical mycobacterium was isolated

Age range	Pulmonary %	Lymphatic %	Soft tissue %
<10 years	0	83	8
10 to 50	23	5	50
>50 years	77	12	42

Table 4. Species or species complexes identified among atypical mycobacteria from pulmonary sources

	State or territory						
	NSW	NT	Qld	SA	Vic.	WA	Total
M. avium complex	164 (4)	47 (4)	106 (24)	37 (12)	119 (16)	113 (12)	**586 (72)**
M. gordonae	28 (0)		1 (0)	1 (0)	48 (0)	18 (0)	**96 (0)**
M. fortuitum complex	26 (0)	5 (0)	8 (1)	1 (0)	25 (0)	8 (0)	**73 (1)**
M. abscessus	13 (0)	1 (0)	20 (7)	1 (0)	5 (0)		**40 (7)**
M. kansasii	6 (6)	1 (0)	10 (9)		8 (1)	6 (5)	**31 (21)**
M. chelonae	8 (0)		4 (0)	1 (0)	8 (0)	1 (0)	**22 (0)**
M. xenopi	5 (0)		1 (0)		3 (0)		**9 (0)**
M. simiae	6 (0)					1 (0)	**7 (0)**
M. asiaticum		6 (0)					**6 (0)**
M. interjectum		1 (0)				3 (0)	**4 (0)**
M. shimoidei	1 (0)	1 (0)			1 (1)	1 (1)	**4 (2)**
M. lentiflavum		1 (0)			1 (0)	1 (0)	**3 (0)**
M. malmoense		1 (0)	1 (0)			1 (1)	**3 (1)**
M. szulgai		1 (0)			1 (1)		**2 (1)**
M. heckeshornense					1 (0)		**1 (0)**
Other *Mycobacterium* sp.	33 (0)	18 (0)	3 (0)		26 (0)	8 (0)	**88 (0)**
Slow-growers (unidentified)		52 (0)	21 (1)	32 (0)	12 (0)	40 (0)	**157 (1)**
Rapid-growers (unidentified)			6 (1)		1 (0)		**7 (1)**
Total	**290 (10)**	**135 (4)**	**181 (43)**	**73 (12)**	**259 (19)**	**201 (19)**	**1,139 (107)**

Numbers in parenthesis refer to cases in which the mycobacterium isolated was judged to be a pathogen

Table 5. Analysis of disease-associated isolates of MAC

	M. intracellulare	*M. avium*	MAC–x
Pulmonary	19	13	4
Lymphatic	15	7	1

Isolation of atypical mycobacteria from soft tissue and other sites

Soft tissue comprises skin, connective tissue, bone and joint but not blood or lymph node. 'Other sites' include pleural fluid, blood, urine and any isolate from an undefined site.

Of the 144 soft tissue isolates, 131 (91.0%) were considered disease associated. For 'other sites', 42 of 103 (40.8%) isolates were considered clinically relevant (Tables 1 and 2).

Queensland reported 88 of all 144 (61.1%) AM isolates from soft tissue, and of these, 78 of 88 (88.6%) were disease associated. *Mycobacterium marinum, M. ulcerans* and the rapidly growing mycobacteria were all isolated more frequently compared with the other jurisdictions. The Northern Territory and South Australia reported the fewest cases of soft tissue disease.

In 2000, *Mycobacterium haemophilum* was isolated only in Western Australia and Victoria, although it has been isolated from the other states and territories previously.

Almost all isolates of rapid growing mycobacteria (e.g. *M. fortuitum complex/ M. chelonae–M. abscessus*) were considered pathogenic. The significance of MAC isolations from other sites was frequently uncertain. *Mycobacterium heckeshornense* was recovered from synovial fluid, blood, and sputum from three different patients in Victoria. It was considered a significant finding in the two sterile sites, but of uncertain significance in sputum.

Isolation of atypical mycobacteria from lymphatic tissue

Most lymphatic disease occurred in children. Almost all (98.4%) AM isolated from lymphatic tissue were considered disease associated, representing an incidence rate of 0.31 isolates per 100,000 population (Table 1). Forty-eight of 60 (80.0%) AM were MAC, and of those that were identified to species level, 15 of 23 (65.2%) were *M. avium*, 7 of 23 (30.4%) were *M. intracellulare*, and there was a single MAC–X strain isolated (Table 5). In Western Australia, *M. haemophilum* was recovered from lymphatic tissue on six occasions (Table 2).

Isolation of atypical mycobacteria from blood

Twenty-nine AM were cultured from blood, and of these 25 of 29 (86.2%) isolates were MAC. Almost half the isolations were from New South Wales. Of the MAC identified to species level, 19 of 20 (95%) isolates were *M. avium* (Table 6). The recently described *M. heckeshornense*[2] was recovered from the blood of a Victorian patient. The HIV status of 12 of 29 (41.4%) patients were known and are shown in bold (Table 6).

Table 6. Isolation of atypical mycobacteria from blood, 2000

	State or territory						
	NSW	NT	Qld	SA	Vic.	WA	Total
M. avium	13		**4**			**2**	**19**
M. intracellulare	1						**1**
MAC		**1**		**2**	**2**		**5**
M. chelonae	1				1		**2**
M. heckeshornense					1		**1**
Rapid grower			1				**1**
Total	**15**	**1**	**5**	**2**	**4**	**2**	**29**

Known HIV positive cases are in bold.

Discussion

The present report, based on laboratory data obtained for the year 2000, is an attempt to describe the association of AM with clinical specimens and clinical disease. Conservatively, in the year 2000, there were 1.8 bacteriologically confirmed cases of disease caused by AM per 100,000 population, compared with 4.0 cases of bacteriologically confirmed tuberculosis per 100,000 population.[3]

In contrast to bacteriological confirmation of tuberculosis, where isolation of the causative agent almost always represents disease, the association between the isolation of AM and clinical disease is less frequent. Australian laboratories undertaking mycobacteriological investigations are increasingly using a broth-based culture system as their primary medium rather than the traditional solid media. The newer systems offer the advantage of reduced time to detection of positive cultures, but they also recover a greater number of AM.[4,5,6,7] Rapid techniques for the preliminary identification of mycobacteria are increasingly available to non-reference laboratories. These tests distinguish between members of the *Mycobacterium tuberculosis* complex and the AM (AccuProbe; Gen-Probe Inc, San Diego, California). A single, AM isolate from a smear negative sputum or urine specimen is, initially of doubtful clinical relevance and as further identification of the AM isolate is not usually warranted, it may not be forwarded to an AMRLN laboratory for additional testing. For this reason the total numbers of recovered AM in this report do not represent all isolations of AM and the calculated rates of AM disease are likely to be under-estimates.

Most AM are ubiquitous, free-living organisms that may be found in a wide variety of environments including soil, dust, air and water. Water appears to be a particularly attractive environment for AM, and they have been recovered from water distribution systems worldwide.[6,7] It is assumed that most people are infected from environmental sources, but isolation of AM from clinical specimens does not necessarily imply disease. The present report found that less than 10 per cent of AM recovered from the respiratory tract was associated with disease and that sputum smear positivity was not a reliable indicator for disease. The American Thoracic Society have developed diagnostic criteria, based on a combination of clinical, radiological and laboratory findings for pulmonary disease caused by AM.[8]

The last AM Survey conducted by the Special Interest Group in 1988 (David Dawson, unpublished data) produced data similar to the current survey. In 1988, there were 334 significant AM cases, with an incidence rate of two cases per 100,000 population. There were 81 pulmonary cases, 88 lymphatic cases but only 51 soft tissue cases. The figures for MAC were also similar in the 1988 survey (70 cases). However, there was a threefold increase in *M. kansasii* in 2000 (21 cases compared to only seven cases in 1988), and twice the number of *M. fortuitum, M. marinum and M. ulcerans* were recovered from soft tissue in 2000. This is probably a reflection of improved technology and skill in recovering AM, rather than an increased infection rate (Table 2).

In an attempt to see if pathogenicity (or lack thereof) could be linked to a particular organism recovered from a pulmonary specimen, data for all slowly growing AM were reviewed (Table 4). *M. gordonae*, recovered from 96 patients, was never associated with pulmonary disease. *M. fortuitum* complex organisms (recovered from 73 patients) were rarely associated with disease. *M. abscessus* (7/40) was more frequently associated with disease than *M. chelonae* (0/22) although the incidence was still low. *M. kansasii*, was usually considered pathogenic (21/31). *M. asiaticum* was isolated only in the Northern Territory and does not appear to be disease associated. Unfortunately the figures are too small to draw conclusions for the other species. As molecular identification methods become more accessible it may be possible to identify species in the many isolates (more than 14% in this survey) that conventional methods cannot identify and to clarify associations between AM species and clinical disease.

Organisms recovered from soft tissue were almost always considered to be clinically significant. There was a higher recovery rate noted from Queensland (61.1% of all isolations) than from other states. *M. haemophilum* was only isolated in Western Australia (from 6 lymph nodes and from 6 soft tissues) and Victoria (3 soft tissue). This is unlikely to be a reflection of geographical differences but differences in

laboratory testing of appropriate tissues for *M. haemophilum*. While reference laboratories routinely culture lymph nodes, bone/joint and skin samples for this pathogen, which has special growth requirements, this however, may not be the case in routine clinical laboratories. Knowledge of *M. haemophilum* ecology is still incomplete but it has been isolated from pulmonary sites in immuno-compromised patients. In a detailed analysis of Australian data covering the period 1977–2000 (presented at the Australian Society for Microbiology's Annual Scientific Meeting in Perth, Western Australia in 2001, by this author) it was shown that *M. haemophilum* was recovered as often from immuno-competent persons as from immuno-compromised (non-HIV) ones, and more often than in HIV+ persons.

The most common isolate from lymphatic tissue was MAC, (48/60, 80%). Not all MAC are fully identified to species level, so complete data for *M. avium* and *M. intracellulare* are unavailable. Where data were available, there were twice as many (lymphatic) *M. intracellulare* isolations as *M. avium* (Table 5). *M. avium* accounted for 23% of pulmonary and 57% of lymphatic MAIS disease in 1988, somewhat different (36%, 30% respectively) for MAC disease seen in 2000.

Atypical mycobacteria cause disease in Australia, with an incidence conservatively estimated at 1.8 cases per 100,000 population. The main sites of disease are in soft tissue, pulmonary and in the lymphatics. Pulmonary AM infections may also present as sputum 'smear positive' requiring further tests to exclude *Mycobacterium tuberculosis*. The *Mycobacterium avium* complex is the most common encountered mycobacteria, isolated from blood, pulmonary and lymphatic sites in both disease and non-disease. The pathogenic rapid growing mycobacteria (*M. fortuitum—M. abscessus—M. chelonae* group) were common in the soft tissue infections.

A better understanding of the ecology and etiology of AM in light of the emerging diversity of the genus (as demonstrated by the number of new species described in the past 10 years) will require reference laboratories to continually improve their laboratory practices and identify clinically relevant isolates to species level.

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