Tuberculosis in Australia: bacteriologically confirmed cases and drug resistance, 2006 A report of the Australian Mycobacterium Reference Laboratory Network

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Abstract

In 2006, the Australian Mycobacterium Reference Laboratory Network identified 905 bacteriologically confirmed cases of disease caused by members of the *Mycobacterium tuberculosis* complex. The annual reporting rate was 4.4 cases per 100,000 population. Of the 905 isolates, 903 were *Mycobacterium tuberculosis* and two were *Mycobacterium bovis*. Fourteen children aged under 10 years (male n=5, female n=9) had bacteriologically confirmed tuberculosis. A total of 100 (11.1%) isolates of *M. tuberculosis* were resistant to at least one first-line anti-tuberculosis agent. Resistance to at least H and R (defined as multi-drug resistant – MDR) was detected in 22 (2.4%) *M. tuberculosis* isolates. Of the 22 MDR-TB isolates, 17 were from the respiratory tract (sputum n=11 bronchoscopy n=5, nasogastric aspirate n=1), three from lymph node, one from a sacral mass, and one sterile site fluid. Smear-positive specimens from the MDR-TB cases were found in sputum (n=6), lymph node (n=2), and one each of bronchoscopy and nasogastric aspirate specimens. The country of birth was known for all 100 cases with a drug-resistant isolate; 10 of whom were born in Australia. The 90 overseas-born cases with drug-resistant disease were from 27 countries. Two Australian-born cases had MDR-TB; one had worked extensively in the Philippines; the other was a contact of a known MDR-TB case. No cases of extensively drug-resistant TB (XDR-TB) were identified in 2006. However, an on-going review of laboratory data identified one case of XDR-TB in 2004.
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Keywords: *Mycobacterium tuberculosis*, *Mycobacterium bovis*, laboratory diagnosis, tuberculosis, drug resistance

Introduction

Several events in 2007 have highlighted the importance of a well resourced, quality assured laboratory service to national tuberculosis (TB) control program in low– and high-income countries. For example, the expanding outbreak in South Africa of extensively drug-resistant TB (XDR-TB; defined as MDR-TB with additional resistance to a fluoroquinolone and a second-line injectable agent) has emphasised that culture and susceptibility testing facilities are necessary in low-income countries where HIV and drug-resistant TB are endemic.[1] The case of a lawyer from the United States of America who travelled on several international flights whilst diagnosed purportedly with XDR-TB but who was subsequently confirmed to 'only' have multidrug-resistant TB (MDR-TB), has reinforced the importance of timely and accurate culture and drug susceptibility test (DST) results in high income countries.[2]

Laboratories and laboratory networks are a fundamental component of TB control, providing testing for diagnosis, surveillance and treatment monitoring at every level of the health-care system. New technologies that provide rapid detection, identification and drug susceptibility testing of *Mycobacterium tuberculosis* have contributed to the decline of TB disease prevalence.[1] Australia has been fortunate to have five Mycobacterium reference laboratories overseeing and supporting a network of public and private pathology laboratories providing high-quality mycobacteriology diagnostic services.

There are two sources of TB-related data for Australia. Since 1991, the National Notifiable Diseases Surveillance System (NNDSS) has provided statistics on TB notifications reported to public health authorities in Australia's states and territories, and includes cases that were identified on the basis of clinical and epidemiological information or on non-bacteriological laboratory investigations. The Australian Tuberculosis Reporting Scheme has been conducted by the Australian Mycobacterium Reference Laboratory Network (AMRLN) since 1986. Statistics compiled by the AMRLN relate to cases of bacteriologically confirmed tuberculosis. This AMRLN report describes the bacteriologically confirmed TB diagnoses for the year 2006.

Methods

The data are based on clinical specimens that were culture-positive for *Mycobacterium tuberculosis* complex (MTBC). No information on infections due to the bacille Calmette-Guérin strain of

Mycobacterium bovis is included in the present report. Isolates of MTBC were referred to one of the five laboratories comprising the AMRLN for species identification and drug susceptibility testing. Comparable methodologies are used in the reference laboratories. Relapse cases, as defined by the *National Strategic Plan for TB Control in Australia Beyond 2000* prepared by the National TB Advisory Committee,[3] were included in the laboratory data as laboratories are generally unable to differentiate relapse cases from new cases. Data include temporary visitors to Australia, illegal aliens or persons detained in Australia in correctional services facilities, and asylum seekers.

For each new bacteriologically confirmed case, the following information was collected where available:

- demography: patient identifier, age, sex, HIV status and state of residence;
- specimen: type, site of collection, date of collection and microscopy result;
- isolate: *Mycobacterium* species and results of drug susceptibility testing;
- nucleic acid amplification testing results; and
- drug-resistant isolates: patient country of origin, and history of previous TB treatment to determine whether resistance was initial or acquired.

Data from contributing laboratories were submitted in standard format to the AMRLN coordinator for collation and analysis. Duplicate entries (indicated by identical patient identifier and date of birth) were deleted prior to analysis. Rates were calculated using mid-year estimates of the population for 2006 supplied by the Australian Bureau of Statistics.[4]

For each case, the nature of the first clinical specimen that yielded an isolate of MTBC was used to record the nominal site of disease. Culture-positive specimens collected at bronchoscopy or by gastric lavage were counted as pulmonary disease. Patients with isolates recovered from multiple sites were counted as pulmonary disease (the most important category for public health purposes) if a sputum, gastric aspirate, bronchoscopy, or lung biopsy specimen was culture-positive.

Drug resistance among new cases (proxy for primary resistance) was defined as the presence of resistant isolates of *M. tuberculosis* in patients, who, in response to direct questioning, denied having received any prior anti-TB treatment (for more than one month) and, in countries where adequate documentation is available, for whom there is no evidence of such a history. Drug resistance among previously treated cases (proxy for acquired resistance) is defined as the presence of resistant isolates of *M. tuberculosis*, in cases who, in response to direct questioning, admit having been treated for one month or more or, in countries where adequate documentation is available, for whom there is evidence of such a history.[5]

Results

There were 905 bacteriologically confirmed cases of tuberculosis in 2006, representing an annual rate of 4.4 per 100,000 population. State-specific reporting rates varied from 1.8 (Tasmania) to 12.9 (Northern Territory) cases per 100,000 population (Table 1).

Causative organism

Almost all isolates were identified as *M. tuberculosis* (n=903), the remaining isolates being *Mycobacterium bovis* (n=2).

Table 1. Bacteriologically confirmed cases of tuberculosis in Australia, 1996 and 2004 to 2006, cases and rate per 100,000 population, by state or territory

State or territory	2006		2005*		2004*		1996*	
	n	Rate	n	Rate	n	Rate	n	Rate
New South Wales†	342	4.8	346	4.9	308	4.4	341	5.3
Northern Territory	27	12.9	24	11.9	21	10.5	23	12.6
Queensland	120	3.0	91	2.3	88	2.3	90	2.7
South Australia	51	3.3	36	2.3	43	2.8	28	1.9
Tasmania	9	1.8	10	2.1	8	1.7	3	0.6
Victoria	263	5.2	261	5.2	262	5.3	214	4.7
Western Australia	93	4.5	42	2.1	57	2.9	51	2.9
Total	905	4.4	810	4.0	787	3.9	750	4.1

* Data from previous reports of the Australian Mycobacterium Reference Laboratory Network.

† Data from the Australian Capital Territory are included with those from New South Wales.

Distribution by gender, age and site of disease

Complete information for gender and age were available for 902 (99.7%) of all patients; 392 (43.5%) were from females, 511 (56.7%) were from males, and gender was unknown for three cases. Fourteen children aged under 10 years (male n=5, female n=9) had bacteriologically confirmed tuberculosis (lymph node n=5, gastric aspirate n=2, sputum n=2, bronchoscopy n=1, cerebrospinal fluid n=2, biopsy n=2).

The site of disease was dependent upon age and gender. The overall male:female ratio was 1.3:1. For respiratory isolates, the male:female percentage was 1.7:1. For TB lymphadenitis, the female:male percentage was 1.7:1. For males, there were two distinct peak age groups in bacteriologically-confirmed rates: a rise to 8.2 cases of TB per 100,000 population in the 25–29 year age group and a second peak in elderly males aged more than 75 years (>13.0 cases per 100,000 population). The age distribution of female cases was similar with 7.5 and 9.3 bacteriologically confirmed TB cases per 100,000 population in the 25–29 years and >84 years age groups, respectively. The median age group for patients with bacteriologically confirmed disease was 30–34 years for males and 35–39 years for females.

The predominant culture-positive specimen type was sputum (n=438, 48.4%); a further 122 (13.5%) were obtained from bronchoscopy, and five were from lung biopsies (Table 2). Fifty-nine pleural specimens (38 fluid, 21 biopsy/tissue) were culture-positive. Of these 59 pleural specimens, five biopsy specimens and one pleural fluid was smear-positive. The most commonly encountered extrapulmonary culture-positive specimen was lymph tissue (n=163, 18.0%) followed by pleural (n=59, 6.5%), peritoneal (n=14, 1.5%), bone/joint (n=29, 3.2%), and genitorurinary tract (n=28, 3.1%).

Table 2. Site of specimens smear– and culture-positive for Mycobacterium tuberculosis complex, 2006

	n	Smear positive (%)*
Sputum	438	241 (55.8)
Bronchoscopy	122	36 (29.5)
Lymph node	163	43 (26.5)
Pleural	59	6 (10.2)†
Genito-urinary	28	ND‡
Bone/joint	29	ND‡
Peritoneal	14	ND‡
Skin	3	ND‡
Cerebrospinal fluid	7	ND‡

* Based on specimens that reported a microscopy result and excludes (i) microscopy not performed or (ii) result unknown.

† 5/6 smear positive specimens were pleural biopsies.

‡ Percentage of specimens smear positive not calculated due to the small number of cases.

Association with HIV

The AMRLN database recorded the HIV status of only 110 (12.2%) patients. Four patients were identified as HIV-seropositive.

Microscopy

Results of microscopy were available for 889 of 905 (98.2%) specimens. Microscopy was not performed on 14 specimens and no results were provided for the remaining two specimens. For specimens where smear results were available, 241 of 438 (55.8%) sputum and 36 of 122 (29.5%) bronchoscopy specimens respectively were positive (Table 2). Of 59 pleural specimens (21 biopsy and 38 fluids) that were culture positive for *M. tuberculosis*, five biopsies and one fluid specimen was smear-positive. Lymph node specimens were smear-positive in only 43 of 163 (26.5%) patients.

Drug susceptibility testing

Results of *in vitro* drug susceptibility testing were available for all 905 isolates for isoniazid (H), rifampicin (R), ethambutol (E), and for 904 of 905 isolates for pyrazinamide (Z). A total of 100 (11.1%) isolates of *M. tuberculosis* were resistant to at least one of these anti-tuberculosis agents. Results of testing for streptomycin (S) were available for 308 of 905 (34.0%) isolates with 46 demonstrating resistance to at least S; 11 had mono-resistance, 17 were resistant to S and H, 15 MDR-TB strains were also S-resistant, and there were three cases of S/E resistance. Resistance to at least H and R (defined as MDR) was detected in 22 (2.4%) isolates. All of the MDR isolates were *M. tuberculosis* (Table 3). Of the 22 MDR-TB isolates, 17 were from the respiratory tract (sputum n=11 bronchoscopy n=5, nasogastric aspirate n=1), three from lymph node, one from a sacral mass, and one fluid (site not stated). Six of the MDR-TB positive sputum specimens were smear-positive, one bronchoscopy specimen and the nasogastric aspirate, and two lymph node specimens.

Five patients with MDR-TB were from the Papua New Guinea–Torres Strait Islands (TSI) cross-border region who access health services in outer TSI and are eligible to receive treatment in Australia. MDR-TB was also isolated from patients born in India (n=6), Australia (n=2), and Indonesia (n=2) with a single case each from England, Lebanon,

Table 3. Drug resistance patterns in multi-drug-resistant strains, Australia 1995 to 2006

Resistance pattern (standard drugs)*	2006	2005	2004	2003	2002	2001	2000
H+R only	16	5	7	4	8	8	3
H+R+E	1	3	2	2	1	1	1
H+R+Z	0	1	1	1	1	3	3
H+R+E+Z	5	3	2	0	2	0	1
XDR-TB	0	0	1	0	0	0	0
Total (%)	22 (2.4)	12 (1.5)	12 (1.5)	7 (0.9)	12 (1.7)	12 (1.6)	8 (1.0)

Resistance pattern (standard drugs)*	1999	1998	1997	1996	1995
H+R only	2	2	6	10	3
H+R+E	1	1	1	1	1
H+R+Z	1	2	5	4	1
H+R+E+Z	0	1	2	0	0
XDR-TB	0	0	0	0	0
Total (%)	4 (0.5)	6 (0.9)	14 (1.9)	15 (2.0)	5 (0.7)

* The streptomycin result was not considered for this table.

† H = isoniazid, R = rifampicin, E = ethambutol, Z = pyrazinamide

Nigeria, the Philippines, Somalia, Thailand, and Uzbekistan. The English-born patient had been a health care worker in South Africa. Of the two Australian-born cases, one had worked extensively in the Philippines, and the other was a cousin to an Indian case of MDR-TB.

Mono-resistance to isoniazid (H) was detected in 53 isolates, three isolates were resistant to ethambutol (E) alone, and one isolate was resistant to pyrazinamide (Z) alone. No rifampicin mono-resistance was observed. Ninety-two isolates demonstrated resistance to H at a concentration of 0.1 mg/L. Of these, 66 (71.2.0%) demonstrated resistance to H at the higher level of 0.4 mg/L. Among MDR-TB strains, 19/22 (86.4%) demonstrated H resistance at the higher concentration (0.4 mg/L). Forty of 100 (40.0%) specimens culture-positive for drug-resistant strains, including 33 of 68 (48.5%) sputum or bronchoscopy specimens, were smear-positive for acid fast bacillus. The two *M. bovis* isolates, which are inherently resistant to pyrazinamide, were not included in the above results.

New case or previously treated, and country of birth

Of the 100 *M. tuberculosis* isolates resistant to at least one of the standard drugs (H,R,E,Z), 80 were from new cases, 14 were from previously treated cases, and treatment information was not available for four cases. The country of birth was known for all cases with a drug-resistant isolate; 10 were born in Australia. The 90 overseas-born cases with drug-resistant disease were from 27 countries, 46 (51.1%) were from four countries: India (n=16). Vietnam (n=11), China (n=10), and the Philippines (n=9).

Discussion

The AMRLN has collected data on bacteriologically confirmed cases of TB since 1986. The results for each year have been published in peer reviewed journals.[6–19] Data from 2006 broke new ground for: (i) the greatest number of bacteriologically confirmed cases of TB/cases per 100,000 population (905/4.4%); (ii) the number of isolates with drug resistance to at least one anti-tuberculous drug; and (iii) the number/percentage of MDR-TB isolates (22/2.4%). Since the AMRLN began collecting data in 1986, the number of bacteriologically confirmed cases per 100,000 population has remained stable at between a low of 3.5 (1992) and a previous high of 4.1 (1996).

Technological advances in laboratory equipment such as automated broth-based culture systems have certainly reduced the time to culture positivity and may have increased the total number of cases.[20] The radiometric broth-based culture system was introduced into Australia in the late 1980's and was the mainstay culture system into the early 2000s. All AMRLN laboratories are now using a non-radiometric automated broth culture system for primary culture.

Drug-resistant TB has emerged as a global problem that threatens TB control programs in many countries. In so many ways, Australia has been the 'lucky country' and the national TB control programs have achieved enviable success. None of the MDR-TB cases from 2006 have been acquired through treatment within Australia, a tribute to the continued high quality of Australian TB clinical services. The finding of 2.4% MDR-TB isolates is the highest recorded since data collection began in 1986. The significance or otherwise of the 2006 data will depend upon future findings but they must not be ignored.

The spectre of MDR-TB makes pre– and post-arrival screening of overseas-born persons even more critical. In particular, health care workers (HCWs) require monitoring; as emphasised by the detection in 2006 of MDR-TB in an English-born HCW who had worked previously in South Africa and similar instances of MDR-TB in overseas-born HCWs in previous reports. Australia also has a role to assist national TB control program and TB laboratory networks in our region.

The 2005 report discussed the global emergence of extensively drug-resistant TB (XDR-TB) and reported that a review of AMRLN data found no cases of XDR-TB.[19] Subsequently, an on-going review of Australian laboratory records found that one confirmed XDR-TB case in an overseas-born person had been identified in 2004 (personal communication, Dr M Hurwitz, Director, Thoracic Unit, The Canberra Hospital). The retrospective diagnosis of XDR-TB has been confounded in Australia and other countries by a change in the definition of XDR-TB in 2006,[21] by changes in laboratory technologies, and by revisions to the critical breakpoints for defining resistance to individual drugs. Retrospective and prospective surveillance of laboratory data will continue in the Australian setting.

The National TB Advisory Committee and the AMRLN have produced complementary publications for over 15 years focusing on the epidemiological and clinical information from the NNDSS database, and on DST results from bacteriologically-confirmed cases in the AMRLN dataset, respectively. This article will be the last standalone MRLN publication because the two TB databases will be combined before the end of 2008 so that more detailed analyses can be performed. For example, Australia will finally be able to report separate 'primary' and 'acquired' drug resistance rates when the clinical information in the NNDSS database, which identifies 'new' and 're-treatment' cases, is combined with the DST results in the AMRLN database. Furthermore, combining the two Australian TB databases will allow drug resistance rates to be calculated by the country of origin for overseas-born patients. These analyses may identify particular migrant groups at increased risk of drug-resistant disease. Australian doctors might consider adding additional agents to the initial treatment regimens of these patients until individual DST results become available. These calculations will also provide WHO and national TB programs with surrogate estimates of the drug resistance rates for some countries where quality assured drug susceptibility testing facilities are not widely available (e.g. Indonesia, Vietnam, Somalia, Sudan, Papua New Guinea).

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The Australian Mycobacterium Reference Laboratory Network comprises the Mycobacterium Reference Laboratories at the following facilities:

- Institute of Medical and Veterinary Science, Adelaide, South Australia
- Queensland Health Pathology Services, Herston Hospitals Complex, Herston, Queensland
- Victorian Infectious Diseases Reference Laboratory, North Melbourne, Victoria
- PathWest Laboratory Medicine WA – QEIIMC, Hospital Avenue, Nedlands, Western Australia
- Institute of Clinical Pathology and Medical Research, Westmead Hospital, Westmead, New South Wales

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