Tuberculosis in Australia: bacteriologically confirmed cases and drug resistance, 2002

A report of the Australian Mycobacterium Reference Laboratory Network

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Abstract

The Australian Mycobacterium Reference Laboratory Network collected and analysed laboratory data on new cases of disease caused by *Mycobacterium tuberculosis* complex in the year 2002. A total of 712 cases were identified by bacteriology, representing an annual reporting rate of 3.6 cases of laboratory-confirmed tuberculosis per 100,000 population. The most commonly encountered culture-positive specimens were sputum (n=325), lymph node (n=142) and bronchoscopy (n=100). Smears containing acid fast bacilli were present in sputum (53.2%), bronchoscopy (37.9%) and lymph node (21.2%). Eight children (male n=3, female n=5) under 10 years of age had bacteriologically-confirmed tuberculosis. A total of 55 isolates (7.7%) of *M. tuberculosis* were resistant to at least one of the standard anti-tuberculosis agents. Resistance to at least isoniazid and/or rifampicin was noted for 53 isolates (7.4%), with multidrug-resistance (MDRTB) observed in 12 (1.9%) isolates. Of the 12 MDRTB isolates, eight were from the respiratory tract and five were from smear positive specimens. Of the patients with drug resistant *M. tuberculosis* isolates, 51/55 (92.7%) were classified as having initial resistance, none had acquired resistance during treatment in Australia. The country of birth was known for 54 of 55 such patients; four were Australian-born, and 50 (90.9%) had migrated from a total of 17 countries. Nucleic acid amplification testing (NAAT) was performed on 139 (19.5%) of the 712 culture-positive specimens. Of smear positive respiratory specimens, 74/80 (92.5%) were NAAT positive. For smear negative respiratory specimens, 12/17 (70.6%) reported a NAAT positive result. Importantly, false-negative NAAT results were obtained from 1/16 and 5/64 of smear positive bronchoscopy and sputum specimens respectively. *Commun Dis Intell* 2003;27:459–465.

Keywords: Mycobacterium tuberculosis, laboratory diagnosis, tuberculosis, drug resistance, nucleic acid amplification test

Introduction

Australia continues to record one of the lowest notification rates (5–6 cases per 100,000 population) of tuberculosis (TB) in the world.[1] As part of the World Health Organization (WHO) Western Pacific Region, Australia's near neighbors have some of the highest burdens of TB in the region. These countries include China, Philippines, Papua New Guinea, Cambodia and Vietnam.[2] Australia also shares a close geographic relationship with the WHO South East Asia Region, in particular, Indonesia which has the third highest burden of TB in the world.[1] These countries also have to deal with drug resistance and co-infection with HIV in a setting of chronically under-funded and under-resourced national TB programs.

There are two sources of TB-related data for Australia. Since 1991, the National Notifiable Diseases Surveillance System (NNDSS) has provided statistics on cases of tuberculosis reported to public health authorities in Australia's states and territories.[3] The second source, the Australian Tuberculosis Reporting Scheme has been conducted by the Australian Mycobacterium Reference Laboratory Network (AMRLN) since 1986.[4] Statistics compiled by the AMRLN relate to cases of bacteriologically-confirmed tuberculosis whereas NNDSS data will have a proportion of cases that are identified on the basis of clinical and epidemiological information, or on non-bacteriological laboratory investigations. This report describes the bacteriologically-confirmed TB diagnoses for the year 2002.

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Methods

The data are based on clinical specimens that were culture-positive for *Mycobacterium tuberculosis* complex (MTBC). Although the bacille Calmette-Guérin strain of *M. bovis* is a member of the MTBC, no information on this organism is included in the present report. Almost all isolates of MTBC were referred to one of the five laboratories comprising the AMRLN, for specific 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, were included in the laboratory data as laboratories are generally unable to differentiate relapse cases from new cases.[5] Temporary visitors to Australia were included as were illegal immigrants within correctional services facilities and asylum seekers located in detention centres or on temporary visas within Australia.

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: species of mycobacterium and results of drug susceptibility testing;
- nucleic acid amplification testing: results of testing; and
- if the isolate was drug resistant: 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 scheme 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 the year 2002, supplied by the Australian Bureau of Statistics.[6]

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 considered to indicate pulmonary disease. Cases with multi-site isolations, provided a sputum or bronchoscopy specimen was culture-positive, were listed as having pulmonary disease, the most important category for public health purposes. Cases for which there were multiple-site isolations were not categorised as having miliary or disseminated disease as differentiation is based on clinical findings that are generally not available to the reporting laboratories. Initial drug resistance was defined as the presence of drug resistant strains of *M. tuberculosis* in cases of tuberculosis in which there was no known history of anti-tuberculosis treatment. Patients who had begun anti-TB treatment and had developed resistance to one or more of the drugs used during treatment were recorded as having acquired drug resistance.[7]

Results

There were 712 bacteriologically confirmed cases of tuberculosis in 2002 (Figure 1), representing an annual rate of 3.6 cases per 100,000 population. State-specific reporting rates varied from 1.7 (South Australia and Tasmania) to 13.0 cases per 100,000 population (Northern Territory) (Table 1). There were 10 patients from Papua New Guinea who were diagnosed in Australia (included in the Queensland data), and most jurisdictions had at least one person visiting from overseas being diagnosed with TB.

Figure 1. Comparison between tuberculosis notifications and laboratory data, Australia, 1990 to 2002

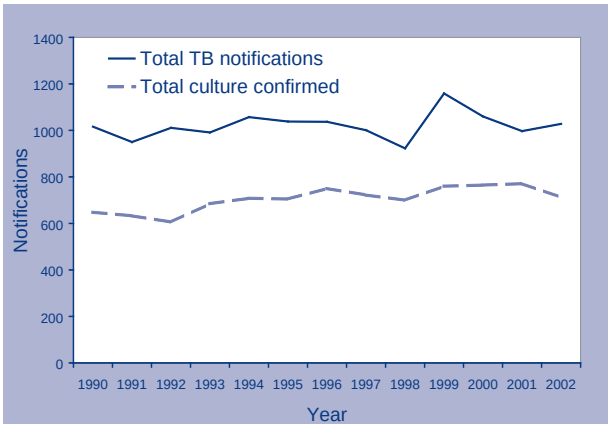


Table 1. Bacteriologically confirmed cases of tuberculosis in Australia, 1992 and 1998 to 2002, cases and rate per 100,000 population, by state or territory*

State or territory	2002		2001[8]		2000[9]		1999[10]		1992[11]	
	n	%	n	%	n	%	n	%	n	%
New South Wales†	301	4.3	327	4.8	307	4.5	291	4.3	252	4.0
Victoria	208	4.3	222	4.6	231	4.8	261	5.5	164	3.7
Queensland	97	2.6	81	2.2	76	2.1	75	2.1	90	3.0
Western Australia	46	2.4	68	3.6	63	3.3	64	3.4	31	1.9
South Australia	26	1.7	38	2.5	41	2.7	46	3.1	41	2.8
Tasmania	8	1.7	12	2.8	2	0.4	2	0.4	7	1.5
Northern Territory	26	13.0	23	11.6	45	23.0	21	10.9	21	12.4
Total	712	3.6	771	4.0	765	4.0	760	4.0	606	3.5

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

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

Causative organism

Almost all isolates were identified as *M. tuberculosis* (710), the remaining two isolates being *M. africanum*. For 2002, there were no bacteriologically confirmed cases of TB caused by *M. bovis*.

Distribution by gender, age and site of disease

Complete information for gender and age were submitted for 697 (97.9%) of the 712 cases. Eight children (male n=3 female n=5) under 10 years of age had bacteriologically confirmed tuberculosis (sputum n=4, gastric aspirate n=2, lymph node n=1, tissue n=1). The relationship of tuberculosis to age and gender is shown in (Figure 2). The overall male: female ratio was 1.05:1. The age and gender rates varied depending upon the site of infection. The predominant culture-positive specimen type was sputum (n=325, 45.6%); a further 100 (14.0%) were bronchoscopy, 15 were biopsy/tissue, and two were aspirate specimens. Thirty-four pleural specimens (31 fluid, 3 biopsy/tissue) accounted for only 4.8 per cent of all culture-positive specimens.

The most commonly encountered extrapulmonary culture-positive specimen was lymph tissue (n=142, 19.9%) followed by those from the genitourinary tract (n=32, 4.5%) and bone/joint (n=23, 3.2%). The female:male ratio of 1.95:1 demonstrated the skewed isolation of extrapulmonary MTBC from females, particularly in the 15–49 year age group (Figure 3). There were 10 isolates from other sites including tissue (spleen, liver, caecum, pericardium, breast, tongue; n=1 for each), pericardial fluid (n=1), and abscess (n=3).

Figure 2. Laboratory diagnosis of *Mycobacterium tuberculosis* complex disease, Australia 2002, by age and sex

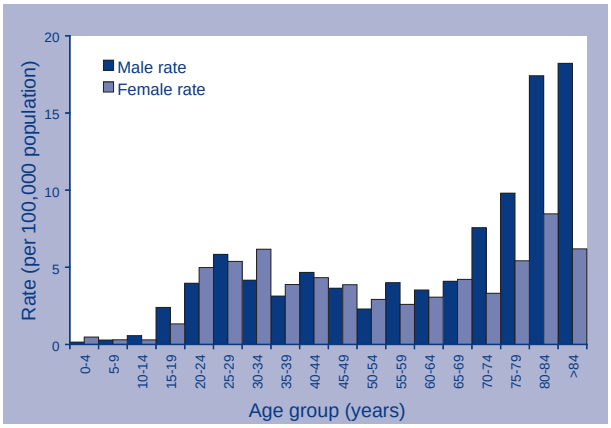
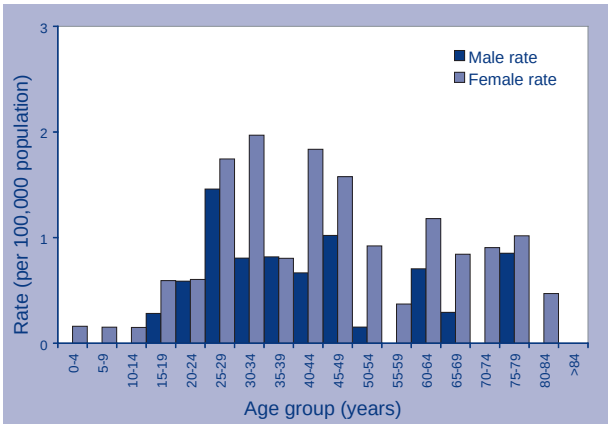


Figure 3. Isolation of MTBC from lymph node, Australia, 2002, by age and sex



Association with HIV

The AMRLN database recorded the HIV status for only 51 (7.2%) patients. A single patient was identified as HIV seropositive; and had a multidrug-resistant strain of *M. tuberculosis* isolated from smear negative sputum.

Microscopy

Results of microscopy were available for 669/712 (94.0%) of specimens; microscopy was not performed on eight specimens and results for a further 35 were unknown. For specimens reporting a microscopy result, smears were positive for 165/310 (53.2%) of sputum and 36/97 (37.9%) of bronchoscopy specimens respectively (Table 2). A total of 34 pleural specimens were culture positive for *M. tuberculosis* with only 3 (9.1%) smear-positive for acid fast bacilli (AFB). Of the 142 specimens of lymph node, microscopy results were available for 133; and 28 (21.2%) were smear-positive for AFB.

Drug susceptibility testing

Results of *in vitro* drug susceptibility testing were available for all 712 isolates for isoniazid (H), rifampicin (R), ethambutol (E), and pyrazinamide (Z). A total of 55 isolates (7.7%) of *M. tuberculosis* were resistant to at least one of the above anti-tuberculosis agents. Results of testing for streptomycin (S) were available for 194/712 (27.2%) of isolates with four demonstrating mono-resistance, and a further five isolates resistant to both S+H. Resistance to at least H and/or R was noted for 53 isolates (7.4%), with resistance to both H and R (i.e. defined as multidrug-resistance) observed in 12 (1.9%) isolates. All of the MDR isolates were *M. tuberculosis* (Table 3). Of the 12 MDRTB isolates, eight were from the respiratory tract (sputum n=7, lung tissue n=1); the remaining four isolates were from lymph node. Five of the MDRTB-positive sputum specimens were smear positive as was the lung tissue. All four lymph tissues were smear negative.

Table 2. Site of specimens smear- and culture-positive for *Mycobacterium tuberculosis* complex disease, Australia, 2002

	Number*	Smear positive (%)†
Sputum	325	53.2
Bronchoscopy	100	37.9
Lymph node	142	21.2
Pleural	34	9.1
Genito-urinary	32	30.8
Bone/joint	23	17.4
Peritoneal	3	ND
Skin	3	ND
CSF	3	ND

* Specimens not tabulated: 15 pulmonary tissue samples, 2 aspirates from upper respiratory tract, from 15 specimens from miscellaneous sites, and 15 of unknown site.

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

ND Percentage of specimens smear positive not calculated due to small numbers.

There was no mono-resistance to either rifampicin or ethambutol, but pyrazinamide mono-resistance was demonstrated in two strains. There were 53 strains that demonstrated resistance to H at a concentration of 0.1 mg/L in the radiometric BACTEC system. Of these, 43 (81.1%) demonstrated resistance at the higher level of 0.4 mg/L. Eighteen of 55 (32.7%) specimens culture-positive for drug resistant *M. tuberculosis* were also smear-positive for AFB.

Table 3. Drug resistance patterns in multidrug-resistant strains, Australia, 1996 to 2002

Resistance pattern (standard drugs)*	2002	2001[8]	2000[9]	1999[10]	1998[10]	1997[12]	1996[13]
H+R only	8	8	3	2	2	6	10
H+R+E	1	1	1	1	1	1	1
H+R+Z	1	3	3	1	2	5	4
H+R+E+Z	2	0	1	0	1	0	0
Total (%)	12 (1.7)	12 (1.6)	8 (1.0)	4 (0.5)	6 (0.9)	14 (1.9)	15 (2.0)

* The streptomycin result was not considered for this table.

H = Isoniazid, R = rifampicin, E = ethambutol, Z = pyrazinamide

Initial or acquired resistance and country of origin

There were 55 *M. tuberculosis* isolates resistant to at least one of the standard drubs (H, R, E or Z). Of these, 51/55 (92.7%) were classified as having initial resistance, none had acquired resistance during treatment in Australia, and no data were available on the presence or absence of previous treatment for four patients. The country of birth was known for 54/55 patients; four were Australian-born, and 50 (90.9%) had migrated from a total of 17 countries. Of the 50 migrants with drug resistant disease, 31 (62.0%) had migrated from one of four countries: Vietnam (n=9), Philippines (n=9), India (n=9), and China (n=4). The four patients with mono-resistance to streptomycin all migrated from Vietnam.

Use of nucleic acid amplification tests

Nucleic acid amplification testing (NAAT) was performed on 139/712 (19.5%) specimens, all of which subsequently grew *M. tuberculosis* on culture. Sputum (n=76), bronchoscopy (n=24), lymph node (n=16) and tissue samples (n=12) were the most frequently tested. Of the 139 specimens, 118 were NAAT positive and 21 were negative. For smear positive respiratory specimens, 74/80 (92.5%) were NAAT positive. For smear negative respiratory specimens, 12/17 (70.6%) reported a NAAT positive result (Table 4). Importantly, 1/16 and 5/64 of smear positive bronchoscopy and sputum specimens respectively that were culture positive for *M. tuberculosis* were NAAT negative. For extrapulmonary specimens such as lymph node, other tissues and urine, smear positives were more likely than smear negatives to yield a positive NAAT, although a small proportion of smear and culture positive specimens produced a negative NAAT result.

Table 4. Results for nucleic acid amplification tests performed on respiratory specimens, Australia, 2002

NAAT result*	Culture positive respiratory specimens	
	Smear positive	Smear negative
Positive	74	5
Negative	6	12
Total (97)†	80	17

* Various NAAT methods were used, depending upon laboratory.

† Three respiratory samples did not record a smear result.

A further five specimens were NAAT positive but culture negative for MTBC. These specimens were not included in the 2002 laboratory data. Specimen types included lymph node (n=2), and one each of sputum, pleural fluid and a bone biopsy. One non-viable culture received into an AMRLN laboratory was NAAT positive.

Discussion

The isolation of 710 *M. tuberculosis* and two *M. africanum* from clinical specimens for 2002 yielded a rate of 3.6 cases per 100,000 population, an outcome consistent with the laboratory data reported for the previous 17 years.[4,8–13] The NNDSS reported 1,028 tuberculosis notifications in 2002,[14] marginally up on the 997 cases reported in 2001.[15] The NNDSS has consistently reported higher notifications than the AMRLN laboratory data (range 24–40%) and for 2002, there was a 44 per cent difference between the two datasets. In 2002, the NNDSS dataset recorded 602 cases from the respiratory tract, 97 pleural, 165 lymphatic, and 52 bone/joint.[14] If the two databases are compared, 70.5 per cent, 35 per cent, 86 per cent and 44 per cent of respiratory, pleural, lymphatic and bone/joint cases respectively were bacteriologically confirmed. In contrast to the 2001 laboratory report where there was almost 90 per cent bacteriological confirmation of respiratory tuberculosis, only 70.5 per cent of respiratory tuberculosis was bacteriologically confirmed in 2002. The consistent finding of a much lower proportion of extrapulmonary disease being confirmed by culture suggests an ongoing reliance being placed upon clinical, histological or radiological diagnoses of these forms of tuberculosis.

In Australia, lymph node tuberculosis is the most common extrapulmonary site and accounts for around 20 per cent of all bacteriologically confirmed disease. The majority of cases occur in overseas-born women in the 15–49 year age range; and these findings have been noted elsewhere.[16,17,18] Specimens submitted for culture included pus from draining sinuses, fine needle aspirates, or lymph tissue. In 2000, 2001 and 2002, smears from lymphatic tissue were positive for AFB on 28.7 per cent, 19.2 per cent and 21.2 per cent of cases respectively.[8,9] Other studies have demonstrated AFB in 25–50 per cent of lymph node biopsy smears.[19] Cultures are positive for MTBC on only 60–70 per cent, in part due to the small population of AFB within the lymph tissue. Interestingly, affected lymph nodes may increase in size, or new nodes may appear whilst on appropriate anti-TB treatment.

Such a paradoxical response is not indicative of inadequate treatment or relapse, and the nodes are sterile on culture.[16] The AMRLN laboratories continue to receive requests for molecular evaluation of formalin-fixed material, notably lymph tissue. These techniques are demanding, time consuming, expensive, and frequently yield a negative result due to the presence of inhibitors within the sample.

A total of 55 isolates (7.7%) of *M. tuberculosis* were resistant to at least one of the standard anti-tuberculosis agents. For 2002, there were 12 (1.9%) isolates of MDRTB. There was no mono-resistance to either rifampicin or ethambutol, but pyrazinamide mono-resistance was demonstrated in two strains. In Australia, the level of drug resistance and MDRTB continues to remain stable. The level of acquired drug resistance in Australia remains low, most drug resistance occurred in patients born overseas and reflects the performance of the TB program of their country of origin.

Data were collected on results of nucleic acid amplification tests. As expected, there was a high level of agreement between NAAT and culture results but a lower level of agreement with smear negative specimens. The level of NAAT lies somewhere between that for culture and smear microscopy. In studies where quantitative culture was performed, the majority of false-negative NAA test results were due to low concentrations of MTBC.[20,21,22] The most important consideration is that culture remains the 'gold standard' for laboratory investigation of mycobacterial disease and a sufficient amount of an appropriate specimen must be used for culture.[23] NAAT should be used to complement 'traditional' laboratory investigations and be limited to situations where the result is likely to influence clinical and/or public health decisions.[24] In 1996, a workshop among clinical, laboratory and public health practitioners considered the clinical suspicion of TB and sputum microscopy in conjunction with the outcomes of NAAT. NAAT was felt to be most useful in situations where there was a smear-negative patient at high risk of TB or a smear-positive patient considered a low risk patient. NAAT results can influence decisions on whether to begin anti-TB treatment, to consider further diagnostic investigations, or to institute public health actions.[25]

Bacteriological confirmation of tuberculosis is important as an isolate is required for identification to species level, drug susceptibility testing and genotyping. The recent agreement among the AMRLN laboratories to a common approach to genotyping based on mycobacterial interspersed repeat units means that the potential to compare all *M. tuberculosis* isolates at a national level is now available.[26] It is proposed to include genotyping data in future AMRLN reports.

Acknowledgements

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, Prince Charles Hospital, Chermside, Queensland
- Victorian Infectious Diseases Reference Laboratory, North Melbourne, Victoria
- Western Australian Centre for Pathology and Medical Research, The Queen Elizabeth II Medical Centre, Nedlands, Western Australia
- Institute of Clinical Pathology and Medical Research, Westmead Hospital, Westmead, New South Wales

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