Tuberculosis in Australia: bacteriologically confirmed cases and drug resistance, 2001

A report of the Australian Mycobacterium Reference Laboratory Network

Richard Lumb,[1,2] Ivan Bastian,[1,2] David Dawson,[2] Chris Gilpin,[2] Frank Haverkort,[2] Greg James,[2] Aina Sievers[2]

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 2001. A total of 771 cases were identified, representing an annual reporting rate of 4.0 cases of laboratory-confirmed tuberculosis per 100,000 population. The predominant specimen type was sputum, (n=369) and a further 111 were collected at bronchoscopy. Smears were positive for 214 of 369 (58.0%) sputum and 42 of 111 (37.8%) bronchoscopy specimens respectively. Seven children (male n=5, female n=2) under 10 years of age had bacteriologically confirmed tuberculosis. A total of 69 isolates (8.9%), comprising 67 *M. tuberculosis*, one *M. africanum*, and one *M. bovis*, were resistant to at least one of the anti-tuberculosis agents. Excluding the *M. bovis* isolate, 61 of 64 (93.5%) were classified as having initial resistance, three had acquired resistance, and no data were available on the presence or absence of previous treatment for four patients. Resistance to at least isoniazid and/or rifampicin was noted for 67 isolates (8.7%), with resistance to both isoniazid and rifampicin (i.e. defined as multidrug-resistant disease) observed in 12 (1.6%) isolates. All of the multidrug-resistant isolates were *M. tuberculosis*, 10 were from the respiratory tract. The country of birth was known for 63 of 68 (92.6%) patients with a drug-resistant strain of *M. tuberculosis* or *M. africanum*; five were Australian-born and 58 (92.1%) had migrated from a total of 22 countries. One hundred and seven respiratory specimens had a nucleic acid amplification testing performed; 89 of 90 (98.9%) smear positives were nucleic acid amplification testing positive, whilst only 13 of 17 (76.5%) smear negative specimens were nucleic acid amplification testing positive. The 2001 laboratory data reveals a stable incidence rate and level of drug resistance in isolates from Australian patients with tuberculosis. *Commun Dis Intell* 2003;27:173–180.

Keywords: Mycobacterium tuberculosis, Mycobacterium bovis, *laboratory diagnosis, tuberculosis, drug resistance*

Introduction

Since 1991, the National Notifiable Diseases Surveillance System (NNDSS) has provided statistics on cases of tuberculosis (TB) reported to public health authorities in Australia's States and Territories.[1] The Australian Tuberculosis Reporting Scheme has been conducted by the Australian Mycobacterium Reference Laboratory Network (AMRLN) since 1986.[2] 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.[3] This report describes the bacteriologically confirmed TB diagnoses for the year 2001.

1. Infectious Diseases Laboratories, Institute of Medical and Veterinary Science, Adelaide, South Australia
2. Australian Mycobacterium Reference Laboratory Network

Corresponding author: Mr Richard Lumb, Principal Medical Scientist, Mycobacterium Reference Laboratory, Infectious Diseases Laboratories, Institute of Medical and Veterinary Science, PO Box 14, Rundle Mall, Adelaide, South Australia 5000, Australia. Telephone: +61 8 8222 3579. Facsimile: +61 8 8222 3543. Email: richard.lumb@imvs.sa.gov.au.

Methods

The data are based on clinical specimens that were culture-positive for *Mycobacterium tuberculosis* complex (MTBC). Although the Bacille Calmette Guérin (BCG) strain of *M. bovis* is a member of the MTBC, no information on this organism is included in the present report. In 2001, nearly 60 laboratories performed culture for mycobacteria.[4] 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,[5] were included in the laboratory data as laboratories are generally unable to differentiate relapse cases from new cases. Temporary visitors to Australia were included as were illegal aliens 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 test (NAAT): 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 2001 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

Total reports and distribution by state or territory

There were 771 bacteriologically confirmed cases of tuberculosis in 2001 (Figure 1), representing an annual rate of 4.0 cases per 100,000 population. State-specific reporting rates varied from 2.2 cases (Queensland) to 11.6 cases per 100,000 population (Northern Territory) (Table 1). There were five patients from Papua New Guinea who were diagnosed in Australia (included in the Queensland data), five persons identified as asylum seekers from Afghanistan (South Australia n=4, Western Australia n=1), and two persons with temporary protection visas from East Timor.

Figure 1. Comparison between tuberculosis notifications and laboratory data, Australia, 1990 to 2001

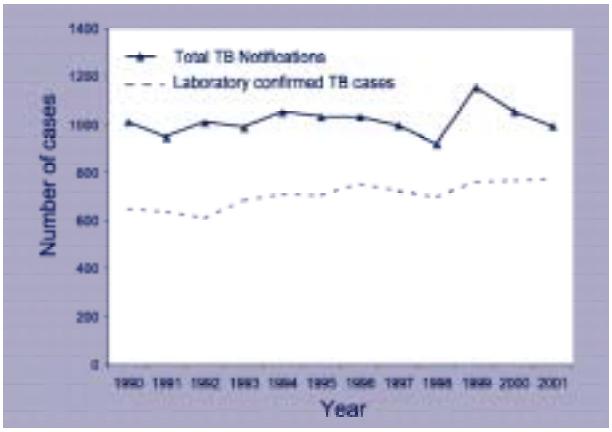


Table 1. Bacteriologically confirmed cases of tuberculosis in Australia, 1991 and 1998 to 2001, cases and rate per 100,000 population by state or territory*

State or territory	2001[8]		2000[9]		1999[9]		1998[10]		1991[10]	
	n	%	n	%	n	%	n	%	n	%
New South Wales†	327	4.8	307	4.5	291	4.3	289	4.4	246	4.0
Victoria	222	4.6	231	4.8	261	5.5	192	4.1	201	4.5
Queensland	81	2.2	76	2.1	75	2.1	85	2.5	79	2.7
Western Australia	68	3.6	63	3.3	64	3.4	66	3.6	46	2.8
South Australia	38	2.5	41	2.7	46	3.1	40	2.7	31	2.1
Tasmania	12	2.8	2	0.4	2	0.4	6	1.3	9	1.9
Northern Territory	23	11.6	45	23.0	21	10.9	22	11.6	21	12.4
Total	**771**	**4.0**	**765**	**4.0**	**760**	**4.0**	**700**	**3.7**	**633**	**3.6**

* Data from previous reports of the AMRLN.

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

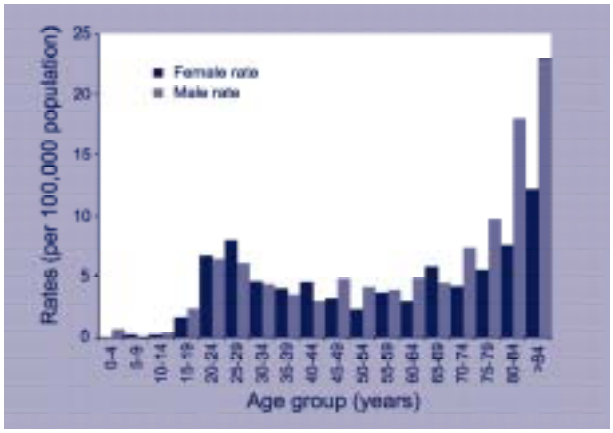
Causative organism

Almost all isolates were identified as *M. tuberculosis* (768) with only two isolates of *M. africanum* and one of *M. bovis*.

Distribution by gender, age and site of disease

Complete information for gender and age were submitted for 767 of the 771 cases. Seven children (male n=5, female n=2) under 10 years of age had bacteriologically confirmed tuberculosis (gastric aspirate n=5, sputum n=1, lymph node n=1). The relationship of tuberculosis to age and gender are shown in Figure 2. The overall male:female ratio was 1.1:1. Age and gender rates varied depending on the site of infection. The male:female ratio for pulmonary disease was 1.3:1. The predominant specimen type was sputum, including eight gastric aspirates (n=369, 47.9%); a further 111 (14.3%) and 14 were bronchoscopy or lung tissue/biopsy samples, respectively. The median age for both males and females with pulmonary disease was 25–29 years. Forty-seven (6.1%) isolates were of pleural origin. There were 139 (18.0%) isolates from lymph node with a male:female ratio of 1:1.6, the median age was 35–39 and 25–29 years respectively for males and females with lymph node disease. There were 16 isolates from other sites including usually sterile fluids (pericardial n=2, blood n=2), abscess (psoas n=2), and tissue (sternal n=2, parathyroid n=1, tonsil n=1).

Figure 2. Laboratory diagnosis of for *Mycobacterium tuberculosis* complex disease, Australia 2001, by age and sex



Association with HIV

The AMRLN database recorded the HIV status for only 81 (10.5%) patients. Two patients were identified as HIV seropositive; both were from South East Asia and both isolates were fully drug susceptible strains of *M. tuberculosis*.

Microscopy

Results of microscopy were available for 712 of 771 (92.3%) specimens; microscopy was not performed on five specimens. Results for a further 54 samples were unknown. Smears were positive for 214 of 369 (58.0%) sputum and 42 of 111 (37.8%) bronchoscopy specimens respectively (Table 2). A total of 47 pleural specimens were culture positive for M. tuberculosis with eight (17.0%) smear-positive for acid fast bacilli (AFB). Thirty-six (11.4%) specimens of pleural fluid and 11 (40.0%) pleural biopsies were smear positive for AFB. Of the 139 specimens of lymph node, microscopy results were available for 125; and 24 (19.2%) were smear-positive for AFB.

Drug susceptibility testing

Results of in vitro drug susceptibility testing were available for all 771 isolates for isoniazid (H), rifampicin (R), ethambutol (E), and pyrazinamide (Z). A total of 69 isolates (8.9%), comprising 67 *M. tuberculosis*, one *M. africanum*, and one *M. bovis*, were resistant to at least one of the above anti-tuberculosis agents. *M. bovis* is inherently resistant to pyrazinamide. Results of testing for streptomycin (S) were available for 228 of 771 (29.5%) isolates with eight (3.5%) demonstrating mono-resistance, and a further 11 isolates resistant to both S+H. Resistance to at least H and/or R was noted for 67 isolates (8.7%), with resistance to both H and R [i.e. defined as multidrug-resistance (MDR)] observed in 12 (1.6%) isolates. All of the MDR isolates were *M. tuberculosis* (MDRTB). Of the 12 MDRTB isolates, 10 were from the respiratory tract (sputum n=6, bronchoscopy n=4); the remaining isolates were from lymph node and pleural fluid (Table 3). One of the four bronchoscopy specimens, and two sputums were smear positive.

Of the 67 *M. tuberculosis* and one *M. africanum*, 43 (5.6%), 1 (0.1%) and one (0.1%) demonstrated mono-resistance to H, R, and E respectively. There was no mono-resistance to pyrazinamide. There were 66 strains that demonstrated resistance to H at a concentration of 0.1 mg/L in the radiometric BACTEC system. One isolate was not tested at the higher concentration of 0.4 mg/L. Of the remaining 65 strains, 43 (66.2%) demonstrated resistance at the higher level.

Thirty-four of 67 (50.7%) specimens culture-positive for drug-resistant *M. tuberculosis* were also smear-positive for AFB.

Initial or acquired resistance and country of origin

There were 67 *M. tuberculosis* and one *M. africanum* isolates resistant to at least one of H, R, E or Z. Of these, 61 of 64 (95.3%) were classified as having initial resistance, three had acquired resistance, and no data were available on the presence or absence of previous treatment for four patients. The country of birth was known for 63 of 68 (92.6%) patients; five were Australian-born, and 58 (92.1%) had migrated from a total of 22 countries.

Of the 58 migrants with drug-resistant disease, 37 (63.8%) had migrated from one of six countries: Vietnam (n=11), Philippines (n=10), India (n=7), Indonesia (n=6), and Papua New Guinea (n=3).

Use of nucleic acid amplification tests

Nucleic acid amplification tests (NAAT) were performed on 136 of 771 (17.6%) specimens which subsequently grew MTBC on culture. Sputum (n=89), bronchoscopy (n=18), and lymph node (n=12) were the most frequently tested. Of the 136 specimens, 110 were NAAT positive and 25 were negative. One specimen of bronchial washings (smear negative) produced a non-interpretable result due to the presence of inhibitors.

Smear positive specimens were more likely to have NAAT performed, (Table 4). Excluding the specimen with a non-interpretable result, 107 culture-positive respiratory specimens had NAAT performed; 89 of 90 (98.9%) smear positive specimens were NAAT positive, whilst only 13 of 17 (76.5%) smear negative specimens were NAAT positive. Importantly, four (2.9%) smear positive specimens (one each of bronchial washings and pleural fluid, two lymph nodes) that were culture positive for *M. tuberculosis*, were NAAT negative.

Table 2. Site of specimens smear– and culture-positive for *Mycobacterium tuberculosis* complex, Australia,2001

	Number*	Smear positive (%)
Sputum	369	58.0
Bronchoscopy	111	39.6
Lymph node	139	19.2
Pleural	47	17.8
Bone/joint	26	19.2
Genito-urinary	20	ND†
Peritoneal	13	ND
Skin	7	ND
CSF	5	ND

* Specimens not tabulated: 14 pulmonary tissue samples, 16 specimens from miscellaneous sites, and 4 of unknown site.

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

Table 3. Drug resistance patterns in multidrug-resistant strains, Australia, 1996 to 2001

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

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

Table 4. Results for nucleic acid amplification tests performed on respiratory specimens, Australia,2001

NAAT result*	Culture positive respiratory specimens	
	Smear positive	Smear negative
Positive	89	4
Negative	1	13
Total (110)	**90**	**17**

* A variety of nucleic acid amplification tests methods were used, depending upon laboratory

Discussion

The isolation of 768 *M. tuberculosis*, two *M. africanum*, and a single *M. bovis* from clinical specimens for the calendar year 2001, yielded a rate of four bacteriologically confirmed cases of tuberculosis per 100,000 population, an almost identical rate as in 2000, and consistent with data reported for the past decade.[8] The NNDSS reported 997 tuberculosis notifications in 2001, the second lowest notification rate on record.[11] The NNDSS has consistently reported higher notifications than the AMRLN laboratory data (range 24–40%) and for 2001, the 22.7 per cent difference between the two datasets was at the lowest end of the range. In 2001, the NNDSS dataset recorded 558 cases from the respiratory tract, 74 pleural, 210 lymphatic, and 43 bone/joint.[11] If the two databases are compared, 88.5 per cent, 66.2 per cent, 63.5 per cent and 60.5 per cent of respiratory, pleural, lymphatic and bone/joint cases respectively, were bacteriologically confirmed. Comparison of two unlinked databases is problematic. However, the data suggests that, with almost 90 per cent of pulmonary cases reported to NNDSS having a bacteriological confirmation of disease, respiratory TB is well investigated in Australia. In contrast, the lower proportion of extra-pulmonary disease confirmed by culture suggests that too much reliance is placed on clinical, histological or radiological diagnoses for these forms of TB.

As expected, the respiratory tract accounted for the majority of culture positive specimens. Of the 494 respiratory specimens, 111 were obtained at bronchoscopy, and of those that reported a microscopy result, 42 of 108 (38.9%) recorded a positive smear. In four cases, MDRTB was isolated from bronchoscopy specimens, including a single specimen that was also smear positive. Bronchoscopy is especially useful for the diagnosis of pulmonary tuberculosis in suspect, sputum smear negative patients and in non-sputum producers.[12] It is of interest that almost 40 per cent of bronchoscopy specimens were reported as microscopy smear positive which might suggest that bronchoscopies are being undertaken in persons who may well have been sputum smear positive but were not tested prior to bronchoscopy. Some bronchoscopies performed may not have been necessary had sputums been submitted,—or smear results retrieved—prior to bronchoscopy. The performance of bronchoscopies on smear-positive cases exposes the patients to a needless invasive procedure and represents an infection control hazard for the bronchoscopist and their support staff. A cost effective alternative to bronchoscopy is to perform three induced sputum tests on consecutive days.[13]

A total of 67 isolates (9.8%), comprising 66 *M. tuberculosis* and one *M. africanum* were resistant to at least one of H, R, E, or Z. One patient had *in vitro* resistance to R only, the 12 other isolates with resistance to R were MDRTB. Molecular tests that can determine presumptive rifampicin resistance directly from specimens are now available and may provide rapid presumptive evidence of MDRTB with a test sensitivity approaching 95 per cent.[14,15] There were 12 (1.6%) isolates with *in vitro* resistance to at least H+R (i.e. multidrug–resistant TB). The rate of MDRTB in Australia has remained steady over the past decade.

The country of birth was known for 63 of 68 (92.6%) patients with a drug-resistant strain of *M. tuberculosis* or *M. africanum*; five were Australian-born and 58 had migrated from a total of 22 countries. Drug-resistant cases among migrants reflect the performance of TB control programs in the countries from which the patients migrated. Determining a history of previous TB treatment among migrants is also problematic and confounds accurate classification of drug resistance as 'initial' or 'acquired'. Drug resistance data is therefore a poor indicator of the performance of Australian TB services. A far more meaningful performance indicator would be the proportion of patients who relapse or fail to respond to treatment within Australia in circumstances where the drug susceptibility profile of the original isolate is known.

For the first time, data were collected on nucleic acid amplification testing performed as a diagnostic test. The data were incomplete with at least one AMRLN member being unable to collect NAAT results for isolates submitted by referring laboratories. All of the 136 specimens where NAAT was performed were cultured for mycobacteria. The different performance of NAAT on smear-positive and smear-negative pulmonary specimens is demonstrated by the data. For smear positive specimens, there was high concordance of NAAT positivity with culture positivity. In contrast, smear negative respiratory specimens had a lower concordance, with

slightly over 75 per cent of culture confirmed specimens also NAAT positive, a finding higher than previous evaluations on NAAT performed in settings with a low incidence of tuberculosis.[16,17,18,19]

The use of NAAT for respiratory specimens from patients with suspected TB should be limited to respiratory smear positive specimens where the result is likely to influence clinical and/or public health decisions, and respiratory smear negative specimens from a patient with a high probability of TB and prompt management and public health decisions are required. The use of NAAT is inappropriate when a patient is respiratory smear positive and has a very high probability of TB, when a patient is respiratory smear negative and has a low probability of TB, and for monitoring treatment.

A further 14 specimens were NAAT positive but culture negative or culture was not performed, and were not included in the 2001 data. In several instances, a NAAT was performed on formalin-fixed tissue after the opportunity to culture diagnostic material had been precluded by the sample having been placed in formalin or another fixative. Whilst this approach may provide a retrospective diagnosis of tuberculosis,[20] it is not practical to identify the organism to species level (within MTBC), perform drug susceptibility testing, or undertake genotyping. When specimens need to be obtained by an invasive technique, the importance of considering mycobacterial disease in the differential diagnosis, especially prior to the procedure, cannot be over-emphasised. Unfortunately, anecdotal evidence suggests that requests for NAAT on fixed specimens are rising, a demanding technique that is time consuming, expensive, and insensitive.

The AMRLN database makes an important contribution to understanding the epidemiology of tuberculosis in Australia. The release of the *National Strategic Plan for TB Control in Australia Beyond 2000* by the Communicable Diseases Network Australia highlights the need to merge the AMRLN and NNDSS databases in order to provide a complete dataset to assess Australia's TB services against agreed performance indicators.

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, The 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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