Tuberculosis in Australia: bacteriologically confirmed cases and drug resistance, 2000:

Report of the Australian Mycobacterium Laboratory Reference Network

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Abstract

The Australian Mycobacterium Reference Laboratory Network collected and analysed laboratory data on new diagnoses of disease caused by *Mycobacterium tuberculosis* complex in the year 2000. A total of 765 cases were identified, representing an annual reporting rate of 4.0 cases of laboratory-confirmed tuberculosis (TB) per 100,000 population. Pulmonary disease was diagnosed in 64.9 per cent of cases with a male:female ratio of 1.5:1. Smears were positive for 209/365 (57.3%) of sputum isolates and 39/117 (33.3%) bronchoscopy isolates. Sputum from males was more likely to be smear-positive (63.3%) than from females (47.5%). Isolates from lymph node accounted for 136 (17.7%) of all cases; only 28.7 per cent were smear-positive. Eighty-four (11.0%) isolates, comprising 82 *M. tuberculosis* and 2 *M. bovis* strains, demonstrated *in vitro* resistance to at least one of the standard anti-TB medications. Resistance to at least isoniazid and rifampicin (defined as multidrug-resistant TB) was observed for only 8 (1.0%) strains, a rate similar to previous years. Almost all (96.3%) of patients with drug resistant strains were classified as having initial resistance. The country of birth was known for 76 (92.7%) of 82 patients with a drug resistant strain of *M. tuberculosis*; 6 were Australian-born and 70 (92.1%) had migrated from a total of 17 countries. Of these 70 migrants with drug-resistant disease, 68.6 per cent had migrated from one of the following countries: Vietnam (n=15), China (n=11), Philippines (n=11), India (n=6), and Indonesia (n=5). *Commun Dis Intell* 2002;26:226–233.

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

Introduction

Australia's notification rate for tuberculosis (TB) has remained stable at between 5-6 cases per 100,000 population since 1991. A rise to 6.1 per 100,000 population in 1999 was largely due to the influx of refugees from Kosovo under the 'Safe Havens' program and evacuees from East Timor to Darwin in September 1999.[1] Data from the World Health Organization (WHO) reveals that Australia has one of the lowest notification rates in the world.[2] Continued collection of accurate, comprehensive and timely statistics for tuberculosis will help ensure strategic directions are identified, that outcomes are achieved, and that Australia's enviable record of TB control is maintained.[3,4]

Since 1991, the National Mycobacterial Surveillance System (NMSS) of the Communicable Diseases Network Australia has provided statistics on cases of tuberculosis reported to public health authorities in Australia's States and Territories.[5] The Australian Tuberculosis Reporting Scheme has been conducted by the Australian Mycobacterium Reference Laboratory Network (AMRLN) since 1986.[6] Statistics compiled by the AMRLN relate to cases of bacteriologically confirmed tuberculosis whereas NMSS data will have a proportion of cases that are identified on the basis of clinical and epidemiological information, or on non-bacteriological laboratory investigations.[7] This report describes the bacteriologically confirmed diagnoses for the year 2000.

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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 Guerin (BCG) strain of *M. bovis* is a member of the MTBC, no information on this organism is included in the present report. Almost 80 laboratories performed culture for mycobacteria in 2000 (Royal College of Pathologists of Australasia Quality Assurance Program) with nearly all isolates of MTBC being referred to one of the five laboratories comprising the AMRLN for specific identification and drug susceptibility testing. Comparable laboratory methodologies are used in the reference laboratories. Relapse cases, as defined in the TB notifications in Australia, 1999 report,[1] were included in the laboratory data as laboratories are frequently unable to differentiate relapses from new cases. 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:

- demography: patient identifier, age, sex, HIV status and State/Territory of residence;
- specimen: type, site of collection, date of collection and microscopy result;
- isolate: species of mycobacterium and results of drug susceptibility testing; and
- drug resistant strain: patient country of origin or risk factors, 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 analysis. Duplicate entries (indicated by identical patient identifier and date of birth) were deleted prior to analysis. Rates were calculated using the respective mid-year estimates of the population supplied by the Australian Bureau of Statistics.[8] For each patient, 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 as pulmonary disease. In cases of multi-site disease, provided a sputum specimen was culture-positive, these cases were listed as pulmonary disease, the most important category for public health purposes. Multiple-site isolations were not categorised as having miliary or disseminated disease as differentiation is based on clinical findings that were frequently not available to the reporting laboratories. Initial drug resistance was defined as the presence of drug resistant strains of *M. tuberculosis* in new cases of tuberculosis. Patients who had begun anti-TB treatment and had developed resistance to one or more of the drugs used during treatment were said to have developed acquired drug resistance.[9]

Results

Total reports and distribution by State or Territory

There were 765 bacteriologically confirmed cases of TB in 2000, representing an annual rate of 4.0 cases per 100,000 population (Figure 1). State-specific reporting rates varied from less than one (Tasmania) to 23.0 cases per 100,000 population (Northern Territory) (Table 1). Of the 45 culture positive cases in the Northern Territory, 13 were associated with one remote Aboriginal community and 3 were foreign nationals working in East Timor transferred to Darwin for diagnosis and initial treatment (Dr Vicki Krause, personal communication). There were 9 patients from Papua New Guinea who were diagnosed in Australia and who are included in the Queensland laboratory data, and 5 persons identified as illegal immigrants (Western Australia n=4, Northern Territory n=1).

Figure 1. Comparison between TB notification rates and laboratory data, Australia, 1990 to 2000

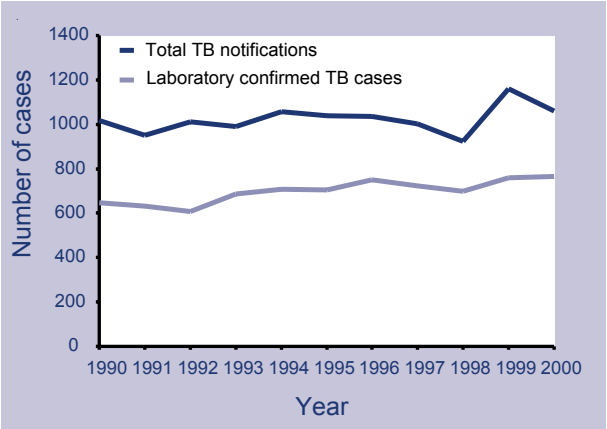


Table 1. Bacteriologically confirmed cases of tuberculosis, Australia, 1990 and 1997 to 2000, cases and rate per 100,000 population, by State or Territory

State/ Territory	1990[11]		1997[7]		1998[10]		1999[10]		2000	
	n	rate	n	rate	n	rate	n	rate	n	rate
NSW*	275	4.5	329	5.0	289	4.4	291	4.3	307	4.5
NT	32	19.4	28	15.0	22	11.6	21	10.9	45	23.0
Qld	78	2.7	74	2.2	85	2.5	75	2.1	76	2.1
SA	31	2.2	39	2.6	40	2.7	46	3.1	41	2.7
Tas	14	3.0	8	1.8	6	1.3	2	0.4	2	0.4
Vic	177	4.1	193	4.2	192	4.1	261	5.5	231	4.8
WA	41	2.5	51	2.8	66	3.6	64	3.4	63	3.3
Total	**648**	**3.8**	**722**	**3.9**	**700**	**3.7**	**760**	**4.0**	**765**	**4.0**

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

Causative organism

Almost all isolates were identified as *M. tuberculosis* (763) with only 2 isolates of *M. bovis*, and no cases of disease caused by *M. africanum*.

Distribution by gender, age and site of disease

Complete information for gender and age were submitted for 756 of the 765 cases. Eleven children under 10 years of age had bacteriologically confirmed tuberculosis (lymph node n=6, CSF n=2, respiratory n=2, pleural n=1). The overall male:female ratio was 1.15:1. The overall age/sex rates are shown in Figure 2. Age and gender rates varied depending on the site of infection (Figures 3 and 4). The male:female ratio for pulmonary disease was 1.5:1.

Figure 2. Laboratory confirmation of MTBC disease, Australia, 2000, by age and sex

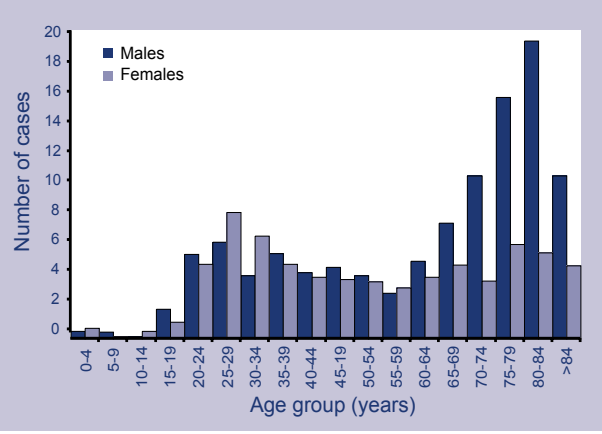


Figure 3. Isolation of MTBC from the respiratory tract, Australia, 2000, by age and sex

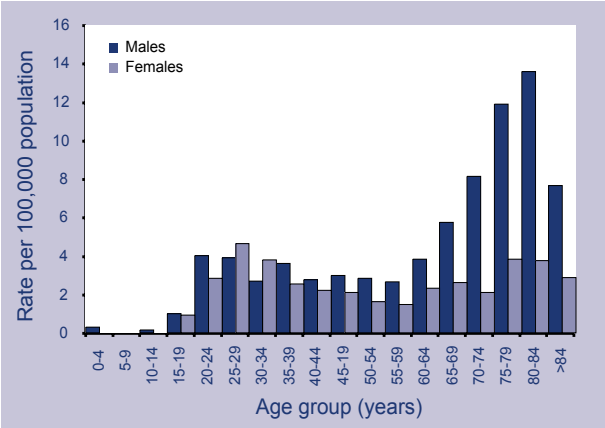
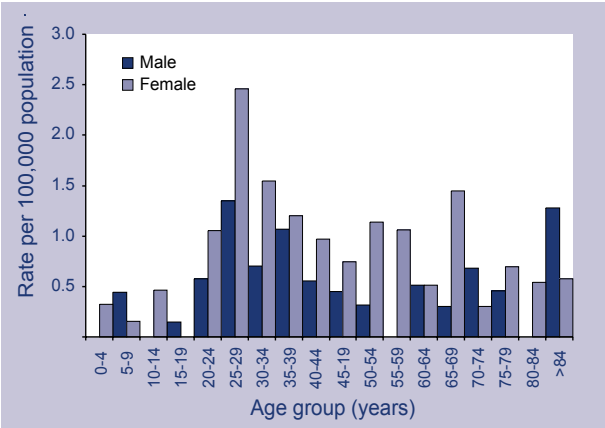


Figure 4. Isolation of MTBC from lymph node, Australia, 2000, by age and sex



Sputum (n=365, 73.7%) was the predominant specimen type; a further 117 (23.6%) were from bronchoscopy specimens and 13 were lung tissue/biopsy samples. Forty-four isolates were of pleural origin: 31 being pleural fluid, 12 from pleural biopsy or tissue, and the source of one pleural specimen was not further identified. Isolates from lymph node accounted for 136 (17.7%) of the total number of isolates with a male:female ratio of 1:1.9. There were 16 isolates from other sites, including usually sterile fluids (pericardial, blood), abscesses (psoas, groin, ischiorectal), faeces, and tissue (colon, caecal, adrenal, pericardium, aorta, subparietal). For 12 isolates, there was insufficient information to determine the site of disease.

Association with HIV

The AMRLN databases had access to the HIV status of only 76 (9.9%) patients. Six patients were identified as HIV seropositive, all were infected with *M. tuberculosis*. One HIV-positive patient was sputum smear-positive with a multidrug resistant strain of *M. tuberculosis*.

Microscopy

Results of microscopy were available for 755 of 765 isolates (Table 2). Microscopy was not performed for 6 specimens and results for a further 4 samples were unknown. Smears were positive for 209 of 365 (57.3%) sputum isolates and 39 of 117 (33.3%) bronchoscopy isolates. Sputum from males was more likely to be smear-positive (63.3%) than from females (47.5%). A total of 44 pleural specimens were culture positive for *M. tuberculosis* with only four (9.1%) smear-positive for acid fast bacilli (AFB). Of the 136 isolates from lymph node, 39/136 (28.7%) were smear-positive for AFB.

Table 2. Site of specimens

	Number*	Smear positive (%)	
All specimens	765	324	(42.4)†
Sputum	365	209	(57.3)
Bronchoscopy	117	39	(33.3)
Lymph node	136	39	(28.7)
Pleural	44	4	(9.1)
Genito-urinary	30	7	(23.3)
Peritoneal	10	3	(30.0)
Skin	8	4	(50.0)
CSF	7	0	(0.0)
Bone/joint	7	3	(42.8)

* Specimens not tabulated: 13 pulmonary tissue samples, 16 specimens from miscellaneous sites, and 12 of unknown site

† Excludes microscopy not performed (6) or result unknown (4).

Drug susceptibility testing

In 2000, results of *in vitro* drug susceptibility testing were available for all 765 isolates for isoniazid (H), rifampicin (R), ethambutol (E), and pyrazinamide (Z). Results of testing for streptomycin (S) were available for 230/765 (30.0%) of isolates. A total of 84 isolates (11.0%), comprising 82 *M. tuberculosis* and two *M. bovis* strains, were resistant to at least one of the above anti-tuberculosis agents. Resistance to H and/or R was noted for 79 isolates (10.3%), with resistance to both H and R (i.e. defined as multidrug-resistant (MDR) disease) observed in eight (1.0%) strains. All of the MDR isolates were *M. tuberculosis* (MDR-TB). Of the eight MDR-TB isolates, seven were from the respiratory tract (sputum 5, bronchoscopy 2); the remaining isolate was from lymph node (Table 3).

The two *M. bovis* isolates were fully susceptible apart from their inherent resistance to Z. Of the 82 *M. tuberculosis* isolates, 56 (7.3%), 4 (0.5%), and 2 (0.3%) demonstrated mono-resistance to H, Z and R, respectively. There was no mono-resistance to ethambutol. Seventy-seven strains demonstrated resistance to H at a concentration of 0.1 mg/L in the radiometric BACTEC system. Twenty strains were not tested at the higher concentration of 0.4 mg/L. Of the remaining 57 strains resistant at 0.1 mg/L, 33 (57.9%) demonstrated resistance at the higher level.

Thirty-six of 82 (43.9%) specimens culture-positive for drug resistant *M. tuberculosis* were also smear-positive for AFB. Importantly, five of the 7 patients with pulmonary MDR-TB were smear-positive.

Initial or acquired resistance and country of origin

There were 82 *M. tuberculosis* isolates resistant to at least one of H, R, E or Z. Of these, 79/82 (96.3%) were classified as having initial resistance, one case had probable acquired resistance, and no data were available on the presence or absence of previous treatment for 2 patients. The country of birth was known for 76/82 (92.7%) of patients with a drug resistant strain of *M. tuberculosis*; six were Australian-born and 70 (92.1%) had migrated from a total of 17 countries.

Of these 70 migrants with drug-resistant disease, 68.6 per cent had migrated from one of the following countries: Vietnam (n=15), China (n=11), Philippines (n=11), India (n=6), and Indonesia (n=5). For the 6 Australian-born persons identified as having a drug-resistant strain, risk factors were not identified for 3 patients, one person was a Torres Strait Islander, one had travelled in South East Asia, and another person was a work contact of a known TB case.

Table 3. Drug resistance patterns in multidrug-resistant strains, Australia, 1996-2000

Resistance pattern (standard drugs)[1]	1996[14]	1997[7]	1998[10]	1999[10]	2000
H+R only	10	6	2	2	3
H+R+E	1	1	1	1	1
H+R+Z	4	5	2	1	3
H+R+E+Z	0	2	1	0	1
Total (%)	**15 (2.0)**	**14 (1.9)**	**6 (0.9)**	**4 (0.5)**	**8 (1.0)**

H = Isoniazid

R = rifampicin

E = ethambutol

Z = pyrazinamide

Discussion

The rate of 4.0 cases of laboratory-confirmed tuberculosis per 100,000 population for the year 2000 falls within the range of 3.7–4.1 cases per 100,000 population reported in the past decade.[10] The annual incidence rates for the States and Territories varied from a low of 0.4 cases per 100,000 population in Tasmania to 4.8 cases per 100,000 population in Victoria, which is also consistent with previous years.[7,10,11–14] However, the Northern Territory incidence rate of 23.0 cases per 100,000 population was higher than in recent years. Almost 30 per cent of the Northern Territory population are Aboriginal or Torres Strait Islanders, a group identified with a far higher incidence rate for TB than the Australian-born, non-Indigenous population.[1]

The respiratory tract was the primary site of disease for 495 (64.7%) patients with only 12 (2.5%) reports noting the isolation of MTBC from other sites concomitantly. Overall, 57.3 per cent of sputum specimens were smear-positive for AFB; a finding consistent with previous reports.[10] Interestingly, a gender difference was noted, with males more likely to be sputum smear-positive than females (63.3% vs 47.5%).

Seven patients (3 male, 4 female) had culture-confirmed TB meningitis, all smear-negative, and caused by *M. tuberculosis*. Three cases were children aged 15 or less. In 2000, there were 12 cases of TB meningitis reported by the NMSS.[15] Laboratory diagnosis of meningeal TB is problematic with smear positivity rates typically reported between 10–40 per cent although higher rates are reported when multiple, large (10–20 mL) volumes of CSF are examined.[16,17] Pleural TB was confirmed bacteriologically in only 44 cases. This diagnosis is seldom confirmed by culture of pleural fluid; the diagnostic yield being increased by pleural biopsy.[18,19] Lymphatic TB was confirmed almost twice as frequently in females than in males, especially in the 25–39 year age group.

Tuberculosis notification data provided by the NMSS have consistently reported incidence data higher than that provided by the AMRLN.[15] A comparison of the two sources of data for the past decade reveals that, on average, there are 34 per cent (range 24–40%) more notifications by NMSS where the bacteriological status was either negative or unknown (Figure 1). Possible reasons for the gap between the two data sources include:

- diagnoses made on clinical and radiological findings only;
- difficulties obtaining specimens from young children and the elderly;
- failure to submit appropriate specimen(s);
- sample(s) being placed into histological fixative; and
- faulty or insensitive laboratory culture techniques.

Eighty-four isolates (11.0%) demonstrated *in vitro* resistance to at least one of the standard anti-TB medications; isoniazid, rifampicin, ethambutol, or pyrazinamide. This figure is marginally higher than that of previous years which, with the exception of 1996 data (11%), has been at less than 10 per cent. For the year 2000, *in vitro* resistance to at least H+R (i.e. multidrug-resistant TB) was observed for only 8 (1.0%) strains, a finding consistent with previous annual reports.[6,7,10–14]

The National Committee for Clinical Laboratory Standards (NCCLS) in the United States of America has recommended that *M. tuberculosis* isolates be tested at two concentrations of isoniazid (e.g. 0.1 and 0.4 mg/L in the BACTEC radiometric system),[20] and this practice has been adopted by the AMRLN. Of 57 isoniazid-resistant isolates tested at both concentrations, 24 (42.1%) demonstrated low-level resistance (i.e. 0.1<MIC<0.4 mg/L). Serum isoniazid levels within this range are obtainable, and continuation of isoniazid treatment in patients with low-level resistance may therefore be beneficial.[21,22] Hence, NCCLS suggests that laboratories reporting *M. tuberculosis* isolates with low-level resistance should append a comment such as, 'These test results indicate low-level resistance to isoniazid. Some evidence indicates that patients who are infected with strains exhibiting this level of resistance to isoniazid may benefit from continuing therapy with isoniazid. A specialist in the treatment of tuberculosis should be consulted regarding the appropriate therapeutic regimen and dosages'.[20] The AMRLN has not reached consensus on explanatory comments for low-level isoniazid resistance and any laboratory proposing to introduce such a comment is advised to consult with their relevant State or Territory TB Control Unit. Regardless, any patient with an isolate which shows resistance to isoniazid or rifampicin should be treated in close co-operation with the State or Territory TB Control Unit.

Previous AMRLN reports have not been able to identify cases of primary or acquired drug resistance because the NMSS and the AMRLN databases remained unlinked. WHO and the International Union Against Tuberculosis and Lung Disease (IUATLD) studies use the term *primary resistance* to define transmission of a drug resistant strain from one person to another, who then develops disease caused by the drug resistant strain. The rates of primary and acquired drug resistance may provide a measure of the past and current quality, respectively, of a tuberculosis control program. International TB authorities therefore prefer that drug resistance data be sub-divided based on the patients' previous exposure to anti-tuberculosis therapy.[9] With the assistance of members of the National TB Advisory Committee, this report has attempted this categorisation for the first time. However, the difficulties in categorising 'primary' and 'acquired' drug resistance from a laboratory database with limited clinical information must be appreciated.

Some patients may not recall previous anti-TB therapy or may not divulge such information. Determining whether drug resistance resulted from previous treatment or from infection by a drug resistant strain then becomes problematic. The WHO/IUATLD now recommend the use of the term *Resistance Among New Cases* when patients deny any prior anti-TB treatment and, in countries where adequate documentation is available, no documented evidence of prior treatment exists. Acquired resistance is defined as the emergence of a drug resistant strain from a person whose initial strain was drug susceptible, which can be determined only in countries with the resources to perform serial susceptibility testing.[9] An alternative approach to estimate acquired drug resistance is obviously necessary. A proxy for acquired drug resistance is to measure *Resistance among previously treated patients*. The WHO/IUATLD recommend that this group be subdivided into 4 subgroups and reported as such whenever feasible:

- patients failing anti-TB treatment (treatment failure);
- patients who become smear positive after completion of treatment and declared cured (treatment relapse);
- patients who interrupt their treatment for more than 2 months after having received a total of at least one month of treatment, then returning with bacteriologically confirmed TB (return after default);
- patients who continue to be smear-positive after completion of a treatment regimen (chronic case).

With the minimal clinical information available in the AMRLN database, the terms 'initial' and 'acquired' drug resistance were used in this report recognising the inherent limitations of these terms. More detailed and worthwhile analyses will only be possible when the AMRLN and NMSS databases are linked.

Of 82 patients with drug-resistant disease, 79 (96.3%) were classified as having initial resistance However, 70 (85.4%) of these 82 patients with drug-resistant TB were overseas born. Determining whether migrants have previously received anti-tuberculosis treatment is problematic. Previous medical records are usually not available and drug susceptibility testing facilities are generally not present in the country of origin. The large predominance of supposed initial resistance in this report is unusual and counter-intuitive, and suggests that a significant proportion of patients categorised as having initial resistance actually have acquired resistance. Since the large proportion of initial drug resistance occurs among the overseas born, a better performance indicator for the Australian National TB Control Program would be to monitor patients who relapse or fail to respond after treatment in Australia where the drug susceptibility profiles of the original and subsequent isolates will also be available.

Acknowledgments

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

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.

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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