Tuberculosis in Australia: bacteriologically confirmed cases and drug resistance, 1997

Report of the Australian Mycobacterium Reference Laboratory Network

David Dawson,[1] WHO Collaborating Centre in Tuberculosis Bacteriology, Queensland Health Pathology Services, Brisbane

Abstract

The Australian Mycobacterium Reference Laboratory Network collected and analysed laboratory data on new diagnoses of infection with *Mycobacterium tuberculosis* complex during 1997. A total of 722 cases were identified, representing an annual incidence of 3.9 cases of laboratory culture-confirmed tuberculosis (TB) per 100,000 population. Historical data shows that Australia's TB incidence rates of culture-confirmed TB have varied little in recent years, ranging from 3.9 to 4.1 cases per 100,000 population. The incidence rate continues to vary between States, reflecting differences in the distribution of persons belonging to high-risk categories for TB. The male to female ratio showed a slight increase to almost 1.3:1, but in keeping with previous years, males with culture-confirmed TB were older (median age group 45-49 years) than females (median age group 35-39 years), and approximately half of all pulmonary diagnoses involved positive microscopy. Lymphatic disease again accounted for almost 20% of the total cases, with 66% of cases being recorded in females. Approximately 9% of isolates, a decrease from 11% in 1996, had *in vitro* resistance to at least one of the four standard anti-tuberculosis drugs. Fourteen isolates were multi-drug resistant in 1997 compared with 15 in 1996. Overall, the data indicates a remarkably stable picture for TB in Australia. *Commun Dis Intell* 1999;23:349-353.

Introduction

Australia's incidence rate for tuberculosis (TB) is among the lowest of any country in the world. Nevertheless, due to the high rates of disease in neighbouring regions, as well as persistent high rates of infection in certain population subgroups such as indigenous Australians and persons born in high-prevalence countries, TB remains a potential threat to the effectiveness of Australia's public health programs. Furthermore, in the past decade, the emergence of resistance to isoniazid and rifampicin, the key anti-TB compounds, has severely compromised TB control efforts in many countries. The opinion of expert authorities is that Australia's TB control programs must be maintained, if not strengthened.[1]

Draft guidelines from the Tuberculosis Working Party of the National Health and Medical Research Council emphasise the importance of surveillance as a strategic tool for elimination of TB.[2] In Australia, surveillance data for TB is available through two sources: the National Mycobacterial Surveillance System (NMSS, conducted by the Communicable Diseases Network of Australia) and the Australian Tuberculosis Reporting Scheme (supported by the Mycobacterium Reference Laboratory Network, MRLN). The NMSS is based on clinical notifications. Data from the reference laboratory network relates to cases confirmed by isolation of the *Mycobacterium tuberculosis* complex (MTBC). The laboratory network has published data for the period 1986 to 1996.[3,4,5,6,7] This report is based on data for 1997.

Methods

The Australian Tuberculosis Reporting Scheme is a joint project of the MRLN and the Department of Health and Aged Care. The data are based on isolates of MTBC (other than the BCG strain) from clinical samples. Due to the specialised nature of TB bacteriology, it can be assumed that the five laboratories that comprise the MRLN account for almost all, if not all, of the bacteriological diagnoses in Australia. Comparable bacteriological procedures are used in the reference laboratories. Relapse patients, that is, those previously diagnosed, treated and considered cured, were included in these data because laboratories cannot usually differentiate them from new cases. Temporary visitors to Australia are also included.

For each new laboratory diagnosis the following information was collected:

- demographic: patient identifier, age, gender, HIV status and State of residence;
- specimen: type, site of collection, date of collection and microscopy result, and
- isolate: species of mycobacterium and results of drug susceptibility tests.

Data for 1997 from contributing laboratories were submitted to the scheme co-ordinator, collated and analysed. Duplicate entries (as indicated by identical patient identifier and age) were deleted before analysis. Incidence rates were calculated using the mid-year estimates of the population supplied by the Australian Bureau of Statistics.

1. Address for correspondence: The Prince Charles Hospital, Rode Road, Chermside, Queensland, 4032, Australia

Table 1. MTBC isolates in Australia, 1995-1997, by State or Territory and year

State	Number of Isolates	1997 Isolates per 100,000 population	1996 Isolates per 100,000 population	1995 Isolates per 100,000 population
New South Wales[1]	329	5.0	5.3	4.8
Victoria	193	4.2	4.7	4.1
Queensland	74	2.2	2.7	2.6
Western Australia	51	2.8	2.9	3.2
South Australia	39	2.6	1.9	2.2
Tasmania	8	1.8	0.6	0.4
Northern Territory	28	15.0	12.6	21.3
Total	722	3.9	4.1	3.9

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

The nature of the first clinical sample that yielded an isolate of MTBC was used to record the site of disease for individual cases. Culture-positive specimens collected at bronchoscopy, as well as gastric washings, were taken to identify cases of pulmonary disease. In most cases of multi-site disease, sputum is the first positive sample. These cases were therefore included among those listed as having pulmonary disease; the most significant category for public health purposes. Although many patients were known to have isolates from more than one body site, such data are of doubtful value for the laboratory-based report and were not collated. Similarly, it is not always possible to accurately categorise cases of miliary and disseminated disease from data available to laboratories.

Results

Total reports and distribution by State

A total of 722 cases were recorded in 1997. This figure represents an annual incidence of 3.9 cases of laboratory culture-confirmed TB per 100,000 population. The distribution of cases by State of residence is shown in Table 1 (data from 1995 and 1996 are included for comparison). State-specific incidence rates varied from 1.8 (Tasmania) to 15 per 100,000 (Northern Territory).

Causative organism

The large majority (706) of the 722 cases were due to *M. tuberculosis*. Nine cases were due to infection with *M. bovis*, and 7 were due to *M. africanum* .

Distribution by gender, age and site of disease

Full information for gender, age and site of disease was submitted for 651 (91%) of the 722 cases recorded. Figure 1 shows the distribution of the 651 cases by age group and gender. The overall male to female ratio was 1.3:1, although this ratio was reversed in the younger age groups. For all cases, the median age group was 40-44 years. The median age group for males was 45-49 years whereas that for females was 35-39 years. Age and gender specific rates varied from less than one in children younger than 15 to almost 30 per 100,000 per year in males over 80 years of age (data not shown). Nine cases related to children younger than 10 years. Five children had disease in pulmonary sites and four had lymph node infections. There were no culture-confirmed cases of tuberculous meningitis in children.

Figure 2 shows the distribution of 651 cases by site of disease and gender. Pulmonary disease accounted for 64% of the total cases (male to female ratio 1.6:1), while

Figure 1. MTBC isolates, 1997, by age group and sex

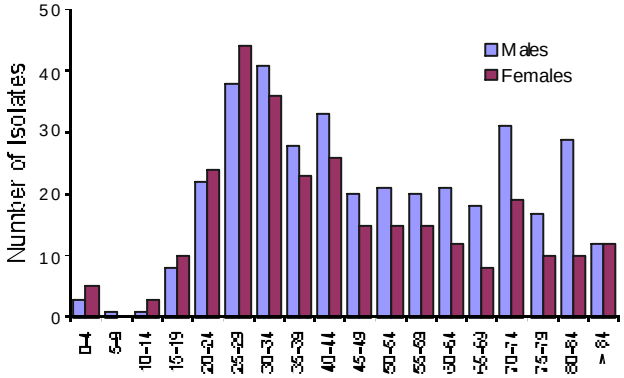

Figure 2. MTBC isolates by site and sex, Australia, 1997

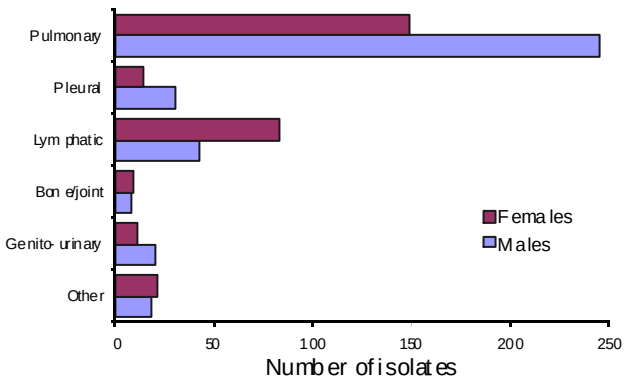

Table 2. *In vitro* resistance of isolates to the standard anti-tuberculosis drugs, Australia, 1995-1997

	Number resistant	1997 % resistant[1]	1996 % resistant[1]	1995 % resistant[1]
Isoniazid (H)	48	6.6	9.7	7.5
Rifampicin (R)	15	2.1	2.1	1.1
Ethambutol (E)	4	0.6	0.3	0.3
Pyrazinamide[2] (Z)	24	3.3	2.3	2.0

1. Percentage of 722 strains tested which were resistant to drug alone or in combination with others
2. All strains of *M. bovis* are naturally resistant to pyrazinamide

disease of the lymph nodes accounted for 19% of the total cases (male to female ratio 0.5:1).

Association with HIV

The reference laboratories recorded seven isolates of MTBC from persons known to be HIV+. Four patients were from Victoria, and one from each of Queensland, South Australia and Western Australia. Two patients had disease in pulmonary sites, and a further two had positive blood cultures.

Smear-positivity in pulmonary disease

A total of 437 (60.5%) of 722 cases were detected from samples of pulmonary origin. In 320 patients (73% of those with pulmonary disease) the diagnosis was made from sputum. A further 93 diagnoses (21%) were made from bronchoscopy samples. Results of microscopy were provided for 242 sputum samples; of these 135 (56%) were positive. Results of microscopy were provided for 72 bronchoscopy samples; of these 24 (33%) were positive.

***In vitro* drug susceptibility**

Each of the 722 isolates were tested against the four drugs recommended for standard treatment of TB in Australia, that is, isoniazid (H), rifampicin (R), ethambutol (E) and pyrazinamide (Z).[1] A total of 65 isolates (9.0% of the total) were resistant to at least one of the standard compounds. The frequency of resistance to H, R, E and Z, alone or in combination, is shown in Table 2 (includes results for 9 isolates of *M. bovis* which are naturally resistant to Z). Resistance to H and/or R was recorded in 49 isolates (6.8% of total). Thirty-four were resistant to H alone, one was resistant to R alone, and 14 (1.9 % of total) were resistant to both H and R in combination (Table 3). Isolates in the latter group are referred to as multi-drug-resistant (MDR). All of the MDR isolates were *M. tuberculosis*.

Table 3. Drug resistance patterns in MDR strains, Australia, 1995-1997

Resistance pattern[1] (standard drugs)	Number of isolates		
	1997	1996	1995
H + R only	6	10	3
H + R + E	1	1	1
H + R + Z	5	4	1
H + R + E+ Z	2	0	0

1. H = isoniazid; R = rifampicin; E = ethambutol; Z = pyrazinamide

Twelve MDR isolates came from sputum or pulmonary samples of which six had positive microscopy. Fifteen isolates identified as *M. tuberculosis* were resistant to Z, and in six cases no other drug resistance was detected. Among seven isolates from patients known to be HIV+, one was MDR (pulmonary disease, negative sputum microscopy), one was resistant to H alone, and one was resistant to Z alone (identified as *M. bovis*). In addition to the standard drugs, streptomycin (S) was tested against 202 isolates (28% of total). Twenty-two (10.8%) were found to be resistant to S, and in all but four cases, resistance to S occurred in combination with resistance to at least one other drug.

Discussion

The data collected from laboratory sources shows that the incidence of bacteriologically confirmed TB in Australia remains at around 4 cases per 100,000 per year. The 722 cases recorded represents only around 75% of cases notified to the NMSS. However, it should be noted that notifications to NMSS can be based on clinical or histological diagnosis alone. The discrepancies in data from the two sources stem from the different criteria for notification and should not been taken to indicate deficiencies in either system. Considering the variety of factors influencing the epidemiology of a disease such as TB, the stability of Australian statistics is somewhat remarkable.

As shown previously, there are differences in annual TB bacteriologically-confirmed incidence rates between States and Territories, ranging from below two in Tasmania to more than 15 per 100,000 in Northern Territory (Table 1). The 1997 data show only minor changes from previous years. The variations in rates between States are almost certainly due to peculiarities in the national distribution of persons in high-risk subgroups, rather than local differences in the risk of acquiring tuberculous infection.

As one would expect, and in keeping with earlier data, the large majority of isolates were *M. tuberculosis*. Seven isolates were identified as *M. africanum* , a species that has previously been recognised rarely in Australia. *Mycobacterium bovis* again accounted for a small but noteworthy number of cases of tuberculosis. Because bovine tuberculosis has been virtually eliminated from Australia's cattle herds, such cases almost certainly represent reactivation of past infection. The importance of *M. bovis* as a human pathogen in Australia during 1970-1994 has been reviewed in a recent publication.[8]

Full data on age, sex and site of disease was available for 651 (90%) of the 722 cases. The Network received full

data for 92% of cases in 1996.[7] All cases with missing data come from New South Wales and Victoria, which record the highest numbers of cases, and where laboratory services are decentralised. The reference laboratories are taking steps to improve this statistic so that in future a more complete analysis can be carried out. Level II laboratories can assist in this process by providing full patient demographics when forwarding isolates to reference laboratories.

Cases of active disease are distributed unevenly between sexes and across age groups (Figure 1). The features of this chart are very similar to what was presented in the 1996 report.[7] The overall male to female ratio is now closer to 1.3:1, which is slightly higher than reported in 1996 and 1995.[6] The median age groups for males and females are static at 45-49 years and 35-39 years respectively. Numbers (and rates) of bacteriologically confirmed disease in children under 15 years remain very low and indicate that the general Australian population is exposed to an almost negligible risk of tuberculous infection. Detailed analyses of cases notified to NMSS have clearly illustrated the different age-distributions of Australian-born patients when compared to persons born overseas.[9] Australians with TB tend to be older than their counterparts born overseas. The latter now account for around 70% of Australian TB notifications and constitute the majority of cases in the young and middle age groups.

The Network's reports have previously identified increases in the relative frequency of lymph node disease among patients with TB in Australia. We have also commented on the significant bias towards females, in contrast with observations for other forms of TB.[7] In this regard, statistics for 1997 are identical to those for 1996; lymph node disease accounted for 19% of all cases, and the male to female ratio was 0.5:1.

The reference laboratories were informed of seven cases associated with HIV infection. Published data suggests that at least 5-10 cases of HIV-TB occur annually in Australia.[10] As no cases were reported from New South Wales, which has the highest prevalence of HIV infection we believe our data for HIV-TB are an underestimate of the true figure. It is noteworthy that two cases were diagnosed from blood culture.

The 1996 report presented results of acid-fast microscopy for the first time; 56% of diagnostic sputum samples had positive microscopy. This year, we have found an identical statistic. Bronchoscopy collections provided 93 diagnoses during 1997, as opposed to 72 during 1996. This year 33% of such samples had positive microscopy; 38% were positive in 1996. Physicians contemplating bronchoscopy for suspected TB should be reminded of the value of a pre-bronchoscopy sputum for measuring a patient's 'infectious risk'.

Methods based on direct detection of MTBC-specific nucleic acids by enzyme-mediated amplification are now regarded as legitimate diagnostic tools. Several Australian laboratories recently carried out an evaluation of the LCx Test for *M. tuberculosis* (Abbott Diagnostics), one of several commercial amplification tests for TB.[11] Commercial nucleic acid amplification tests (NAAT) have high sensitivity and specificity when applied to microscopy-positive samples. In addition, NAAT will return a positive result with around 50% of samples that are smear-negative but culture positive. Nucleic acid amplification tests should now be viewed as indispensable adjuncts to conventional microscopy and culture; they are available in all Australian reference laboratories. A negative NAAT with smear-positive samples is useful as the infecting organism is most likely an atypical mycobacterium. And because NAAT on sputum will invariably remain positive for a period following treatment in pulmonary disease, they have the potential to confirm TB in certain categories of patients, for example, those who have received treatment prior to sample collection.

Collation of surveillance data for *in vitro* drug resistance is an important activity of the MRLN. Such information is not available through the NMSS. This year we have shown that a total of 65 isolates (9%) had *in vitro* resistance to at least one of the standard anti-TB drugs, H, R, E and Z. The corresponding figures for previous years were: 1996 (11%); 1995 (9%); 1994 (7%). Forty-nine isolates (6.8% of the total) were resistant to one or both of H and R, the most effective anti-TB compounds. The corresponding figures for previous years were: 1996 (9.9%); 1995 (7.5%); 1994 (6.1%). The majority of strains resistant to H and/or R are resistant to H alone, but in 1997 we found 14 (1.9%) were resistant to H and R, that is, were MDR. The corresponding figures for previous years were: 1996 (2%); 1995 (0.7%); 1994 (0.3%). We have already reported that a small number of strains identified as *M. tuberculosis* have been found resistant to Z alone.[6] This year we found six strains in this category. This finding conflicts with the long-held belief that 'wild' strains of *M. tuberculosis* are susceptible to Z.

More detailed study of H-resistant strains and a better understanding of the genetic mechanisms for H-resistance have suggested that the current breakpoint (0.1 mcgm/mL) for determining resistance to H by the standard BACTEC proportion method is too low. A level of around 0.4 mcgm/mL would seem to be a better indicator of *in vivo* resistance to H. Many strains judged as 'resistant' under the 0.1 mcgm/mL criterion are likely to respond to standard therapy. Until the issue is resolved through appropriate clinical evaluation, reference laboratories would be advised to test H at both 0.1 and 0.4 mcgm/mL, or alternatively ensure that strains resistant at 0.1 mcgm/mL are retested at 0.4 mcgm/mL.

Our previous reports made reference to the WHO and International Union Against Tuberculosis and Lung Disease Global Project on Anti-tuberculosis Drug Resistance Surveillance.[12] The project requires that patients be stratified on the basis of previous treatment for TB to allow *in vitro* drug resistance to be categorised as either *primary* resistance (where the patient is known not to have received chemotherapy) or *acquired* resistance (where the patient is known to have received chemotherapy). We have as yet, been unable to stratify Australian data, and WHO reports to date have listed drug resistance for Australian isolates as *combined* (denoting that treatment history is unknown). The MRLN data remains undervalued, nationally as well as internationally, without linkage to the NMSS database. Use of a common identifier such as laboratory accession number should enable matching of drug resistance data with patient ethnicity, treatment history, and other factors. Some Australian States already collect such data, but the need for a uniform national approach cannot be overstated.

Acknowledgements

The Mycobacterium Reference Laboratory Network comprises:

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

The willing assistance of William Chew, Prof Lyn Gilbert, Frank Haverkort, Regina Lasaitis, Richard Lumb, Tina Parr and Aina Sievers is acknowledged with thanks.

References

1. Tuberculosis in Australia and New Zealand into the 1990's. Patel A, Streeton J. Canberra. Australian Government Publishing Service, 1990.
2. Tuberculosis Working Party of the National Health and Medical Research Council. Towards Elimination of Tuberculosis II (Final Draft). May 1998.
3. Dawson D, Anargyros P, Blacklock Z et al. Tuberculosis in Australia: An analysis of cases identified in reference laboratories in 1986-88. *Pathology* 1991;23:130-134.
4. Dawson DJ, Cheah DF, Chew WK, Haverkort FC, Lumb R, Sievers AS. Tuberculosis in Australia, 1989-1992: Bacteriologically confirmed cases and drug resistance. *MedJ Aust* 1995;162:287-290.
5. Curran M, Dawson D. Tuberculosis in Australia: Bacteriologically confirmed cases and drug resistance, 1993. *Commun Dis Intell* 1995;19:343-345.
6. Dawson D. Tuberculosis in Australia: Bacteriologically confirmed cases and drug resistance, 1994 and 1995. *Commun Dis Intell* 1997;21:245-249.
7. Dawson D. Tuberculosis in Australia: Bacteriologically confirmed cases and drug resistance, 1996. *Commun Dis Intell* 1998;22:183-187.
8. Cousins DV, Dawson DJ. Tuberculosis due to *Mycobacterium bovis* in the Australian population: cases recorded during 1970-1994. *Int J Tuberc Lung Dis* 1999; 3:715-721.
9. Gilroy N, Oliver G and Harvey B. Tuberculosis notifications in Australia, 1996. *Commun Dis Intell* 1998;22:173-183.
10. HIV/AIDS and related diseases in Australia: Annual Surveillance Report 1997. Sydney. National Centre in HIV Epidemiology and Clinical Research, 1997.
11. Lumb R, Davies K, Dawson D et al. Multicenter evaluation of the Abbott LCx*Mycobacterium tuberculosis* ligase chain reaction assay. *J Clin Microbiol* 1999; 37:3102-3107.
12. Cohn DL, Bustreo F, Raviglione MC. Drug resistant tuberculosis: review of the worldwide situation and the WHO/IUATLD Global Surveillance Project. *Clin Infect Dis* 1997;24: S121-30.