Tuberculosis in Australia: bacteriologically confirmed cases and drug resistance, 2008 and 2009

A report of the Australian Mycobacterium Reference Laboratory Network

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Abstract

There were 886 and 1,062 bacteriologically-confirmed cases of tuberculosis (TB) in 2008 and 2009, representing an annual rate of 4.1 and 4.9 cases per 100,000 population respectively. Over the 2 years, a total of 23 children aged under 10 years (male n = 13, female n = 10) had bacteriologically confirmed tuberculosis, including 3 children with TB meningitis. Results of *in vitro* drug susceptibility testing were available for 885 of 886 and 1,060 of 1,062 isolates for isoniazid (INH), rifampicin (RIF), ethambutol (EMB), and pyrazinamide (PYZ) in 2008 and 2009 respectively. In 2008, a total of 94 (10.7%) isolates of *Mycobacterium tuberculosis* complex were resistant to at least one of the anti-tuberculosis agents. Any resistance to INH was noted for 76 (8.7%), 23 (2.6%) for RIF, 10 (1.1%) for EMB and 9 (1.0%) for PYZ. Resistance to at least INH and RIF (defined as multidrug-resistant TB (MDR-TB) was detected in 21 (2.4%) isolates. None of the 21 MDR-TB isolates had resistance to either ofloxacin or the injectable agents. In 2009, a total of 168 (15.9%) were resistant to at least one of the anti-TB agents. Any resistance to INH was noted for 150 (14.2%) isolates, 37 (3.5%) for RIF, 5 (0.5%) for EMB and 13 (1.2%) for PYZ. A total of 31 (2.9%) isolates were MDR-TB. In 2009, there were 2 cases of quinolone resistance in MDR-TB from persons born overseas. Mono-resistance to INH was the most commonly detected resistance with 33 and 80 isolates in 2008 and 2009, respectively. Mono-resistance to RIF was infrequently encountered with 2 and 5 isolates in 2008 and 2009 respectively. There were six and 11 MDR-TB patients from the Papua New Guinea (PNG) – Torres Strait Islands (TSI) cross-border region in 2008 and 2009 respectively. The PNG-TSI zone now contributes a substantial proportion of MDR-TB cases to the database. In addition, there were 24 isolates of *Mycobacterium bovis* bacille Calmette Guérin (BCG), 15 were cultured from males (4 aged ≤ 5 years) and from 9 females (5 aged ≤ 5 years). The predominant site of isolation was from vaccination abscess. Eight males (range: 57–87 years) had *M. bovis* BCG isolated from urine or blood culture. *Commun Dis Intell* 2011;35(2):154–161.

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

Introduction

Australia continues to benefit from an effective national tuberculosis (TB) control program delivered through state- and territory-based TB services. The incidence of TB cases is low at between 5–6 cases per 100,000 population.[1] In contrast, the Western Pacific and South East Asia regions of the World Health Organization report far more incident cases (estimated) at 108 (2007) and 181 (2007) per 100,000 population, respectively.[2,3] These two regions account for almost 60% of the global burden of TB.

In Australia, drug resistance is mainly associated with people born in high-burden TB countries within the Western Pacific and South East Asian regions and reflects the performance of national TB programs in these regions.[2,3] Multidrug-resistant TB (MDR-TB) has remained within a low range of 0.5%–2.0%, although recent rises above 2.0% in 2006 (2.4%) and 2007 (2.8%) demand vigilance.[4,5] For the Western Pacific Region, the proportion of new cases with MDR-TB was estimated to be 4% and rising to 24% in re-treatment cases. Cases from China, the Philippines and Vietnam accounted for 97% of the total estimated MDR-TB (new and re-treatment).[2]

There are two sources of TB-related data for Australia. Since 1991, the National Notifiable Diseases Surveillance System (NNDSS) has provided statistics on TB notifications reported to public health authorities in Australia's states and territories. The Australian Tuberculosis Reporting Scheme has been conducted by the Australian Mycobacterium Reference Laboratory Network (AMRLN) since 1986. Statistics compiled by the AMRLN relate to cases of bacteriologically-confirmed tuberculosis whereas NNDSS data also include 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 years 2008 and 2009.

Methods

The data are based on clinical specimens that were culture-positive for *Mycobacterium tuberculosis* complex (MTBC). Almost all isolates of MTBC were referred to

one of the five laboratories comprising the AMRLN for species identification and drug susceptibility testing. Comparable methodologies are used in the reference laboratories. Relapse cases, as defined by the *National Strategic Plan for TB Control in Australia Beyond 2000* prepared by the National TB Advisory Committee,[6] were included in the laboratory data as laboratories are generally unable to differentiate relapse cases from new cases. Data include temporary visitors to Australia, immigrants or persons detained in Australia in correctional services facilities, and asylum seekers. For each new bacteriologically-confirmed case, the following information was collected where available: demography: patient identifier, age, sex, HIV status and state of residence; specimen: type, site of collection, date of collection and microscopy result; isolate: *Mycobacterium* species and results of drug susceptibility testing; nucleic acid amplification testing results; and for drug resistant isolates: patient country of origin, and history of previous TB treatment to determine whether resistance was initial or acquired. Data from contributing laboratories were submitted in standard format to the AMRLN coordinator for collation and analysis. Duplicate entries (indicated by identical patient identifier and date of birth) were deleted prior to analysis. Rates were calculated using mid-year estimates of the population for 2008 and 2009 supplied by the Australian Bureau of Statistics.[7,8] For each case, the nature of the first clinical specimen that yielded an isolate of MTBC was used to record the nominal site of disease. Culture-positive specimens collected at bronchoscopy or by gastric lavage were counted as pulmonary disease. Patients with isolates recovered from multiple sites were counted as pulmonary disease (the most important category for public health purposes) if a sputum, bronchoscopy, or lung biopsy specimen was culture positive. Drug resistance among new cases (proxy for primary resistance) was defined as the presence of resistant isolates of *M. tuberculosis* in patients who, in response to direct questioning, denied having received any prior anti-TB treatment (for more than 1 month) and, in countries where adequate documentation is available, for whom there is no evidence of such a history.[9] Drug resistance among previously treated cases (proxy for acquired resistance) is defined as the presence of resistant isolates of *M. tuberculosis* in cases who, in response to direct questioning, admit having been treated for 1 month or more or, in countries where adequate documentation is available, for whom there is evidence of such a history.[9]

For 2009 onwards, the AMRLN has been requested by the National Tuberculosis Advisory Committee to provide laboratory data on bacteriologically confirmed isolation of *Mycobacterium bovis* (bacille Calmette Guérin) (BCG).

Results

There were 886 and 1,062 bacteriologically-confirmed cases of tuberculosis in 2008 and 2009, representing an annual rate of 4.1 and 4.9 cases per 100,000 population respectively. State-specific reporting rates varied from 0.6 (2008: Tasmania) to 11.4 (2008: Northern Territory) cases per 100,000 population. In 2009, all jurisdictions except South Australia and the Northern Territory recorded increased notification rates over 2008 levels (Table 1).

Causative organism

The overwhelming majority of the MTBC isolated were *M. tuberculosis* with a small number of *Mycobacterium africanum*, *M. bovis* and 'oryx' bacillus identified (Table 2).

Distribution by gender, age, and site of disease

Complete information for gender and age was available for 884 (99.8%) patients and 1,052 (99.1%) in 2008 and 2009 respectively. The distribution of bacteriologically confirmed cases by site is presented in Table 3.

Table 1: Bacteriologically confirmed cases of tuberculosis in Australia, 1998 and 1999, and 2007–2009, cases and rate per 100,000 population, by state or territory

State or territory	2009		2008		2007*		1999*		1998*	
	n	Rate	n	Rate	n	Rate	n	Rate	n	Rate
New South Wales†	409	5.5	327	4.5	343	5.0	291	4.3	289	4.4
Victoria	331	6.1	299	5.6	279	5.4	261	5.5	192	4.1
Queensland	153	3.4	111	2.6	118	2.8	75	2.1	85	2.5
Western Australia	87	3.9	72	3.3	45	2.1	64	3.4	66	3.7
South Australia	51	3.1	49	3.1	46	2.9	46	3.1	40	2.7
Tasmania	7	1.4	3	0.6	8	1.6	2	0.4	6	1.3
Northern Territory	24	10.9	25	11.4	33	15.4	21	10.9	22	11.6
Total	1,062	4.9	886	4.1	872	4.1	760	4.0	700	3.7

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

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

The site of disease was dependent upon age and gender. The overall male:female ratio was 1:0.7 and 1:0.9 for 2008 and 2009 respectively for respiratory isolates. Males were predominant but females accounted for the greatest number of lymphadenitis (Table 4). In 2009, for males, there were two distinct peak age groups in bacteriologically-confirmed rates: a rise to 11.6 cases of TB per 100,000 population at 25–29 years of age and a second peak in the elderly (males aged more than 84 years (up to 20.5 cases of TB per 100,000 population). A similar age distribution in female cases occurred with 12.9 and 3.6 bacteriologically-confirmed TB cases per 100,000 population at the 25–29 and greater than 84 years age groups, respectively. The median age group for patients with bacteriologically-confirmed disease was 30–34 years for both males and females.

Respiratory samples were the predominant culture-positive specimen type with sputum and bronchoscopy specimens being the 2 most common specimen types (Table 3). The most commonly encountered

Table 2: Members of the *Mycobacterium tuberculosis* complex isolated in the years 2008 and 2009

Organism	2009	2008
M. tuberculosis	1,052	881
M. bovis	3	4
M. africanum	3	0
'Oryx' bacillus	4	1
Total	1,062	886
M. bovis (BCG)	24	N/A

N/A Not available

Table 3: Site of specimens smear- and culture-positive for *Mycobacterium tuberculosis* complex, 2008 and 2009

	2009			2008		
		Smear positive			Smear positive	
	N*	n*	%	N*	n*	%
Sputum	472	240	50.8	407	203	49.9
Bronchoscopy	137	39	28.5	119	36	30.3
Lymph node	250	44	17.6	156	31	19.9
Pleural	48	2	4.2†	41	2	4.9‡
Genito-urinary	28		§	27		§
Bone/joint	36		§	21		§
Peritoneal	25		§	29		§
Skin	9		§	8		§
Cerebrospinal fluid	10		§	7		§

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

† One pleural biopsy and 1 fluid were smear positive.

‡ Two pleural biopsies only were smear positive.

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

Table 4: Distribution and site of disease, 2008 and 2009, by age and sex

	2009		2008	
Male/female	n	ratio	n	ratio
All	568/493	1:0.9	525/359	1:0.7
Respiratory	356/272	1:0.8	354/189	1:0.5
Lymph node	108/142	1:1.3	62/94	1:1.5
Median age – male				
All	30–34		30–34	
Respiratory	35–39		35–39	
Lymph node	25–29		30–34	
Median age – female				
All	30–34		30–34	
Respiratory	30–34		30–34	
Lymph node	30–34		30–34	

extrapulmonary culture-positive specimen was lymph tissue followed by pleural, peritoneal, bone/joint, and genitourinary tract (Table 3).

In 2008 and 2009, a total of 23 children aged under 10 years (male n = 13, female n = 10) had bacteriologically confirmed tuberculosis (sputum n = 4, gastric aspirate n = 4, lymph node n = 5, bone/joint n = 3, cerebrospinal fluid (CSF) n = 3, and one each from oropharyngeal aspirate, pleura, pus and bronchoscopy.

Association with HIV

For 2008 and 2009, the AMRLN database recorded the HIV status of only 77 (8.7%) and 96 (9.0%) patients. One bacteriologically-confirmed patient was HIV positive in 2009.

Microscopy

Results of acid-fast microscopy were available for 866 of 886 (97.7%) and 1,054 of 1,062 (99.2%) specimens in 2008 and 2009 respectively.

For 2008, smears were positive in 203 of 407 (49.9%) sputum and 36 of 119 (30.3%) bronchoscopy specimens respectively (Table 3). Of 41 pleural specimens (17 biopsy and 24 fluids) that were culture-positive for *M. tuberculosis*, 2 biopsies only were smear-positive. Lymph node specimens were smear-positive in only 31 of 156 (19.9%) cases. The corresponding figures for 2009 were 240 of 472 (50.8%) sputum and 39 of 137 (28.5%) bronchoscopy specimens. Of 48 pleural specimens (17 biopsies and 31 fluids), two (4.2%) were smear positive. Only 44 of 250 (17.6%) lymph node specimens were smear positive (Table 3).

Drug susceptibility testing

Results of *in vitro* drug susceptibility testing (DST) were available for 885 of 886 and 1,060 of 1,062 isolates for isoniazid (INH), rifampicin (RIF), ethambutol (EMB), and pyrazinamide (PYZ) in 2008 and 2009 respectively. A single strain failed to grow on subculture in both years. In 2009, a single strain was mixed with *Mycobacterium fortuitum* and could not be isolated as a pure growth. None of the *M. bovis* isolates (which are inherently PYZ-resistant) were included in the following results. Therefore, a total of 881 and 1,057 isolates were available for DST in 2008 and 2009 respectively. For the first time, streptomycin (STR), when available, was included in the drug resistance profiles (Table 5).

In 2008, a total of 94 (10.7%) isolates of *M. tuberculosis* complex were resistant to at least one of the above anti-tuberculosis agents. Any resistance to INH was noted for 76 (8.7%), 23 (2.6%) for RIF 10 (1.1%) for EMB and 9 (1.0%) for PYZ. Resistance to at least INH and RIF (defined as MDR) was detected in 21 (2.4%) isolates (Table 5). Of the 21 MDR-TB isolates, 16 were from the respiratory tract (sputum n = 13, bronchoscopy n = 3), lymph node (n = 4) and a single isolate from fluid (site not stated). Eight of the MDR-TB-positive sputum specimens were smear-positive as were 2 bronchoscopy samples. The most common drug resistance profile among MDR-TB strains was resistance to INH/RIF only, a trend continuing from previous years (Table 6). Please note that Table 5 has STR-resistance included but that Table 6 does not include STR-resistance as historical data cannot be accessed. Therefore, data distortions

Table 5: Drug resistance profiles, 2008 and 2009

	2009	2008
Total isolates	1,062	886
Total isolates and DST	1,057*	881*
Fully susceptible	889†	787†
Any resistance		
S	61	53
H	150	76
R	37	23
E	5	10
Z	13	9
Mono-resistance		
S	7	16
H	80	33
R	5	2
E	1	0
Z	3	0
Multidrug-resistant tuberculosis		
HR	12	3
HRE	1	0
HRZ	3	1
HREZ	1	1
SHR	9	7
SHRE	0	3
SHRZ	4	2
SHREZ	1	4
Poly-resistant		
SR	1	0
SH	38	19
SE	1	0
HE	0	1
HZ	1	0
SHE	0	1
SHZ	0	1

* Excludes no drug susceptibility testing (DST) available and no *Mycobacterium bovis.*

† Includes streptomycin resistant strains.

Streptomycin (S), isoniazid (H), rifampicin (R), ethambutol (E), pyrazinamide (Z)

occur inevitably and MDR-TB data for 2008 is an excellent example. In Table 5, there were 3 INH/RIF-resistant strains and 7 STR/INH/RIF-resistant strains but 10 INH/RIF-resistant strains recorded in Table 6 where STR-resistance was not considered. None of the 21 MDR-TB isolates in 2008 had concomitant resistance to ofloxacin or a second-line injectable agent (kanamycin, amikacin, capreomycin).

In 2009, a total of 168 (15.9%) isolates were resistant to at least one of the anti-tuberculosis agents. Any resistance to INH was noted for 150 (14.2%), 37 (3.5%) for RIF, 5 (0.5%) for EMB and 13 (1.2%) for PYZ. A total of 31 (2.9%) isolates were MDR-TB. Twenty-three isolates were from the respiratory tract (sputum n = 21, bronchoscopy n = 2), lymph node n = 3, peritoneal n = 2, and one each from pleural biopsy, bone, and CSF. Ten of the MDR-TB-positive sputum specimens were smear-positive and all of the specimens from extrapulmonary sites were smear negative. In 2009, there were 2 cases of quinolone resistance in MDR-TB from persons born overseas (Indonesia, China). In 2008 and 2009, no cases were detected of extensively drug resistant tuberculosis (XDR-TB), defined as MDR-TB strains with additional resistance to a quinolone and a second-line injectable agent.

Overall, mono-resistance to INH was the most-commonly-detected resistance profile with 33 and 80 isolates in 2008 and 2009 respectively (Table 5). Mono-resistance to RIF was infrequently encountered with 2 and 5 isolates in 2008 and 2009 respectively. For 2009, three of the 5 mono-RIF resistant strains were found in patients from the Papua New Guinea–Torres Strait Islands (PNG–TSI) zone (Table 7). Resistance to STR/INH was the most frequent form of poly-resistance with 19 and 38 isolates in 2008 and 2009 respectively (Table 5).

In 2008, 6 MDR-TB patients were PNG nationals from the Western Province and who are able to access the PNG–TSI cross-border region. These patients access health services in outer TSI and receive treatment in Australia. In 2009, another 11 DR-TB patients were from the PNG–TSI zone. The impact of MDR-TB arising from the PNG–TSI zone is demonstrated in the Figure. In 2009, only 10 of 30 PNG–TSI patients had a fully susceptible strain; mono-resistance, including three with RIF-mono-resistance was found in 5 patients, STR/INH-resistance in 4 patients, and 11 had MDR-TB (Table 7).

New or previously treated cases, and country of birth

The majority of drug resistance was considered to be primary acquisition of a drug resistant strain; 47/78 (60.3%) in 2008 and 124/168 (73.8%) in 2009.

Table 6: Drug resistance patterns in multidrug-resistant strains, Australia, 1995 to 2009

Resistance pattern (standard drugs)*	2009	2008	2007	2006	2005	2004	2003	2002	2001	2000	1999	1998	1997	1996	1995
H+R only	21	10	16	16	5	7	4	8	8	3	2	2	6	10	3
H+R+E	1	3	2	1	3	2	2	1	1	1	1	1	1	1	1
H+R+Z	7	3	5	0	1	1	1	1	3	3	1	2	5	4	1
H+R+E+Z	2	5	1	5	3	2	0	2	0	1	0	1	2	0	0
XDR-TB	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Total (%)	31 (2.9)	21 (2.4)	24 (2.8)	22 (2.4)	12 (1.5)	12 (1.5)	7 (0.9)	12 (1.7)	12 (1.6)	8 (1.0)	4 (0.5)	6 (0.9)	14 (1.9)	15 (2.0)	5 (0.7)

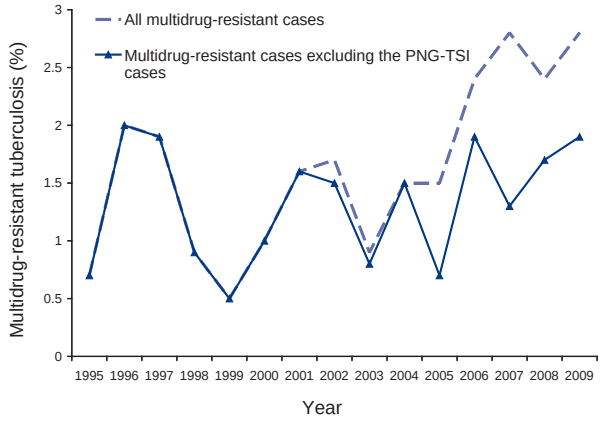
* The streptomycin result was not considered for this table.
H = isoniazid, R = rifampicin, E = ethambutol, Z = pyrazinamide
XDR-TB Extensively drug resistant tuberculosis

Table 7: Drug resistance profile of *Mycobacterium tuberculosis* isolates from the Papua New Guinea–Torres Strait Islands cross-border region, 2009

Drug resistance	N
Fully susceptible	10
Mono-resistance	
S	1
H	1
R	3
Poly-resistance (not MDR-TB)	
SH	4
Multidrug-resistant tuberculosis	
HR	3
HRZ	1
SHR	6
SHRZ	1
Total	30

Streptomycin (S), isoniazid (H), rifampicin (R), ethambutol (E), pyrazinamide (Z)

Figure: Percentage of multi-drug resistant tuberculosis in Australia: the impact of cases from the Papua New Guinea–Torres Strait Island zone



There were 6 and 12 Australian-born cases of drug-resistant tuberculosis in 2008 and 2009 respectively. The overseas-born persons with drug-resistant disease were from 20 and 27 countries respectively for 2008 and 2009. The countries of birth were predominantly from 5 countries; India, Papua New Guinea, Vietnam, China, The Philippines (data not shown).

Isolation of *Mycobacterium bovis* BCG

There were 24 isolates of *M. bovis* BCG in 2009. Fifteen were cultured from males (4 aged ≤ 5 years) and from 9 females (5 aged ≤ 5 years). The predominant site of isolation was from the vaccination site or axilla (n = 13). Nine of these 13 patients were less than 5 years of age. Eight males (age range: 57–87 years) had *M. bovis* BCG isolated from urine or blood culture (n = 2). The isolation of *M. bovis* BCG from the sputum of a 23-year-old patient was associated with a neck node and draining sinus.

Discussion

The detection of 886 laboratory-confirmed cases of TB in 2008 (i.e. 4.1 cases per 100,000 population) is consistent with previous AMRLN reports with the incidence of bacteriologically confirmed TB generally between 3.5–4.4 cases per 100,000 population (see previous AMRLN reports). In contrast, in 2009, the AMRLN recorded 1,062 cases of bacteriologically confirmed tuberculosis with an incidence of 4.9 cases per 100,000 population, the highest figure recorded since laboratory data were first collected nationally in 1985. All jurisdictions, except the Northern Territory and South Australia had increases in the incidence rate of bacteriologically confirmed disease. Increases in the laboratory diagnosis of respiratory- and lymph node- disease accounted for the majority of additional cases in 2009.

As expected, the number of cases notified to NNDSS was higher than for bacteriologically confirmed TB. There were 1,212 and 1,334 notifications of tuberculosis in 2008 and 2009 respectively compared with 886 (73%) and 1,062 (80%) of cases confirmed bacteriologically.[10] The most frequent reasons postulated for the extra cases reported in the NNDSS database include: diagnosis of childhood and extrapulmonary TB based on clinical, radiological and epidemiological information; and submission of extrapulmonary samples in formalin precluding bacteriological investigations.

The format for documenting drug resistance has changed from previous reports and is now more consistent with requirements of the World Health Organization. For the first time, streptomycin has been included more formally in the drug resistance data and has resulted in some changes to the proportion of strains with drug resistance. In 2008 and 2009, there were an additional 7 and 16 isolates respectively with mono-resistance to streptomycin. This change in analytic methodology results in modest increases in total drug resistance reported (e.g. for 2009, overall drug resistance increased from 15.3% to 15.9% when mono-streptomycin resistance was included).

The rise in the isolation of drug resistance to 15.9% for 2009 was the highest percentage since the MRLN began data collection in 1985. The previous highest had been in the years 1989–1992 where 14.4% of isolates were resistant to at least one of the four anti-

tuberculosis drugs. That report did not consider streptomycin resistance indicating that the overall resistance would have been slightly higher.[11]

MDR-TB remains at a low level but there are reasons for concern. Since 2006, the proportion of MDR-TB isolates has risen above the long-term range of 0.5%–2.0%, due to patients in the PNG–TSI zone presenting for treatment. In 2009, there were 31 (2.9%) cases of MDR-TB but when the 11 PNG–TSI patients were excluded, the proportion decreased to 1.9%. However, the level of drug resistance in the PNG–TSI zone patient group is most disturbing. Of a total of 30 patients identified as being from PNG–TSI, only 10 patients had a fully susceptible strain. Importantly, 3 patients had mono-resistance to rifampicin meaning that 14 of 30 PNG–TSI patients had *in vitro* resistance to at least rifampicin. A further 4 patients had resistance to streptomycin and isoniazid; considered to be a precursor to MDR-TB.[12] Although there is highly likely to be patient bias in the limited data, the figures are of great concern for PNG TB control and for Australia.

The recognition of *M. tuberculosis* isolates with low level rifampicin is another matter of concern. All Australian laboratories are now using the Becton Dickinson MGIT 960 automated liquid culture (MGIT) system for primary isolation and for first- and second-line drug susceptibility testing. A recent paper by Van Deun and colleagues highlighted that isolates with low-level resistance to rifampicin may not be detected by the MGIT system.[13] The *rpoB* mutations associated with low level resistance included Leu511Pro, Asp516Tyr ,His526Leu, His526Ser, Ile572Phe, and 533Pro.[10] Met515Ile has also been associated with low level rifampicin resistance.[14] The authors concluded that low-level but probably clinically relevant rifampicin resistance linked to specific *rpoB* mutations, is easily missed by standard growth-based methods, particularly the automated broth-based systems. The critical rifampicin concentration for the MGIT system is 1.0 mg/L but the problematic strains had minimal inhibitory concentrations 0.13–0.38 mg/L. The frequency of these low level rifampicin resistant isolates is presently unknown. The bacteriologically unfavourable treatment outcomes for most of the borderline rifampicin resistant strains suggest that these specific mutations may have clinical significance. Laboratories undertaking DST using the MGIT system should consider performing *rpoB* sequencing in the following circumstances: (i) all rifampicin resistant strains and (ii) for isolates where isoniazid resistance is reported.

An alternative strategy to sequencing would be wider use of one of the commercially-available molecular tests, Hain Genotype MTBDR*plus*[15,16] or the GeneXpert MTB/RIF.[17,18] Both are able to detect MTBC nucleic acid and to detect gene mutations associated with rifampicin resistance, including low level resistance. The Hain Genotype MTBDR*plus* assay is able to detect mutations associated with resistance to rifampicin (*rpoB*) and isoniazid (*katG*, *inhA*) either directly from processed sputum specimens or from culture.[15,16] The laboratory testing protocol is based on a conventional multiplex nucleic acid amplification followed by reverse hybridisation on a solid phase. For smear positive respiratory specimens, the sensitivity and specificity for rifampicin approaches 100% for both assays. For the Genotype MTBDR*plus* assay, the sensitivity and specificity for isoniazid resistance may also be high but appears to vary between geographic regions depending on the proportion of isolates that are phenotypically isoniazid resistant, but for which no resistance mutation is found in *katG* or *inhA*.[16] The recently released Genotype MTBDR*sl* assay uses the same technology to detect mutations associated with injectable agents (kanamycin, capreomycin, amikacin), quinolones, and ethambutol.[19,20] Using isolates of *M. tuberculosis*, the sensitivity and specificity respectively for quinolones, kanamycin, amikacin, capreomycin, was 87%–90.2% and 90.2%–100%, 77% and 100%, 83.3%–100% and 100%, and 80%–86.8% and 98%–99.1%. The results for ethambutol were disappointing with both studies reporting sensitivities less than 60%.[19,20] For both Genotype assays, the time to obtain results is less than 2 days.

In contrast to the conventional molecular protocols of the Genotype assay, the GeneXpert MTB/RIF is a radical departure with all reactions occurring within a single-use disposable cartridge loaded into a module and controlled via a computer. A technical knowledge of molecular protocols is not required nor are specialised molecular laboratory facilities. Test samples may be direct or processed sputum, or positive cultures. The lysis buffer used early in the testing protocol will inactivate more than 95% of viable tubercle bacilli meaning that the platform may be used outside of the TB laboratory. The assay is stand-alone once the cartridge has been placed into the machine and testing is completed in less than two hours. In a multi-country evaluation, the sensitivity for detecting MTBC nucleic acid in smear positive and smear negative specimens was 98.2% and 72.5% respectively and with 100% specificity. For rifampicin resistance, the assay achieved a sensitivity of 97.6% and specificity of 98.1%.[19] Most of the AMRLN laboratories now have a GeneXpert platform in their laboratory.

The much anticipated merging of the AMRLN and NNDSS databases has experienced further delays due to information technology limitations and transitions in various states. A combined database will not be available before the 2012 dataset at the

earliest. In the interim, Australia must continue to provide a combined prevalence of drug resistance and remains unable to provide comprehensive data to the World Health Organization global reports sub-classifying drug-resistance between new cases and previously-treated patients.

Acknowledgements

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

SA Pathology, Adelaide, South Australia

Queensland Health Pathology Services, Herston Hospitals Complex, Herston, Queensland

Victorian Infectious Diseases Reference Laboratory, North Melbourne, Victoria

PathWest Laboratory Medicine WA – QEIIMC, Hospital Avenue, Nedlands, Western Australia

Institute of Clinical Pathology and Medical Research, Westmead Hospital, Westmead, New South Wales.

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