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TB and not TB: Mycobacteriology in Australia in the 21st century

Introduction
In the past, non-mycobacteriologists may well have viewed the specialty as a backwater where the science and scientists moved slowly, and the organisms grew even more slowly! Little changed over nearly a century as mycobacteriologists employed the classic microscopy and culture techniques that had been developed and refined over the 2 decades following Koch’s description of the tubercle bacillus in 1884 (Figure 1).

However, mycobacteriology has undergone a renaissance in the last decade following a resurgence of tuberculosis (TB) in the United States, the increased recognition of the clinical significance of non-tuberculous mycobacteria (NTM), and the introduction of new molecular technologies such as nucleic acid amplification tests (NAAT). This brief review and other articles in this edition will highlight some of the exciting changes and challenges in the field of mycobacteriology in Australia.

Tuberculosis in Australia and other high-income countries
Following three decades of steady decline, the annual rate of TB cases in the United States (US) increased from 22,201 in 1985 to 26,283 in 1991 – an increase of 18.4%1. This increase was mainly due to down-sizing of public health services though migration and the HIV epidemic, and outbreaks of multidrug-resistant tuberculosis (MDRTB) also contributed2. Rates of TB are again falling in the US but this success has required billions of dollars investment in various public health measures, hospital infrastructure, and laboratory technology. Australia was fortunate not to suffer a similar increase in TB cases because some of the factors contributing to the US epidemic were also present (e.g. migration and reduced government support for TB control programmes). The national notification rate has remained stable since 1985, with 997 cases reported in 2001 (i.e. 5.1 cases per 100,000 population)3. Australian mycobacteriology laboratories have nonetheless benefited from the technological improvements introduced to control the resurgence of TB in the US and other high-income countries.

The US Centers for Disease Control & Prevention (CDC) demanded dramatic improvements in mycobacteriology laboratory turnaround times (TATs): smear examination reports within 24 hours, positive culture reports of Mycobacterium tuberculosis (M. tuberculosis) within 10-14 days, and drug susceptibility testing (DST) results within 15-30 days of specimen collection1. These TATs required the development and widespread adoption of broth-based culture methods. The radiometric BACTEC system has been used for more than 20 years and remains the ‘gold standard’ method for the rapid detection and susceptibility testing of M. tuberculosis, allowing laboratories to attain CDCs challenging TATs.

Unfortunately, the handling and disposal of radioactive broth cultures has proved problematic and has forced the introduction of non-radiometric systems, such as the Mycobacterium Growth Indicator Tube (MGIT) system. These non-radiometric systems provide comparable performance to the BACTEC radiometric method but with a higher contamination rate to which mycobacteriology laboratories have had to adapt.
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International collaborative efforts to validate second-line drug susceptibility testing, which had been commenced for solid media and the BACTEC radiometric method, will have to be repeated and extended for the non-radiometric methods.

Many hoped that the introduction of NAATs to the clinical mycobacteriology laboratory would provide a rapid diagnosis of TB that would far outstrip even the 1-3 week TAT for positive results provided by the broth-based culture systems. This initial enthusiasm has been followed by disappointment and confusion, particularly for our clinical colleagues. Though theoretically able to detect a single copy of M. tuberculosis nucleic acid with high specificity, NAAT has proven to have variable sensitivity compared with TB culture, particularly for smear-negative respiratory specimens where the reported sensitivities range as low as 17.2%.

These imperfect performance characteristics relegate NAAT to being a supplemental test. NAAT cannot replace microscopy or culture, and should not be performed on every TB specimen or suspect. An accompanying paper by Richard Lumb considers the appropriate use of NAAT and the recommendations could be summarised as follows: a smear-positive respiratory specimen in a patient at low-risk of TB, a smear-negative respiratory specimen in a patient at high-risk of TB, and selected non-respiratory specimens (e.g. CSF) where a prompt management decision is necessary (recognising that the performance of NAAT on non-respiratory specimens has not been properly validated). The appropriate use of NAAT and the correct interpretation of NAAT results therefore requires close liaison between clinicians and mycobacteriology laboratories - a fact which this article will demonstrate is true for many facets of clinical mycobacteriology.

Other molecular techniques, such as restriction fragment length polymorphism (RFLP) typing, spoligotyping, variable number tandem repeats (VNTR) and mycobacterial interspersed repetitive units (MIRU), have entered the mycobacteriology laboratory and revolutionised our understanding of the epidemiology of TB disease. Recent transmission has been shown to account for a higher proportion of cases in low-incidence countries than expected. For example, studies in Denmark, New York, San Francisco and the Netherlands found an average of 43% of TB cases were clustered, suggesting recent transmission. Furthermore, exogenous reinfection after curative treatment has been documented to occur more frequently than anticipated in high incidence countries.

An accompanying article by Gilpin & Fyfe in this issue announces the establishment of an Australian TB genotyping project using the MIRU method. The benefits of a national genotyping project include: rapid identification of laboratory cross-contamination events (that account for 0.9-3.5% of M. tuberculosis isolates in other high-income countries), recognition of clusters that cross State borders, assistance for routine contact tracing, and programme evaluation. However, a national TB genotyping project presents several challenges to the mycobacteriology laboratory: in data handling, in choosing a combination of techniques with adequate discriminatory power, and in providing ‘real-time’ results that can assist public health decisions. Public health authorities will also be confronted with laboratory results that suggest up to 40% of cases are clustered.

Recent transmission though, is not the only explanation for M. tuberculosis isolates sharing the same genotype. Migrants present with common strains acquired in their home countries and elderly Australians may develop re-activation disease with strains common in our community in the early-mid 1900s. Molecular genotyping methods may also lack the discriminatory power to differentiate strains within large clusters, such as the Beijing family. Again, close collaboration between the mycobacteriology laboratory, clinicians and public health officers will be required to interpret and respond appropriately to the results of these new molecular methods.
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**Tuberculosis in the region**

While high-income low-incidence countries such as Australia benefit from the introduction of the new TB diagnostics, 95% of the 8.9 million new TB cases occurring worldwide annually live in under-resourced low-income countries. Over 50% of this global TB burden falls on Australia’s neighbours in SE Asia and the Western Pacific. Our neighbours are also increasingly confronted by the major problems of HIV/TB co-infection and MDR-TB. The National Tuberculosis Advisory Committee (NTAC) has recognised that Australia must respond to this situation not only for humanitarian reasons but also in self-interest. The need to liaise with regional partners to assist TB control programmes in neighbouring countries has been included among the eight key recommendations in Australia’s national strategic plan for TB control.

Australian mycobacteriology laboratories have accepted this challenge. For example, the Queensland mycobacterium reference laboratory as a World Health Organisation (WHO) collaborating centre has a long history in supporting TB initiatives in the Western Pacific region. The Western Australian and Victorian reference laboratories have assisted TB programmes in Indonesia and the Pacific islands, respectively. The Institute of Medical & Veterinary Science has also been supporting various TB programmes in Indonesia, which ranks third on WHO’s list of high-burden countries. The official TB incidence estimates for Indonesia are 282 cases/100,000 but experience from eastern Indonesia suggests that the true incidence may be as high as 1,000/100,000 (or 1%) 13.

Working in countries such as Indonesia not only highlights the inequalities of the global TB epidemic but also provides challenges and opportunities not seen in high-income countries. Perkumpulan Pemberantasan Tuberkolusis Indonesia (PPTI) is Indonesia’s principal TB non-government organisation (NGO) and uses charitable donations to run two clinics in poor urban suburbs of Jakarta. Richard Lumb and the author reviewed one of the PPTI laboratories as part of a TB training programme in 2002 (Figure 2). The clinic serviced by this laboratory treats more than 1,400 TB cases per year (i.e. more than Australia’s total TB burden). In these difficult conditions, the laboratory provides a high-quality TB microscopy service (as demonstrated by the tray of perfectly prepared slides shown in Figure 3) and the clinic provides expert care using directly observed therapy and short-course treatment funded by donations.

The five-point protocol recommended by WHO for TB control (known as ‘DOTS’) recommends microscopy as the principal diagnostic for TB in low-income countries. Hence, while high-income low-incidence countries benefit from modern diagnostics and molecular epidemiological techniques, the large majority of the world’s TB suspects are being investigated using a test introduced over 100 years ago (Figure 1). Nonetheless, microscopy for acid-fast bacilli (AFB) is cheap, relatively easy to perform, requires minimal equipment, and detects smear-positive cases who are most infectious and represent the greatest public-health risk, and is therefore the appropriate diagnostic in low-income settings. The strengths of AFB microscopy are exemplified by the results obtained in laboratories such as PPTI, Jakarta. Unfortunately, many TB suspects in Australia’s neighbouring countries do not have access even to this basic diagnostic test because local laboratories lack a functioning microscope, reagents, adequately-trained staff, or a method manual.

To address one of these problems in Indonesia, IMVS has collaborated with Dr. Gunawan Yamin (Head of Microbiology & Parasitology, Indonesian Ministry of Health) and the Indonesia Australia Specialised Training Project (funded...
through AusAID to produce 15,000 copies of a TB microscopy handbook in Bahasa for distribution to every TB laboratory in the country (Figure 4). Experiences such as the PPTI Laboratory in Jakarta demonstrate that small investments such as providing laboratory manuals can reap huge benefits.

**Non-tuberculous mycobacteria**

In addition to the improvements in culture- and molecular-based methods for TB diagnosis, clinical mycobacteriology laboratories in high-income countries have witnessed a revolution in the appreciation of the clinical significance of NTM, their detection, and species identification. This revolution impacts on the routine microbiology laboratory, who are now increasingly expected to recognise clinical syndromes associated with NTM infection (e.g. chronic non-healing skin ulcers refractory to standard antibiotic therapy), to recognise potential mycobacteria on Gram stains, and subsequently to incubate plates at appropriate temperatures and for longer periods to isolate NTM. Three articles in this issue on skin and soft tissue infections, identification of NTM in the routine laboratory, and M. haemophilum provide advice on these matters for the general clinician and microbiology laboratory.

One driver of the burgeoning interest in NTM has been the improvement in the methods for species identification. The article by Crighton & James summarises the switch from slow, arcane, phenotypic identification methods to the modern rapid methods of high-performance liquid chromatography (HPLC) and 16S rRNA gene sequencing. These improvements have presented the mycobacteriology laboratory with their own challenges. 16S rRNA sequences of clinical NTM isolates are usually compared against GenBank and other public-access databases, whose data are not quality controlled and where the original speciation of sequenced organisms may be incorrect. Better quality databases are obviously required.

Improved methods of NTM identification have raised other questions. What is the clinical significance of each novel NTM? What criteria must be met to define a new species? What is the recommended treatment for each novel NTM? Again, clinicians and mycobacteriologists must collaborate to answer these questions. A shared knowledge base must be developed that matches accurate NTM identification with information on the likely clinical significance of the isolate and the outcome of various antibiotic therapies. The management of future patients with NTM can then be guided by this accumulated knowledge base.

Drug susceptibility testing of NTM is another problematic area for clinicians and the mycobacteriology laboratory. The laboratory methods for NTM DST are often not standardised and the results generally do not predict clinical outcome. The situation with the susceptibility testing of M. avium complex (MAC) exemplifies the problems. Only macrolide susceptibility testing has been shown to predict clinical response and standardised susceptibility testing methods have only recently been published. Leo McKnight’s article in this issue nicely summarises the current situation with NTM DST, the recent recommendations from the American Thoracic Society for determining the
clinical significance of NTM, and the approved methods for performing DST from the National Committee for Clinical laboratory Standards (NCCLS)17-19.

Finally, the article by Johnson et al. outlines the long and proud history of Australian laboratories in research on one particular NTM, M. ulcerans. This NTM, which causes skin ulcers, contractures and significant morbidity in sub-Saharan Africa, is also present in limited foci in Victoria and Queensland. Australian mycobacteriologists have been involved from the first description of the disease and organism in the 1940s19, through the development of PCR techniques for the clinical and environmental detection of the organism20, to involvement in the full genomic sequencing of M. ulcerans.

**Conclusion**

Space has not permitted the activities of all mycobacteriology laboratories to be mentioned nor the work of Australian mycobacteriologists in the veterinary field, who are well represented in this issue by excellent articles on M. bovis, ‘canine leprosy’ and Johne’s disease. Mention of this latter disease reminds one of the putative link between M. paratuberculosis and Crohn’s disease not addressed in this edition of Microbiology Australia but for which there are enthusiastic protagonists and antagonists21,22. Again, an Australian study of anti-mycobacterial therapy in patients with Crohn’s disease may help clarify the possible association of M. paratuberculosis with Crohn’s disease23.

This introductory article has attempted to highlight the recent, significant and exciting changes in the field of mycobacteriology and to show that Australian laboratories are at the forefront of this work. The Australian Mycobacterium Reference Laboratory Network, comprising the five State mycobacterium reference laboratories, form the nucleus of the Mycobacterium Special Interest Group (SIG), and are well complemented by contributions from other Australian mycobacteriology laboratories. This edition of Mycobacteriology Australia should demonstrate the vitality of this SIG and assure the reader that the field of mycobacteriologyin Australia is advancing faster than a ‘slow grower’!

**Addendum**

Readers considering donating to the PPTI clinics in Jakarta may contact:
Dr Halim Danusantoso MD FCCP
Medical Supervisor, TB Control Clinic
Perkumpulan Pemberantasan Tuberkulosis Indonesia-Jakarta (PPTI-J)
Indonesian Tuberculosis Control Association, Jakarta branch
Jl. Baladewa 34
Jakarta 10540 Indonesia
E-mail: halim39@cbn.net.id

**References**