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Policy and guidelines

The use of whole genome sequencing for tuberculosis public health activities in Australia: a joint statement of the National Tuberculosis Advisory Committee and Communicable Diseases Genomics Network

Ellen J Donnan, Ben J Marais, Chris Coulter, Justin Waring, Ivan Bastian, Deborah A Williamson, Norelle L Sherry, Katherine Bond, Vitali Sintchenko, Ella M Meumann, Kristy Horan, Louise Cooley, and Justin T Denholm, for the National Tuberculosis Advisory Committee and the Communicable Diseases Genomics Network

Summary

The public health utility of whole genome sequencing (WGS) for *Mycobacterium tuberculosis* includes genome-wide mutation analysis for genotypic drug susceptibility testing (DST), strain identification, differentiation of tuberculosis (TB) relapse from re-infection, transmission cluster detection to guide targeted public health responses and detection of laboratory cross contamination. This has the potential to optimise individual patient care and provide better targeted TB control measures compared to previous MIRU-VNTR genotyping methods. High resolution genotyping of TB should allow better targeting of contact tracing interventions and prevention strategies. WGS will be useful in programmatic evaluation of clinical and public health TB programs through the identification of relapse and cluster analyses to assess effectiveness of contact tracing activities.

Characterising pathogens using WGS is usually less laborious and less expensive than traditional typing methods, and laboratory costs will likely further decrease as it is probable that WGS will soon reduce the need for routine phenotypic DST. However, resource, infrastructure and workforce (including information technology infrastructure and bioinformatics support) has limited implementation of WGS in some jurisdictions, though all mycobacterial reference laboratories in Australia have implemented WGS to some extent. Pathogen genomic data need to be integrated with clinical and public health data to realise their full value. Effective reporting strategies still need to be established, including analyses of key clinical variables and reporting of clusters over time, as well as processes for communicating clusters in a timely manner for public health benefit.

NTAC and CDGN consider it a future requirement for all jurisdictions to have:

- access to prospective WGS of TB isolates;
- WGS results incorporated into routine laboratory reporting systems;
- WGS results reported to clinicians and public health programs;
- WGS results integrated into the routine TB surveillance system;
- routine cross jurisdictional comparison of WGS data; and
- continued education for public health staff and clinicians.

Through increasing capacity for identification of transmission risk and opportunities to intervene and prevent reactivation, WGS will be an increasingly valuable tool for TB control and elimination in Australia.

Background

Bacterial pathogen characterisation using whole genome sequencing (WGS) has four main applications: high-identification and assessment of strain relatedness; genotyping; detection of mutations conferring drug resistance; and virulence gene detection.[1] WGS-based genotyping offers opportunities for enhanced detection and tracking of outbreaks and transmission events. The array of information from genomic sequencing has been described as having potential for 'precision medicine', and is already leading to 'precision public health'.[2] Within the public health sphere, the key applications of interest for *Mycobacterium tuberculosis* are antimicrobial resistance prediction and transmission surveillance. The basic biology of *M. tuberculosis*, with its lack of horizontal gene transfer, slow growth in culture, and the nature of tuberculosis (TB) disease with extended duration of treatment, make it an ideal target pathogen for utilising WGS given both the clinical and public health value.[3]

In Australia, molecular epidemiological typing of *M. tuberculosis* by the Australian Mycobacterium Reference Laboratory Network (MRLN) is considered standard for the initial isolate identified from every patient diagnosed with tuberculosis.[4] At the time of publication of the current Australian laboratory guidelines for mycobacteriology testing, the approved method was mycobacterial interspersed repetitive unit–variable-number tandem repeat (MIRU-VNTR) typing.[5] MIRU-VNTR genotyping in Australia can be used to rule out transmission links if types are different, but is limited by poor strain discrimination, especially among strains of *M. tuberculosis* commonly found among migrants, who account for 85–90% of TB cases.[4] This makes accurate cluster identification problematic with strains that are highly monomorphic and poorly differentiated using MIRU-VNTR, such as Beijing lineage strains.[6]

The Communicable Diseases Genomics Network (CDGN) and the National Tuberculosis Advisory Committee (NTAC) have recognised *M. tuberculosis* as a priority organism for WGS in 2021–2022. A working group has been established comprising WGS personnel from each state and territory (and from New Zealand); two NTAC representatives; and the coordinator of the Australian Society of Microbiologists Mycobacterium Special Interest Group, representing the five Australian Mycobacterium Reference Laboratories (MRLs) and other mycobacteriology facilities. The working group has performed a scoping study of *M. tuberculosis* WGS capabilities and methodologies across Australia; has shared reporting formats for WGS results; and is organising quality assurance programs (QAPs) for participating laboratories. As of 2022, all jurisdictions either perform or have access to WGS capacity for *M. tuberculosis*, with extensive experience and capability in the larger eastern states. The working group aims to optimise reporting formats to local needs in each jurisdiction; to standardise methodologies where necessary; and to investigate interjurisdictional TB transmission using an integrated national genomics platform for rapid sharing and analysis of genomics data, such as Austrakka which has been used successfully for SARS-CoV-2 typing during the coronavirus disease 2019 (COVID-19) pandemic.

Public health utility of WGS for TB

The key strength of WGS for *M. tuberculosis* is that it allows genome-wide mutation analysis for genotypic drug susceptibility testing, as well as lineage identification and cluster detection (using single nucleotide polymorphism (SNP) or core genome multi-locus sequence typing (cgMLST)), facilitating targeted public health responses. WGS also has high discriminatory power to recognise mixed infections with several strains of *M. tuberculosis*,[3,7,8] and can detect laboratory cross contamination. This has the potential to optimise individual patient care and provide better targeted TB control measures.[2,9]

Accurate identification of transmission clusters, differentiating endogenous relapse from exogenous re-infection during disease recurrence, and detecting cases of laboratory

cross-contamination using WGS has led to more precise disease classification and transmission detection compared to previous MIRU-VNTR genotyping methods.[2,10] It has also been shown to have increased specificity over using MIRU-VNTR.[2,3,11] Increased specificity conveys advantages of being better able to define local epidemiology and transmission, to identify previously unrecognised transmission, and to exclude suspected transmission.[3] It also provides greater confidence when investigating potential laboratory cross contamination. WGS has also provided increased awareness of transmission of TB amongst Aboriginal and Torres Strait Islander communities, and of cross-border transmission, allowing for prioritised access to enhanced clinical and programmatic support in local communities.[12,13]

Currently, WGS is being undertaken on all primary *M. tuberculosis* isolates in some jurisdictions in Australia. Direct sequencing of *M. tuberculosis* from sputum smear positive samples is being undertaken in research settings, but it remains too expensive and cumbersome for routine clinical and public health use.[2] Direct sequencing for clinical diagnosis and rapid drug susceptibility, with potential to significantly reduce turn-around times and the requirements for biosafety facilities,[3,11] is likely to be a game changer for prompt effective treatment, but has many technical hurdles to overcome.

Genomic drug susceptibility testing (DST)

Mutation analysis of *M. tuberculosis* bacteria, against a catalogue of known mutations conferring resistance to antibiotics used to treat TB, has the potential to give more rapid and complete information on drug resistance.[3] A study in New South Wales that compared WGS with conventional phenotypic DST found WGS identified an additional 1% of isolates which were likely drug resistant; however, WGS provided a 20% increase in drug resistance detection in comparison with commercial genotypic assays by identifying mutations outside of the classic resistance determining regions in *rpoB*, *inhA*, *katG*, *pncA* and *embB* genes.[14] Satta et al suggested genomic susceptibilities can be available within nine days of a positive culture compared with an average of three weeks with traditional phenotypic methods.[11] More recent papers have suggested even faster turn-around times, with WGS information from early positive cultures where sequencing is routinely performed demonstrating result availability in a matter of days rather than weeks (particularly when second line drug susceptibility testing needs to be set up when initial resistance is detected from an isolate).[2,3]

Early identification of resistance patterns in patients with drug-resistant (DR)-TB will guide initiation of effective treatment, therefore reducing the infectious period of the patient and the risk of community transmission. It should allow a reduction in time spent in negative pressure isolation in hospital facilities, given that infectivity is rapidly terminated with the provision of highly effective treatment. Earlier effective treatment will also decrease the risk of development of further resistance whilst on treatment.

Cluster investigation and transmission

Both Australian and international experience confirms that WGS supports enhanced investigations by more accurately defining clusters, and should be considered as the gold standard for assessment of transmission and strain relatedness.[2] Using previous Australian standard methods, if two patients who recently migrated from a high TB incidence country presented with the same common MIRU-VNTR profile, genotyping has not been able to differentiate between recent transmission in Australia and reactivation of distant infection with a common strain from their homeland.[4] Similarly, Bainomugisa et al demonstrated local community outbreaks with high genomic clustering among patients in the Torres Strait Protection Zone with WGS, including two probable cross-border transmission events of multidrug-resistant TB.[12]

The increased specificity of WGS clusters results in a reduced number of clusters to investigate. Gurjav et al found that the overall rate of MIRU-24 clustering was 20.1% (340/1,692) and was highest among Beijing lineage strains (35.7%; 168/470), and that there was limited transmission within identified clusters in New South Wales.[15] In comparison, in New South Wales in 2018, the overall cluster rate using WGS was 9%, with 82% of clustered cases being born overseas.[16] Early evidence suggested minimal transmission in most Australian settings;[15] however, local transmission events amongst overseas-born people in Australia is being increasingly recognised.

WGS can provide evidence for probable transmission pathways, including a molecular clock of accumulation of random mutations,[1,3] though sometimes is still insufficient to resolve transmission networks in TB outbreaks at the time.[11] *M. tuberculosis* is an ideal organism for this purpose, as estimates for the genome mutation rate vary from 0.3 to 1.1 single nucleotide polymorphisms (SNPs) per genome per year.[17] WGS thus can provide additional insights into transmission dynamics and can sometimes suggest missing or previously unidentified cases in clusters.[2] SNP trees and SNP matrices can assist clinicians in understanding transmission pathways. This has included operational definitions of ≤ 5 SNP difference as a threshold for highly related strains (and therefore, potential transmission), although genomic information must always be interpreted in light of epidemiological and clinical context.[18]

Investigation of TB recurrence

Sequencing of isolates from patients who have had a recurrence of TB can differentiate relapse from reinfection, which provides evidence to assess treatment adherence and efficacy, and overall information for evaluating program effectiveness.[2,3,11] Shanmugam et al found that WGS provided increased resolution, but differentiation between relapse and reinfection was broadly consistent with MIRU-VNTR and spoligotyping in Indian TB patients.[19] Given the significant proportion of Beijing lineage isolates with common MIRU-VNTR patterns in Australia, it has been shown programmatically that WGS is more useful for strain differentiation.[20]

Laboratory cross contamination

Laboratory cross contamination can have significant costs and implications for individuals and the health system. False positive *M. tuberculosis* results can delay diagnosis and treatment of other conditions.[9,21] It can also lead to unnecessary hospitalisation, laboratory testing, radiography, use of negative pressure rooms, and risks from potentially harmful treatments.[9,21] This can also have significant psychological and social pressures on patients and their families. Fortunately, cross-contamination events remain uncommon in Australia. An international meta-analysis found two percent of all culture positive results were false positives secondary to laboratory contamination.[21] It also found 9.2% of patients receive unnecessary and potentially harmful treatments. A Victorian study found a contamination rate of 0.7% in 2,298 samples using MIRU-VNTR.[22]

Genotyping using MIRU-VNTR has been used as the method to detect potential laboratory cross contamination; however, this method has been confounded by the common Beijing strains as for cluster detection. WGS has high discriminatory power to identify contamination events, and has enabled the detection of likely laboratory cross-contamination events with much greater precision than previous typing methods.[23–25]

Genomic public health laboratory surveillance

A key surveillance objective for TB in Australia is to monitor the epidemiology of TB in Australia to better inform prevention strategies.[4] High resolution genotyping of TB could/should allow better targeting of interventions to stop transmission.[2] The increased specificity and indicators for transmission dynamics

often allow the identification of local and inter-jurisdictional transmission, which can be used to implement prevention strategies and to guide contact tracing initiatives. WGS potentially allows for more targeted public health actions, preventing unnecessary investigations of false clusters.[17] The international harmonisation of WGS methodologies and sequence reporting formats allows the analysis of Australian data in the local, national and global context and sharing of WGS with public health partners overseas when required.

The use of WGS and other methods of genomic DST are being incorporated into clinical care. The advantage of WGS over existing methods such as Xpert MTB-RIF Ultra assay (Cepheid), GenoType MTBDR*plus* and GenoType MTBDR*sl* assays (Hain Lifescience GmbH), is that WGS can investigate resistance to a broader range of drugs, as well as a wider spectrum of resistance-conferring mutations. However, these assays can currently be performed directly on clinical samples, providing rapid identification of resistance to key agents when used.

Characterising pathogens using WGS is usually less laborious and less expensive than traditional typing.[2] Laboratory costs will likely further decrease in both materials and staffing, as it is probable that WGS will soon reduce the need for routine phenotypic DST. WGS has been shown to have high sensitivity and specificity for the first-line drugs isoniazid and rifampicin.[11] Whilst routine phenotypic surveillance has not yet been ceased in Australia, it has in several overseas countries.[2,26,27] It is important to note that any sequence-based method is unlikely to completely replace direct susceptibility testing for drug-resistant strains, as inferring drug susceptibility must rely on the continuous updating of databases correlating genotypic and phenotypic data.[2]

Potential use for program evaluation

WGS is useful for elements of programmatic evaluation of clinical and public health TB programs. Identification of relapse in patients previously treated in Australia can flag review of regimen, duration and adherence which can provide important information in treating the relapsed disease. Cluster analysis can be used to assess effectiveness of retrospective identification, screening and use of preventive therapy in contact tracing activities, with systematic and pre-planned evaluation providing valuable opportunities for service strengthening.[28]

Communicating WGS evidence of recent transmission in migrant groups can be used to support arguments for use of preventive therapy over chest X-ray (CXR) surveillance in these groups. Investigation of potential laboratory cross contamination events can highlight breakdowns in laboratory processes,[3] which can be used to implement more robust procedures to reduce potential contamination events. Identification of laboratory cross contamination provides an effective evaluation of laboratory processes.[3]

Current challenges and limitation of WGS for TB

Resource, infrastructure and workforce limitations currently restrict WGS to jurisdictional public health laboratories, reference laboratories, and hospital infection control-affiliated laboratories.[1] Information technology infrastructure and bioinformatics support are key components required for output that is useful to public health.[2,11] Mycobacterial reference laboratories in Australia have all implemented WGS to some extent, though processes for prospective and retrospective sequencing differ. The use of WGS by diagnostic laboratories is likely to expand in the future.

Pathogen genomic data need to be integrated with clinical and public health data to realize their full value.[2] Routine integration of WGS data into surveillance systems requires infrastructure for laboratory and public health systems to transmit and receive sequencing results; epidemiologists with the knowledge to interpret the laboratory data and integrate with the other available data; and clinicians to understand

and communicate relevant results.[2] Delivery of WGS information to TB doctors and nurses in a timely and understandable format is a key issue.[11] Whilst WGS has been implemented to some degree across Australia, the ability of WGS to reduce TB transmission remains unproven.[11] Therefore, ongoing monitoring and evaluation of the public health impact of genomic sequencing on TB control is warranted.

Effective reporting strategies for WGS still need to be established, including analyses of key clinical variables and reporting of clusters over time. Public reporting of WGS for TB in Australia is currently limited. WGS data has been included in the Tuberculosis in New South Wales Surveillance Reports since 2018, though challenges of communicating the data remain.[16] Processes need to be implemented for communicating clusters in a timely and accurate manner, so as to be of public health benefit. One study detailed the contribution of real-time feedback between the microbiology laboratory and the TB control program prompting further contact investigations and identification of additional epidemiologically linked cases.[10] As WGS becomes more routinely integrated into programmatic responses, it will be important to document and share the evidence of WGS impact on contact tracing activities in Australia.

Finally, as the technological and logistic integration of WGS becomes routine in programmatic TB management in Australia, there are a series of evolving ethical and legal questions which must also be considered. As discussed throughout this paper, WGS brings increasing capability for demonstrating evidence of the direction and timing of transmission, and for optimising performance of public health and clinical services. The community and organisational expectations that arise in parallel to these developments will likely necessitate updating public health practice, including considerations of access and use of personal and health data. Transparency and broad consultation will be critical to ensure that public health practices are aligned with both established and emerging community norms.

Future requirements

NTAC and CDGN consider it a future requirement for all jurisdictions to have:

- access to prospective WGS of TB isolates;
- WGS results incorporated into routine laboratory reporting systems;
- WGS results reported to clinicians and public health programs;
- WGS results integrated into the routine TB surveillance system;
- routine cross jurisdictional comparison of WGS data; and
- continued education for public health staff and clinicians.

WGS results need to be incorporated into routine laboratory reporting systems; to be considered contextually through multidisciplinary review; and to be communicated clearly to clinicians and public health programs. Reporting schedules may vary jurisdictionally depending on the TB burden; appropriate reporting time frames from laboratory to public health would be considered to be weekly to a maximum of one month. Continued education for public health staff and clinicians is necessary to ensure the output of WGS is being maximised. WGS results need to be integrated into surveillance systems so analyses and reporting can be multifaceted and contemporaneous. Routine cross jurisdictional comparison of WGS data to detect Australia-wide clusters should be standard practice.

There needs to be continued collaboration between NTAC, CDGN, the Australian MRLs, and jurisdictional TB and public health programs through the various channels available. Communication of experiences (with implementation, utilisation for DST and cluster detection, and evaluation) has the potential to strengthen WGS throughout Australia.

Closer partnerships between genomics service providers, TB control programs and relevant clinicians are essential for achieving the vision outlined in this Statement. The ongoing translational research in developing and implementing culture-independent and metagenomic methods of drug resistance detection and subtyping should be supported. The Committees also support the development of sustained capacity for genomic testing in Asia-Pacific region.

Progression towards TB elimination is a stated goal for both the Australian and international TB communities.[29,30] An important contribution towards elimination in low incidence countries is an aim for zero local transmission.[31] Through increasing capacity for identification of transmission risk and opportunities to intervene and prevent reactivation, WGS will be an increasingly valuable tool for TB control and elimination in Australia.

Notes

Ellen Donnan (Chair), Justin Denholm (Deputy Chair), Ben Marais, Chris Coulter, Justin Waring and Ivan Bastian are members of the National Tuberculosis Advisory Committee.

Ivan Bastian (Co-chair), Vitali Sintchenko (Co-chair), Chris Coulter, Deborah Williamson, Norelle Sherry, Katherine Bond, Ella Meumann, Kristy Horan, Louise Cooley, Ellen Donnan and Justin Denholm are members of the Communicable Diseases Genomics Network Tuberculosis working group.

This paper is endorsed by the Communicable Diseases Network Australia.

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