DukeNUS Medical School | Centre for Outbreak Preparedness

ASIA PATHOGEN GENOMICS Initiative

Ministry of Health Indonesia and Asia Pathogen Genomics Initiative Tuberculosis Genomics Meeting Report

Recent advances and in-country experiences
Ayodya Resort Nusa Dua, Bali, Indonesia
11 November 2024

Executive Summary

On 11 November 2024, the Ministry of Health Indonesia and the Asia Pathogen Genomics Initiative (Asia PGI) hosted a meeting which convened country representatives, international donors and next generation sequencing (NGS) manufacturers to discuss advances and country experiences in using NGS for Tuberculosis (TB) surveillance and management. This was the first in-person meeting following the establishment of the Asia PGI TB Genomics Regional Expert Working Group, with 63 representatives from 15 countries present. The discussions highlighted progress in implementing TB genomics across various settings as well as barriers to adoption - including technical, financial and operational hurdles.

Asia PGI aims to advance TB genomics in the region through several strategic initiatives. The following priorities emerged, considering practical considerations outlined in the recently updated WHO consolidated guidelines and insights from this meeting:

1. **Coordinating regional validation studies and cross-country knowledge exchange to facilitate TB genomics integration within national strategic planning**
    - Assess performance, cost-efficiency and feasibility of NGS platforms through multi-country validation studies in high burden settings in Asia
    - Leverage regional expertise through cross-country knowledge exchange to discuss operational barriers, harmonize protocols and develop implementation guidance

2. **Expanding the availability of DR-TB genomics assays through sustainable cost and supply chain models**
    - Collaborate with manufacturers on preferential pricing access schemes and explore cost-saving mechanisms
    - Support countries in multi-pathogen use of genomics infrastructure to optimize resources

3. **Building capacity in TB genomics workflows and data interpretation for clinical and public health management**
    - Support piloting streamlined TB genomics bioinformatics tools to aid with clinical decision making and public health management
    - Asia PGI Academy to serve as a hub for cross-country training in the various platforms to be assessed, supported by major genomics manufacturers and TB genomics specialists from high-income countries in the Expert Working Group.

Justification and Evidence for TB Genomics

Extensive research has been conducted to generate evidence to inform policies for uptake of new TB diagnostics. With the support of FIND, the updated WHO Consolidated Guidelines for Tuberculosis (2023-2024) now includes a class-based recommendation for the use of targeted Next Generation Sequencing (tNGS) as a diagnostic tool for drug resistant TB (DR-TB).[1] Targeted NGS enables fast and comprehensive DR-TB testing, with strong genotype to phenotype correlation for TB, representing a paradigm shift in fast and efficient DR-TB diagnosis.

A multicenter, cross-sectional diagnostic accuracy study of GenoScreen Deeplex Myc-TB and Oxford Nanopore Technologies Am-PORE TB for rifampicin, isoniazid, fluoroquinolones (moxifloxacin, levofloxacin), amikacin, kanamycin, capreomycin, pyrazinamide, ethambutol, bedaquiline, linezolid, clofazimine, and streptomycin was conducted in three countries: Georgia, India, and South Africa.

Independent analytical validation demonstrated reproducibility and single nucleotide polymorphism (SNP) calling accuracy. Both tNGS solutions provide accurate and reproducible results directly from clinical samples, with ONT having lower sensitivity and specificity for detecting resistance to Linezolid, Clofazimine, Bedaquilline.[2] These findings are critical in the context of global TB treatment priorities, which are 1) shorter, all oral treatments (eg, BPaL/M [bedaquiline, pretomanid, linezolid, and moxifloxacin]) more targeted treatments 3) timelier treatment decision making.

The next stage of the study is to assess NGS implementation models and prepare for scaling up NGS interventions in countries. This will require generating evidence to boost in-country capacity for use of NGS for comprehensive diagnosis of DR-TB.

Regional Developments in TB Genomics and Implementation Progress

To date, a large proportion of TB genomics implementation has been in low burden settings, with evidence building that can support further investments in TB control and inform use in high burden settings. Genomic surveillance can play a critical role in identifying imported TB cases that might evade detection by rapid tests such as GeneXpert. Routine WGS can provide a comprehensive view of local TB transmission dynamics, aligning with WHO End TB Strategy for TB elimination.

In Australia, routine whole genome sequencing (WGS) is performed for all rifampicin resistant TB (RR-TB) cases. Sequencing shows that most cases in New South Wales (NSW) are imported (80%), which has important implications on TB control policies. WGS can help with tracking clusters of transmission and identifying transmission pathways. It can identify large or multi-year clusters and provide a better understanding the geographic and spatial mapping of cases for targeted public health response. Taiwan has also used WGS for TB since 2022, mainly for the surveillance of DR-TB cases, whereas high risk populations undergo NGS for diagnosis.

TB remains a significant challenge in Indonesia, which is the second largest contributor of TB cases in the world and faces delay DR-TB diagnosis and treatment. For 2024, case notification is expected to be 77% out of a total estimated 1 million cases. The Biomedical and Genome Science Initiative (BGSi) was launched in 2022 under the Binomika branch of the Ministry of Health (MOH). The program aims to accelerate access to genomic testing, support the integration of genomics into MOH strategic programs, and improve the safe and appropriate use of medicines (*pharmacogenomics*), all with the end goal of reducing burden of disease in Indonesia. Stakeholders include 10 BGSi Hub Hospitals and the Genomic Sequencing Lab Network. These partnerships were established and strengthened during the COVID-19 pandemic.

A roadmap for implementation of NGS was developed in 2023, and a pilot study for the use of tNGS is planned for 7,600 patients across 34 provinces. Results will be compared with drug susceptibility testing (DST) from culture and with WGS. This study will have important findings that compare the benefits of a decentralized tNGS strategy compared to a centralized WGS strategy. Despite the integration of recruitment and testing workflows into the existing national TB program, there have been challenges in aligning across multiple stakeholders and in sample recruitment. Other factors affect study outcomes, including quality of sputum samples and laboratory capacity.

There have also been concerns that the current rapid molecular diagnostic test for DR-TB, GeneXpert XDR, does not detect resistance to BPaL. As a result, Project TARGET, funded by Gates Foundation and FIND, has been launched to conduct tNGS for DR- TB detection in Indonesia. This study is being conducted in West Java among seven collaborating Xpert hospitals and aims to sequence RR-TB cases using Illumina ISeq100-Deeplex. In this project, sample flow is from primary care level to hospital and then to reference laboratory. The study has faced challenges with low sample quality; only 48% of samples eligible for tNGS. The country team has also highlighted that the laboratory process is detailed and time intensive, involving DNA extraction, RT-PCR, library preparation and then sequencing. As a result, the median turnaround time is currently 9 days, with a target time of one week. The high cost for tNGS has also been highlighted, at a total cost per sample of around $352, which includes facility, equipment, personnel training and supplies.

Thailand has also faced continued challenges with TB control, with incidence of 157 per 100,000 population and treatment coverage at 71%. The Thailand Operational Plan to End Tuberculosis (2023 – 2027) has emphasized research and innovation in TB prevention and control. With JICA collaboration, the Thailand TB Medical Science Excellency Center (TB-MSEC) is currently rolling out WGS interventions in the North and North-East of Thailand. From 2022 – 2023, 1,710 samples were sequenced and demonstrate differences in lineage across regions.[3] WGS was used to conduct TB lineage and sub-lineage surveillance, investigate transmissibility and in the event of an identified school outbreak was used to conduct targeted active case finding. Several clusters are related to transportation routes from Bangkok to North/NE of Thailand. It was also found that patients previously incarcerated have higher odds ratio for TB and have been identified as a high-risk population. Implementation challenges include the high initial cost of equipment and infrastructure, the need for technical expertise for sequencing and data interpretation and need for standardization and validation of NGS protocols.

In Malaysia, 2023 data show that TB incidence is 122 per 100,000 population and MDR-TB is 1.7 per 100,000 population, although this is likely under-detected due to low use of GeneXpert. WGS was conducted to investigate the lineages and resistance genotypes of the RR-TB population. From an analysis of 56 DR-TB cases, most were of East-Asian (Beijing) lineage, with 12 clusters identified. Results showed that West and East Malaysia have different TB genotypic diversity, with West Malaysia TB genotypic patterns more closely resembling West Java, Indonesia. Genotyping revealed new possible epidemiological links undetected by conventional investigation and detected 66 total mutations across 20 genes and for 9 anti-TB drugs. Another key finding was that some mutations for RR were not detected by the rapid molecular diagnostic tool. This raises a major concern around the selective potential of current TB diagnostic tools. This selective diagnostic escape can potentially result in in silent strains conferring TB resistance, which can be minimized through detection with NGS. Use of both phenotypic and genotypic DST would give more reliable results for TB treatment and management.

Asia PGI Strategic Directions for Advancing TB Genomics in the Region

Programmatic implementation of NGS for DR-TB was assessed in Brazil, Indonesia, South Africa, Eswatini, Bangladesh and Kyrgyzstan to inform the most recent WHO Consolidated Guidelines for TB. From these experiences, several practical considerations were raised for the use of tNGS. These included assessing country readiness for NGS implementation, accounting for technical and operational guidelines to support roll out, strength of diagnostic/surveillance networks, training for partners and key stakeholders, cost effectiveness and impact modelling, and manufacturer engagement for market shaping. Countries must also consider which delivery model is best suited to country context, and whether a centralized, decentralized or outsourced service model would better achieve national sequencing goals.

Furthermore, Asia PGI conducted a review on regional initiatives related to genomic sequencing of tuberculosis (findings available at http://tb.asiapgi.net). This review showed most organizations conducting TB genomics sequencing in the region use Illumina short reads (21) while a few (5) used ONT long reads. Regarding public health impact, most initiatives studied diagnostic testing effectiveness or to assess anti-tuberculosis drug resistance profiles. The review also identified three key focus areas for TB genomics in the region: (1) Integrating sequencing within national strategic planning, (2) expanding the availability of lower cost genomics assays, and (3) upskilling clinicians and public health professionals in using TB genomics.

The practical considerations in the WHO updated guidelines and insights from this meeting will guide Asia PGI's strategic priorities in addressing the barriers and opportunities towards accelerating TB genomics in the region, as described below.

Coordinating regional validation studies and cross-country knowledge exchange to facilitate TB genomics integration within national strategic planning

The integration of TB genomics within national strategic planning depends on the technologies adopted (WGS vs. tNGS) and their application in clinical workflows and public health surveillance. From the meetings discussion, it was proposed that tNGS would be most applicable to drug resistant cases due to faster turnaround time and the potential to be applied directly from sputum specimens. However, WGS may be more applicable for surveillance, outbreak identification and supporting epidemiological investigation. In high burden settings, financial constraints hinder advocating for the routine use of genomics, despite clear examples of its contributions to TB surveillance.

Therefore, there is a pressing need to validate and compare the performance of existing NGS platforms across diverse settings in Asia. Validation efforts should focus on assessing analytical accuracy, cost-efficiency and implementation feasibility of NGS platforms for DR-TB profiling. Multi-country studies in high TB burden countries could generate evidence to inform platform selection and guide adoption strategies tailored to specific needs of national TB programs.

Furthermore**,** the Asia PGI Regional Expert Working Group serves as a platform for fostering cross-country collaboration and knowledge exchange. Periodically convening, either virtually or in-person, will allow for a diverse group of TB genomics specialist to discuss operational barriers, harmonize protocols and develop contextual implementation guidance on institutionalizing genomics into national TB programs.

Expanding the availability of DR-TB genomics assays through sustainable cost and supply chain models

Expanding the availability of lower cost genomic assays will depend strongly on advances in technology and costing options provided by manufacturers. The Illumina Genoscreen Deeplex TB Combo Kit allows for culture-free detection of resistance to 15 anti-TB drugs. Additional technology applicable to TB genomics includes Illumina's Respiratory Pathogen ID/AMR Panel (RPIP) which is culture free and can detect multiple pathogens including 8 TB resistant genotypes. Most recently, Illumina has also released the MiSeqi100, which is cartridge based like GeneXpert and allows for ambient shipping and storage of consumables. This new platform can also run 1 sample at a time for greater flexibility of sequencing needs.

MGI provides a similar solution with ATOplex MTB tNGS, which also detects drug resistance for 15 first- and second-line anti-TB drugs. MGI offers easy operation through two amplification steps in one tube, a 24–29-hour turnaround time and automatic generation of reports. To improve access to these assays for TB genomics implementers in the region, both manufacturers have set up NGS access scheme programs that provides fixed, transparent and preferential pricing on sequencers and DR-TB products for Asia PGI partners via the Illumina Global Health Access Initiative (GHAI) and MGI's Pandemic Preparedness Program (PPP).

ONT's Am-PORE TB kit, which will be commercially available in early-2025, is a GridION locked solution that allows for sequencing to be conducted in around 6 hours with 16 TB drug mutations screened. It does not require cloud-based data upload and one Am-PORE TB kit can accommodate 88 samples in four batches of 22 samples per sequencing run. Recently, ONT has partnered with bioMérieux, which will be distributing Am-PORE TB through its distributor channels globally.

Asia PGI is involved in ongoing discussion with NGS manufacturers to develop procurement models that address the unique economic and logistical barriers of countries in the region for the implementation of TB genomics. These models will explore opportunities to reduce genomics cost through fixed, transparent and preferential pricing as well as leasing agreements. Asia PGI also aims to explore mechanisms for integrating genomics sequencing into existing diagnostic networks by shared use of genomic sequencing infrastructure for other pathogen and decentralized workflows that minimize logistical burdens.

Building capacity in TB genomics workflows and data interpretation for clinical and public health management

Another key area in TB genomics involves training for clinicians and public health professionals in the application of genomics data for TB control. Clinicians are concerned with drug resistant results from GeneXpert XDR and advocate for tNGS to have a more comprehensive picture of TB drug resistance profiles in their communities. Questions were also raised among meeting participants regarding how best to respond to heteroresistance found through tNGS. Additional tools, such as WGS, are needed for further investigation. These cases should be reported at the national level for expert opinion and policy guidance. However, the question remains, if identified TB sequences are not related to phenotypic resistance, then what percentage of variance is needed to report resistance? The complexity of bioinformatics data produced can make it difficult to interpret tNGS results to guide clinical decision making.

Currently, there are tools that exist which aid in bioinformatics interpretation. Sequentia (www.sequentiabiotech.com) is a bioinformatics organization whose mission is to make complex bioinformatics accessible to laboratories and users. One of their products, MICK MAGMA, produced in collaboration with the University of Antwerp, is a streamlined NGS software for the analysis and interpretation of TB genomic data. The software is linked to the WHO variant catalog and can produce easy to interpret red-yellow-green profiling to indicate the potential of resistance to certain anti-TB drugs. Based on results, the software can also provide optimized treatment regimens for each patient. The software is platform and method agnostic, and results can easily be communicated with other stakeholders, which can facilitate expert discussion around treatment recommendations. This type of analysis allows for surveillance of resistance, especially to anti-TB medications such as BPaL/M which are not detected by current rapid molecular tools and can facilitate clinical action as well as inform wider public health policies.

Asia PGI seeks to explore solutions that streamline complex DR-TB bioinformatics analysis. Tools like MICK MAGMA, which aid in clinical decision making, represent a promising approach towards making genomics data interpretation more accessible to clinicians and public health policy makers. Piloting such tools in high impact settings will harness innovation around TB genomics in the region and promote data sharing to the WHO TB Mutation Catalogue for mutual benefit.

Furthermore, a key factor in advancing TB genomics in the region will be through training bioinformatician, clinicians and laboratory technicians to ensure that DR-TB genomic data can be effectively generated, interpreted and applied. The establishment of regional training hubs, such as the Asia PGI Academy at the Duke-NUS Medical School, Singapore, and developing capacity development modules that include standard operating procedures and protocols for TB genomics wet labs and bioinformatics will be essential going forward. This will serve as a hub for cross-country training in the various platforms to be assessed, supported by both the major genomics manufacturers based in Singapore alongside TB genomics specialists in the Expert Working Group.

Conclusions

With recent advances in global and regional genomic capacity following the COVID-19 pandemic, TB has been identified as a clear use case for pathogen genomic surveillance. Genomic data can transform TB control policies and practices, giving health practitioners a deeper understanding of individual patient infection to inform treatment therapy for precision patient care, while also informing public health action through information on circulation of community infection. Genomic surveillance can enable monitoring of the TB epidemic, identifying changes in TB burden, geographic spread, and TB transmission. There is also the potential to look across country data over time to view cross-border transmission events.

Asia PGI is committed to accelerating genomics for disease prevention and control, including driving implementation research, facilitating capacity building, and supporting collaborative efforts in making genomics a cornerstone of TB prevention and control. Future work will focus on the need to better understand where and when to sequence TB cases, how to complement existing molecular diagnostics and how to streamline NGS implementation within broader national health plans.

References

1 WHO consolidated guidelines on tuberculosis. Module 3: diagnosis - rapid diagnostics for tuberculosis detection, third edition., (World Health Organization, Geneva, 2024).

2 Colman, R. E. *et al.* Evaluating culture-free targeted next-generation sequencing for diagnosing drug-resistant tuberculosis: a multicentre clinical study of two end-to-end commercial workflows. *Lancet Infect Dis* (2024). https://doi.org/10.1016/S1473-3099(24)00586-3

3 Nuttavuthisit, K. *et al.* Whole-genome sequencing of Mycobacterium tuberculosis sublineage L1.1.1.7 from Chiangrai, Thailand. *Microbiol Resour Announc*, e0066324 (2024). https://doi.org/10.1128/mra.00663-24