Implementing molecular tuberculosis diagnostic methods in limited-resource and high-burden countries

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Next-generation sequencing has the potential to drastically change the landscape of tuberculosis diagnosis worldwide. Considerate implementation in low-resource settings should ensure access and sustainability, especially through new portable platforms. https://bit.ly/3iiWFyo

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Abstract

Tuberculosis (TB) is one of the deadliest infectious diseases in the world with more than a million people dying of TB each year. Accurate and timely TB diagnosis has the potential to alleviate the global TB burden; therefore, one of the pillars of the End TB Strategy developed by the World Health Organization (WHO) is the early diagnosis of TB, including universal drug-susceptibility testing (DST). The WHO emphasises the importance of DST before treatment initiation, using molecular WHO-recommended rapid diagnostic tests (mWRDs). Currently available mWRDs are nucleic acid amplification tests, line probe assays, whole genome sequencing, and targeted next-generation sequencing. However, implementing the sequencing mWRDs in routine laboratories in low-income countries is constrained by the existing infrastructure, high cost, the specialised skills needed, data storage, and the current delay in results compared with other routine methods. These limitations are pronounced in resource-limited settings, which often have a high TB burden and need for innovative TB diagnostic technologies. In this article we propose several possible solutions, like adapting infrastructure capacity to needs, advocating for lowering costs, building bioinformatics and laboratory capacity, and increasing the use of open-access resources for software and publications.

Introduction

Tuberculosis (TB) represents an enormous burden on global health, with 9.9 million new people developing TB, and 1.3 million dying from TB in 2020 [1]. In the first high-level meeting on TB, the United Nations released commitments to four global targets, one of which is diagnosing and treating 40 million people with TB in 2018–2022 [2, 3]. An important barrier to reaching these targets is the availability of innovative methods for TB diagnosis. Moreover, one of the key components of the End TB Strategy issued by the World Health Organization (WHO) is the early diagnosis of TB, including universal drug-susceptibility testing (DST) [4]. The WHO guidelines stress the importance of DST before treatment by molecular WHO-recommended rapid diagnostic tests (mWRDs) [2]. However, DST coverage for first-line drugs only ranges from 41% to 93%, and there is suboptimal DST coverage for second-line drugs [2]. Notably, there are no WHO-recommended rapid methods for DST of newer and repurposed drugs, which is worrisome as the use of these drugs is increasing.

This viewpoint discusses the currently available mWRDs and the challenges of their implementation, especially in resource-limited settings, providing possible solutions.

Currently available mWRDs

Table 1 shows the current mWRDs with their methods, advantages, and limitations.

To facilitate the application of these mWRDs, the WHO issued the first catalogue of drug-resistance mutations in *Mycobacterium tuberculosis* (MTB) [5]. It was built from high-quality phenotypic DST data,

TABLE 1 Molecular tuberculosis (TB) diagnostic methods

	Nucleic acid amplification tests (NAATs)	Line probe assays (LPAs)	Whole genome sequencing (WGS)	Targeted next-generation sequencing (tNGS)
Mechanism	Amplify the DNA using PCR, and detect a particular nucleic acid sequence	PCR-based tests that use LPA as detection (detect the binding pattern of DNA amplification products to probes that target specific parts of the *Mycobacterium tuberculosis* genome, resistance-associated mutations to anti-TB drugs, or the wild-type DNA sequence)	Analyse the entire genomic DNA sequence at a single time	Focus on amplicons (DNA amplification products) or targets known to have strong associations with mutations
Advantages	Detect specific mutations associated with resistance to selected anti-TB drugs Can be used directly on clinical specimens Short turn-around time	Detect resistance to a wide range of first-line and second-line agents and provide mutation-specific data for common variants Can be used directly on clinical specimens Short turn-around time (5 h)	Can identify low frequency variants Improve detection of various types of mutations Assess a broader range of drugs compared with phenotypic DST Provide unbiased detection of mutations mediating low or moderate MIC Can also be used for surveillance and source investigation	Can identify low frequency variants in targeted regions with high confidence Can be used directly on clinical specimens Shorten turn-around time if performed from clinical specimens
Limitations	Cannot identify low frequency variants Sometimes follow-up actions (*e.g.* sequencing) are needed to guide appropriate TB treatment		Low availability of supply chain Need high technical and analytical skills Need high storage size and security High cost (capital investment costs, running costs, data storage costs) Need for culture Longer turn-around time than other mWRDs methods Difficulty interpreting whole-genome variation data in the context of the high number of rare variants	High cost, but less than WGS; tNGS requires adding the PCR step; the decrease in cost could be linked to the running of many samples in the same flow cell
Example of platforms	Xpert MTB/RIF and Xpert MTB/RIF Ultra (Cepheid); Truenat (Molbio); Abbott RealTime MTB and Abbott RealTime MTB RIF/INH (Abbott); BD MAX MDR-TB (Becton Dickinson); cobas MTB and cobas MTB-RIF/INH (Roche); FluoroType MTBDR and FluoroType MTB (Hain Lifescience/Bruker)	GenoType MTBDR*plus* v1 and v2, and GenoType MTBDR*sl* (Hain Lifescience/Bruker); Genoscholar NTM+MDRTB II, and Genoscholar PZA-TB II (Nipro)	Miseq, MiniSeq, NextSeq, HiSeq (Illumina); Personal Genome Machine (Ion Torrent); PacBio RS II (Pacific Biosciences); MinION (Oxford Nanopore Technologies)	

DST: drug-susceptibility testing; MIC: minimum inhibitory concentration; mWRD: molecular WHO-recommended rapid diagnostic tests.

standardised whole genome sequencing (WGS) for generation of unbiased, raw sequence data, a standardised bioinformatics pipeline for variant detection and annotation, and a standardised and validated methodological approach for associating genotype with phenotype. This catalogue contains 1200 variants associated with resistance and 246 variants not associated with resistance [5]. However, this catalogue has difficulty classifying rare mutations, probably does not classify the mutations conferring modest minimum inhibitory concentration (MIC), and does not consider the lineage effects, resistance levels and co-occurrence of mutations. An updated version of the catalogue will be issued in the following months, addressing most of these shortcomings.

Challenges in implementing sequencing mWRDs in resource-limited settings

The implementation of sequencing mWRDs in routine laboratories in low-income countries (LICs) is hampered by the currently available technology, its high cost, the very specialised skill set needed to process and analyse the outputs, data storage, and the current delay in results availability compared with other current routine methods. These limitations are more pronounced and consequential in resource-limited settings, which often have a high TB burden and a crucial need for innovative TB diagnostic technologies.

Existing infrastructure

A major challenge in sequencing methods is that they generate vast amounts of data that require strong expertise and powerful machines to extract and analyse. The required infrastructure to effectively obtain results using WGS is not available in many laboratories in LICs. The data obtained from sequencing methods need to be properly stored in data warehouses that are yet to be developed in most LICs. Cloud storage represents another storage solution; however, the existing internet capacity needs to be improved before this option can be realistically accessible in LICs.

Cost of the machines and reagents

The cost of WGS varies by the type of assays, equipment, and software used. The commercial cost of sequencing an MTB genome is between USD 150 and USD 300 [6]. This cost includes the process of resequencing, where the MTB genome sequence is compared to a reference genome.

The costs are increased when establishing a sequencing facility in low-income settings due to the costs of shipments, customs, and the eventual profit margin for local companies. Lessons learnt from implementing WGS in Kyrgyzstan show that the main cost drivers were not using library preparation and sequencing kits to their full capacity during initial training, and partly by prices charged by the regional distributor exceeding those charged to laboratories in Europe and North America [7]. In addition to hardware costs, data manipulation and analysis are done using software licensed and commercialised by companies, representing an additional cost to institutions in LICs.

Training and capacity building

Local capacity needs to be built in both the laboratory and bioinformatics aspects of MTB WGS as many institutions continue to suffer from an insufficient number of experienced personnel that can perform, supervise, and train others in bioinformatics and sequencing research [8]. The fields of sequencing and novel TB diagnostic methods are interdisciplinary, requiring knowledge and training in biology, chemistry, and bioinformatics. In addition to training laboratory staff, there is a need to build capacity for clinicians to interpret the results of sequencing and adequately incorporate them into their routine clinical practice.

Possible solutions

Adapting infrastructure capacity to needs

Decision makers at the country level need to be aware of and adapt the sequencing capacity to the country's needs. National programmes should avoid obtaining the sequencing infrastructure and not using it, because when not in use the maintenance of the machines is even higher, with implicit cost consequences. Therefore, LICs could opt for a national referral hub where all samples would be processed and then returned to health centres. Moreover, several countries could share a regional referral hub for sequencing TB strains, with shared infrastructure and human resources. Another effective strategy would be to mutualise resources with other pathologies (*e.g.* coronavirus disease 2019 (COVID-19), malaria, or even cancer) for surveillance, antimicrobial resistance control and research [9–11]. The lessons learnt from the COVID-19 pandemic response could inform TB sequencing technologies and inform local and regional stakeholders on the best strategies to implement [9, 12, 13].

Advocating for lowering costs

When sequencing methods are widespread and used at a large scale, there are compelling arguments to advocate with companies to lower the cost of machines and reagents. Using next-generation sequencing (NGS) (USD 50–100) instead of WGS (USD 150–300) as a diagnostic tool would help reduce costs without reducing the advantages of these innovative tools [6]. Mutualising costs with other countries for third-generation sequencing techniques like nanopore might address some of the existing challenges regarding infrastructure or cost, and could be a competitor for current sequencing companies in low-income and high-burden countries [14].

Building bioinformatics and laboratory capacity

An increasing number of universities are offering training in bioinformatics in low-resource settings. Bioinformatics education in Africa is currently being shaped through initiatives such as H3ABioNet, a project funded through the US National Institutes of Health [15]. One of the network's main remits is to build human capacity in bioinformatics in Africa. Proposed solutions include continuous mentorship and support, train-the-trainer programmes, internships, knowledge transfer programmes and e-learning platforms [16]. While being a much-needed initiative in the current landscape, the long-term sustainability of this kind of project remains of concern, with countries needing to take ownership of bioinformatics training and capacity building. In the future, most of the technology could be automated, leaving less of the processing burden to laboratory staff.

Data interpretation and sharing

Data analysis and visualisations should be available in real-time, and be simplified and easy to interpret by laboratory staff and clinicians to facilitate clinical decisions. Access to knowledge and results sharing should be available to researchers and decision-makers in resource-limited settings through open access publications and open license software.

Conclusion

The landscape of genome sequencing for TB diagnosis and DST is rapidly evolving, with promising technologies being developed. Nevertheless, access to these innovative diagnostic methods should be granted in all types of settings, with an emphasis on high-burden countries.

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References

1 World Health Organization. Global Tuberculosis Report. Geneva, World Health Organization, 2021. https://www.who.int/publications/i/item/9789240037021

2 World Health Organization. WHO consolidated guidelines on tuberculosis – module 3: rapid diagnostics for tuberculosis detection. Geneva, World Health Organization, 2021. https://www.who.int/publications/i/item/9789240029415

3 Sakamoto H, Lee S, Ishizuka A, *et al.* Challenges and opportunities for eliminating tuberculosis – leveraging political momentum of the UN high-level meeting on tuberculosis. *BMC Public Health* 2019; 19: 76.

4 Lönnroth K, Raviglione M. The WHO's new end TB strategy in the post-2015 era of the sustainable development goals. *Trans R Soc Trop Med Hyg* 2016; 110: 148–150.

5 Walker TM, Miotto P, Köser CU, *et al.* The 2021 WHO catalogue of *Mycobacterium tuberculosis* complex mutations associated with drug resistance: a genotypic analysis. *Lancet Microbe* 2022; 3: e265–e273.

6 Dlamini MT, Lessells R, Iketleng T, *et al.* Whole genome sequencing for drug-resistant tuberculosis management in South Africa: what gaps would this address and what are the challenges to implementation? *J Clin Tuberc Mycobact Dis* 2019; 16: 100115.

7 Vogel M, Utpatel C, Corbett C, *et al.* Implementation of whole genome sequencing for tuberculosis diagnostics in a low-middle income, high MDR-TB burden country. *Sci Rep* 2021; 11: 15333.

8 Rivière E, Heupink TH, Ismail N, *et al.* Capacity building for whole genome sequencing of *Mycobacterium tuberculosis* and bioinformatics in high TB burden countries. *Brief Bioinform* 2021; 22: bbaa246.

9 Leite JA, Vicari A, Perez E, *et al.* Implementation of a COVID-19 genomic surveillance regional network for Latin America and Caribbean region. *PLoS One* 2022; 17: e0252526.

10 Akoniyon OP, Adewumi TS, Maharaj L, *et al.* Whole genome sequencing contributions and challenges in disease reduction focused on malaria. *Biology (Basel)* 2022; 11: 587.

11 Argimón S, Masim MAL, Gayeta JM, *et al.* Integrating whole-genome sequencing within the National Antimicrobial Resistance Surveillance Program in the Philippines. *Nat Commun* 2020; 11: 2719.

12 Meredith LW, Hamilton WL, Warne B, *et al.* Rapid implementation of SARS-CoV-2 sequencing to investigate cases of health-care associated COVID-19: a prospective genomic surveillance study. *Lancet Infect Dis* 2020; 20: 1263–1271.

13 Mboowa G, Mwesigwa S, Kateete D, *et al.* Whole-genome sequencing of SARS-CoV-2 in Uganda: implementation of the low-cost ARTIC protocol in resource-limited settings. *F1000Res* 2021; 10: 598.

14 Wang Y, Zhao Y, Bollas A, *et al.* Nanopore sequencing technology, bioinformatics and applications. *Nat Biotechnol* 2021; 39: 1348–1365.

15 Mulder NJ, Adebiyi E, Alami R, *et al.* H3ABioNet, a sustainable pan-African bioinformatics network for human heredity and health in Africa. *Genome Res* 2016; 26: 271–277.

16 Tastan Bishop Ö, Adebiyi EF, Alzohairy AM, *et al.* Bioinformatics education – perspectives and challenges out of Africa. *Brief Bioinform* 2015; 16: 355–364.