Use of targeted next-generation sequencing to detect drug-resistant tuberculosis

Rapid communication, July 2023
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Background

In 2021, more than 10 million people fell ill with tuberculosis (TB), including almost half a million with multi-drug or rifampicin-resistant TB (MDR/RR-TB) (1). Only one third of people with MDR/RR-TB were known to have been diagnosed and enrolled on treatment. The number of individuals falling ill with TB that is susceptible to rifampicin but resistant to isoniazid is estimated to be more than two times higher than RR-TB and remains largely undetected and untreated (2). Globally, the prevalence of fluoroquinolone resistance among MDR/RR-TB is approximately 20%. Recently the World Health Organization (WHO) endorsed the use of a novel all-oral 6-month regimen composed of bedaquiline, pretomanid, linezolid and moxifloxacin (BPaLM) in people suffering from MDR/RR-TB, including those with additional resistance to fluoroquinolones (pre-XDR-TB). The newly recommended BPaLM regimen offers better outcomes, remarkably shortens the duration of treatment, and thus significantly improves quality of life for people with MDR/RR-TB (3). An emerging concern is bedaquiline and linezolid resistance, as these two drugs are the backbone of the shorter MDR/RR-TB regimens, and it is anticipated that these drugs will remain essential for treatment of drug-resistant TB in future regimens.

To end the global TB epidemic by 2030, we must drastically expand access to drug-resistance testing, including to the drugs that constitute the best available regimens recommended for drug-susceptible and drug-resistant TB. Presently there are no WHO-recommended rapid diagnostics (WRDs) that can detect resistance to all the drugs in these two types of regimens in a single test to inform treatment decisions, nor are there any WRDs that detect resistance to new and repurposed drugs such as bedaquiline, linezolid, delamanid and pretomanid (4). Current WRDs only detect resistance to a limited number of drugs and cover only a single or few resistance-associated gene regions.

Targeted next-generation sequencing (NGS) technology couples amplification of selected genes with next-generation sequencing technology to detect resistance to many drugs with a single test. Furthermore, since targeted NGS can interrogate entire genes to identify specific mutations associated with resistance, targeted NGS may provide improved accuracy compared with existing WRDs. In addition, new targeted NGS-based tests can detect resistance to new and repurposed drugs not currently included in any other molecular assays. Targeted NGS-based tests therefore offer great potential to provide comprehensive resistance detection matched to modern treatment regimens.

In 2022, WHO therefore commissioned a series of systematic reviews of published and unpublished data on the class of targeted NGS products that are commercially developed for TB drug resistance detection. The WHO assessment process for TB diagnostics has evolved into a mechanism that focuses on evaluating classes of TB diagnostic products rather than specific products. For this guideline process, the class of targeted NGS products was defined as one that uses massively parallel sequencing to detect resistance to TB drugs, starting from a processed clinical sample and ending with an end-user report that relates detected Mycobacterium tuberculosis mutations to the presence (or absence) of drug resistance, based on the interpretation of a standard catalogue of mutations. Three products met the inclusion criteria for evaluation. The systematic reviews included data on diagnostic accuracy, economic information, and qualitative evidence on feasibility, acceptability, equity and end-user values and preferences. Based on available data, detection of resistance to the following drugs was reviewed: rifampicin, isoniazid, levofloxacin, moxifloxacin, ethambutol, pyrazinamide, bedaquiline, linezolid, clofazimine, amikacin and streptomycin. WHO convened a Guideline Development Group (GDG) on 2–5 May 2023 to discuss the findings of the systematic reviews and make recommendations on this technology.
This rapid communication aims to inform national TB programmes and other stakeholders about the key findings and considerations on using targeted NGS technologies for detecting drug-resistant TB following the assessment of available evidence. Detailed recommendations will be presented in the 2023 update of the *WHO consolidated guidelines on tuberculosis. Module 3: diagnosis – rapid diagnostics for tuberculosis detection.*

**Key findings**

**Targeted next-generation sequencing was found to be accurate**

*Among people with bacteriologically confirmed pulmonary TB*

The performance of targeted NGS on respiratory samples for determining resistance to rifampicin, isoniazid, levofloxacin, moxifloxacin, pyrazinamide and ethambutol was assessed. The available evidence varied by drug, from 12 studies with 1440 participants for isoniazid to 3 studies with 346 participants for pyrazinamide.

The test performance was determined to be accurate for all drugs included in the assessment, with a pooled sensitivity of ≥ 95% for rifampicin, isoniazid, moxifloxacin and ethambutol, 94% for levofloxacin and 88% for pyrazinamide. The specificity was ≥ 96% for all drugs. The reference standard was culture-based phenotypic drug susceptibility testing (DST) for isoniazid, levofloxacin and moxifloxacin and a combination of phenotypic DST and whole-genome sequencing (WGS) for rifampicin, pyrazinamide and ethambutol. The percentage of tests with indeterminate results ranged from 9% (levofloxacin and moxifloxacin) to 18% (pyrazinamide), with higher indeterminate rates in samples with lower bacterial load. The overall certainty of the evidence for test accuracy ranged from low to moderate.

*Among people with bacteriologically confirmed RR-TB*

The accuracy of targeted NGS performed on respiratory samples for resistance to isoniazid, levofloxacin, moxifloxacin, pyrazinamide, bedaquiline, linezolid, clofazimine, amikacin, streptomycin and ethambutol was assessed. The available evidence varied by drug, from 12 studies with 1440 participants for isoniazid to 3 studies with 346 participants for pyrazinamide.

The test performance among people with RR-TB was determined to be accurate for isoniazid, levofloxacin, moxifloxacin, pyrazinamide and ethambutol (pooled sensitivity ≥ 95%), and acceptable for bedaquiline (68%), linezolid (69%), clofazimine (70%), amikacin (87%) and pyrazinamide (90%). The specificity was ≥ 95% for all drugs except streptomycin (75%). The reference standard was culture-based phenotypic DST for all drugs except ethambutol and pyrazinamide, where a combination of phenotypic DST and WGS was used. The percentage of tests with indeterminate results ranged from 9% (levofloxacin and moxifloxacin) to 21% (ethambutol) and depended on the bacterial load. The overall certainty of the evidence for test accuracy ranged from low to high.

There were no data on the impact of targeted NGS on patient outcomes such as time to treatment or treatment outcome.

**Targeted next-generation sequencing was found to be cost-effective depending on context**

The cost and cost–effectiveness data for targeted NGS were assessed through a systematic review of the published literature and a WHO-commissioned generalized model-based cost–effectiveness analysis,
representing three settings with different TB epidemiologies (Georgia, India and South Africa). Among the studies included in the systematic review, three were on targeted NGS only, three were on targeted NGS and WGS and four were on WGS only. Based on the review, the most significant cost component was the sequencing step, and the largest component costs were reagents and consumables. Modelling was performed for the use of targeted NGS among all people with bacteriologically confirmed TB in Georgia, and among all people with bacteriologically confirmed RR-TB in all three settings. Based on modelling results, targeted NGS as an initial test for DST among all people with bacteriologically confirmed TB in Georgia (a high MDR/RR-TB burden setting) was considered cost-effective at a willingness-to-pay threshold (WTP) of three times the country GDP per capita. When considering targeted NGS as a replacement for universal phenotypic DST among all people with RR-TB, targeted NGS was cost-effective in India irrespective of the WTP and in South Africa at three times the WTP threshold but was not cost-effective in Georgia. When opportunities for multi-disease testing or high patient volumes exist, targeted NGS approaches are also expected to be more cost-effective if they result in earlier effective treatment initiation.

**Targeted next-generation sequencing was found to be acceptable and implementable under routine conditions, despite inherent complexity**

A review of qualitative evidence of the use of targeted NGS found no eligible studies, therefore WHO commissioned a qualitative study consisting of semi-structured interviews primarily with laboratory staff and management personnel directly involved with implementing targeted NGS, as well as with three global experts involved in TB care and diagnostics (17 respondents in total). Data from these stakeholder interviews found the acceptability of targeted NGS technology was high. There was an overwhelmingly positive sentiment for the potential utility of targeted NGS, and it was seen as a ‘major advancement’ in drug resistance detection. The main reasons for the high level of acceptability were simultaneous detection of resistance to multiple drugs and the speed of direct testing on clinical samples instead of waiting for culture-based testing (3–5 days compared with 4–6 weeks respectively). Conversely, the tests were noted to be highly complex, requiring specialized infrastructure and skilled human resources. Furthermore, challenges were identified relating to the installation, training, ensuring a stable supply chain of consumables, robust data management, data storage and reliable internet connectivity. Nonetheless, the technology was considered feasible to implement in centralized settings.

**Overall conclusions**

Available evidence supports the use of targeted NGS to detect drug resistance after TB diagnosis, to guide clinical decision-making for drug-resistant TB treatment. This class of tests does not replace WRDs that are more accessible, cheaper and easier to perform for detecting resistance to rifampicin, isoniazid and fluoroquinolones. However, this class can be considered an alternative for prioritized patient populations requiring comprehensive DST with faster results compared with phenotypic DST, or where access to phenotypic DST is limited. Although targeted NGS could provide important early DST results, which may impact treatment decisions in some patients, the suboptimal sensitivity for selected new and repurposed drugs implies that phenotypic DST is still required. Extrapolation of these findings to other brand-specific tests cannot be made, and any new in-class product will need to be evaluated by WHO before clinical use.

The products and drugs for which eligible data met the class-based performance criteria are listed below.

- **Deeplex® Myc-TB (GenoScreen):** for rifampicin, isoniazid, pyrazinamide, ethambutol, fluoroquinolones, bedaquiline, linezolid, clofazimine, amikacin and streptomycin.
- NanoTB® (Oxford Nanopore Technologies): for rifampicin, isoniazid, fluoroquinolones, linezolid, amikacin and streptomycin.
- TBseq® (ShengTing Biotech): for ethambutol.

Where a product has not yet met the requirements for a specific drug (i.e. the drug is not listed), further improvements to the product and a review of the evidence will be necessary before clinical use.

**Next steps**

- The updated policy guidelines on targeted NGS for TB drug resistance detection will be released before the end of 2023, as part of the *WHO consolidated guidelines on tuberculosis. Module 3: diagnosis – rapid diagnostics for tuberculosis detection, 2023 update*. The summary of findings and the evidence-to-decision tables will be produced in conformity with the GRADE (Grading of Recommendations Assessment, Development and Evaluation) method and made available on the WHO Global TB Programme website.

- The updated guidelines will be accompanied by the 2023 update of the *WHO operational handbook on tuberculosis. Module 3: diagnosis – rapid diagnostics for tuberculosis detection*. The handbook will provide guidance on the technologies currently recommended, steps to introducing the new class of tests into a health programme and the model algorithms.

- The release of the new guidance will be followed by a series of WHO webinars for different regions to disseminate the new guidelines. The updates will also be included on the online WHO TB Knowledge Sharing Platform providing easy access to the guidelines, implementation aids and eLearning tools, all in one place. The webinars and the platform will support countries to update their national guidelines, train staff, inform programme budgets and facilitate the transition to the use of the new interventions. National TB programmes and other stakeholders are encouraged to seek advice from WHO before introducing the latest technologies recommended in the revised guidelines.

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**References**
