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EDITORIAL

Rapid molecular diagnostics to detect resistance to second-line anti-TB drugs

Since the 1990s, drug-resistant TB (DR-TB) has been a priority for the WHO global TB programme. Outbreaks of multidrug-resistant TB (MDR-TB) first occurred in hospitals, involving people living with HIV and healthcare workers, and quickly expanded to the community.[1,2] At the same time, disruption to the health systems in countries of the former Soviet Union favoured an increase in MDR-TB cases.[3] These countries remain among those with the highest MDR- and rifampicin-resistant (RR-) TB prevalence in the world.[3] In 2019, 465,000 patients were estimated to have RR-TB, 78% with MDR-TB and 9% with extensively-resistant TB (XDR-TB). Among patients with MDR-TB and XDR-TB, favourable treatment outcome has been as low as 57% and 35%, respectively.[4] In 2020, the COVID-19 pandemic resulted in a drop in MDR/RR-TB detection and treatment worldwide.[5] Also in 2020, the WHO provided global estimates of the incidence of isoniazid resistance (INH^R) for the first time: there were 1.4 million incident cases of INH^R-TB, of which 1.1 million were susceptible to rifampicin.[4] Most of these patients were not diagnosed with DR-TB and did not receive appropriate treatment. There is little data available on the TB treatment outcomes among patients with INH^R-TB.[6] More recently, M/XDR-TB has been detected in TB treatment-naïve patients due to transmission of M/XDR-TB to healthy individuals in the community,[7,8] but little attention has been given to the adoption of infection control measures at healthcare units or at household level. Since the emergence of COVID-19, the Chinese and Russian governments (both are high DR-TB burden countries) have highlighted the need to improve TB infection control measures in these settings.[9,10]

Since 2007, the WHO has published several guidelines (using the Grading of Recommendations Assessment, Development and Evaluation [GRADE] approach), to gather and summarise the scientific evidence that support policy recommendations.[11] The majority of recent WHO recommendations for TB diagnosis are based on accuracy studies carried out in research settings,[11–15] underlining the fact that the implementation and impact of any new diagnostic algorithms should be strictly evaluated and monitored. In 2010, the WHO recommended that rapid molecular diagnostics (RMDs) be performed at the peripheral and/or intermediate level of TB laboratory networks to detect TB and RR-TB. The aim of this recommendation was to improve the early detection of MDR/RR-TB and significantly reduce the time to initiation of appropriate treatment.[12] In 2020, for cases reported with bacteriologically confirmed TB, only 61% had been tested using RMDs, and for patients with MDR/RR-TB only 43% were diagnosed and reported, and 37% started treatment.[4] In addition, there is insufficient scientific data on the clinical impact, pre- and post-analytical barriers, and the implementation of RMDs under field conditions for MDR/RR-TB diagnosis in high-burden countries.[16,17]

The WHO's End TB Strategy (aiming to end the global TB epidemic as part of the UN Sustainable Development Goals) stressed the importance and challenges of TB and M/XDR-TB early detection, prevention and care. The appropriate use of molecular diagnostics to increase M/XDR-TB case-detection rates and the coverage of quality-assured second-line drug susceptibility testing (DST) among these cases have been emphasised by several 2016–2020 TB action plans in different regions (West and East Europe, South Africa, Americas, India).[18–21] In 2016, the WHO endorsed the use of line-probe assays (LPAs) such as GenoType® MTBDR*sl* (Hain Lifescience, Nehren, Germany) for the detection of mutations associated with resistance to second-line drugs (SL-LPA) and recommended the use of fluoroquinolones (FQs) and second-line injectable drugs (SLIDs) in treating patients with MDR/RR-TB.[14] In the last decade, there have been significant changes in the diagnosis and management of MDR/RR-TB, including the use of new and repurposed drugs and novel therapeutic approaches. Since 2019, when FQs were introduced as the backbone of the new injectable-free (shortened) MDR-TB treatment regimen, MTBDR*sl* has been used for the rapid exclusion of FQ and SLID resistance, a major cause of unfavourable TB treatment outcomes.[22,23] In 2021, WHO consolidated the recommendations into a single guideline[24] and recommend that the level of complexity (low, moderate or high – based on requirements for infrastructure, equipment and technical skills) be used to guide nucleic-acid amplification testing (NAAT) (Table). Of the 29 studies on SL-LPA (MTBDR*sl*) identified, 26 evaluated the performance of MTBDR*sl* v1.0. Among published studies

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Table NAAT classes endorsed by the WHO[24] according to level of complexity based on the requirements for infrastructure, equipment and technical skills.

Complexity level	Technique	Commercial examples
Low	Automated NAATs for the detection of resistance to isoniazid and second-line anti-TB agents	Xpert® MTB/XDR (Cepheid, Sunnyvale, CA, USA)
Moderate	Automated NAATs	Abbott RealTi*m*e MTB and Abbott RealTi*m*e MTB RIF/INH (Abbott Laboratories, Chicago, IL, USA); BD MAX™ MDR-TB (BD, Franklin Lakes, NJ, USA); Cobas® MTB and Cobas MTB-RIF/INH (Roche, Basel, Switzerland); FluoroType® MTBDR and FluoroType® MTB (Hain Lifescience, Nehren, Germany).
High	Non-automated reverse hybridisation-based NAATs	Genoscholar™ PZA-TB (Nipro, Osaka, Japan)

Previously recommended technologies were also revised: Xpert® MTB/RIF and Xpert® MTB/RIF Ultra (Cepheid) Truenat™ (Molbio, Verna, India); line-probe assays, including GenoType® MTBDR*plus* v1 and v2; GenoType® MTBDR*sl* (Hain Lifescience/Bruker) and Genoscholar™ NTM+MDRTB II (Nipro).
NAAT = nucleic acid amplification test.

that evaluated the MTBDR*sl* v2.0 performance in the indirect testing of *Mycobacterium tuberculosis* cultures compared with phenotypic culture-based DST reference, MTBDR*sl* sensitivity ranged from 84% to 100% and specificity from 99% to 100%. The analysis confirmed that, similar to first-line LPA (GenoType® MTBDR*plus*; Hain Lifescience), resistance-conferring mutations detected using SL-LPA are highly correlated with phenotypic resistance to FQs and to SLIDs. Given its high specificity, positive results on SL-LPA could be used to guide the implementation of appropriate infection control precautions.[24] SL-LPAs are therefore recommended to detect additional resistance to second-line anti-TB drugs in patients with confirmed MDR/RR-TB or INH^R. These recommendations apply to the direct testing of sputum specimens from patients with MDR/RR-TB/INH^R, irrespective of smear status, admitting a higher indeterminate rate in smear-negative sputum specimens when compared with smear-positive ones.[24]

In this context, the article by Lutchminarain et al.[25] in this issue of the *Journal* is welcome. The authors compared the performance of MTBDR*sl* v2.0 on the direct testing of smear-positive and smear-negative specimens of all patients with newly diagnosed RR-TB and characterised the mutation patterns identified. They found that the SL-LPA successfully identified *M. tuberculosis* complex and the susceptibility pattern in 70.7% (1325/1873) of the specimens analysed. SL-LPA improved the rapid diagnosis of pre-XDR-TB and XDR-TB, identified in respectively 145 (7.7%) and in 72 (3.8%) of patients. However, the SL-LPA showed a higher inconclusive rate in smear-negative specimens than in smear-positive specimens (67.2% vs. 6.6%). The authors therefore suggest that, according to South African National Department of Health guidelines, the SL-LPA should be performed directly only on smear-positive clinical specimens in settings where smear-negative TB remains a challenge.[21] The authors also highlighted that even with the low prevalence of FQ resistance in South Africa (1.2%), there is a possibility of primary transmission of drug-resistant TB strains, as previously described by others,[7,8] because in the study only newly diagnosed TB patients had been included. Lutchminarain et al. also underline the fact that FQ resistance was higher than resistance to SLIDs, at 65.5% and 34.5%, respectively, in all pre-XDR-TB isolates, as previously described.[26–29]

In case of FQ resistance, the most frequent mutations occurred in the *gyr*A region at codon 90 (most prevalent A90V), followed by codon 94, similar to other study findings.[26,27] In case of SLIDs mutation patterns, the authors observed that the predominant mutation was *rrs* (A1401G) in 71 (65.7%) patients, and only 3 (2.8%) in the *eis* region, consistent with other published data.[29] FQ heteroresistance was identified in 10% of the studied strains, generally due to mutations in the *gyr*A region (the clinical relevance of this is unknown); these results are similar to those described by Gardee et al., who identified FQ heteroresistance in 16.5% of ofloxacin-resistant strains.[26] Lutchminarain et al. point out that the lack of comparisons with drug-susceptible testing and/or genome sequencing as one of the limitations of the study. They also underline that resistance was inferred for the undefined FQ and SLID mutations found, as some mutations causing resistance were not covered by the mutation probes included in the assay.

The results of this study by Lutchminarain et al.[25] reinforce the need for the evaluation of new rapid genotypic tests in clinical samples under field conditions in high-burden countries using the shortened treatment for DR-TB. This would allow for the early detection of mutations that cause resistance to new drugs (i.e., bedaquiline, delamanid, pretomanid), which may be associated with phenotypic resistance,[24,29,30] in addition to FQ, as injectable drug regimens are no longer an option. This approach is especially important post-COVID-19 because of the expected rise in the incidence of M/XDR-TB cases in the coming years.[5]

A. L. Kritski[1]
M. Viveiros[2]
A. C. C. Carvalho[3]

[1]Academic Tuberculosis Program, Faculty of Medicine, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil;
[2]Global Health and Tropical Medicine, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisbon, Portugal;
[3]Laboratory of Innovations in Therapies, Education and Bioproducts, Oswaldo Cruz Institute, Fiocruz, Rio de Janeiro, RJ, Brazil
Correspondence to: Anna Cristina C Carvalho, Laboratory of Innovations in Therapies, Education and Bioproducts, Oswaldo Cruz Institute, Fiocruz, Rio de Janeiro, RJ, Brazil. e-mail: anna.carvalho@ioc.fiocruz.br

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