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Commentary

The case for expanding worldwide access to point of care molecular drug susceptibility testing for isoniazid

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The Xpert MTB/XDR assay (*Mycobacterium tuberculosis*/extensively drug resistant; Cepheid, Sunnyvale, CA, USA) is a recent addition to the repertoire of tuberculosis (TB) drug susceptibility testing (DST) technologies and is set to bolster the early detection of drug-resistant TB [1]. Following on from the Xpert MTB/RIF (rifampin) Ultra assay, already used extensively to detect the presence of *Mycobacterium tuberculosis* complex and resistance to rifampicin, the Xpert MTB/XDR assay is designed to detect resistance to isoniazid, fluoroquinolones, ethionamide, and second-line injectables [1]. It has a turnaround time of approximately 90 minutes and has shown high accuracy for expanded resistance detection so far [1]. Isoniazid-resistant rifampicin-susceptible TB (Hr-TB) is the world's most common TB drug resistance pattern, approximately double that of rifampicin resistance and estimated at 7.4% of new and 11.4% of previously treated TB cases [2]. We argue that the Xpert MTB/XDR should be used in tandem with the Xpert MTB/RIF Ultra as it could improve the timely detection of Hr-TB. Moreover, we emphasize the need for prioritizing the development of point-of-care rapid molecular DST that not only addresses the canonical isoniazid resistance-conferring mutations already covered by current molecular assays. Instead, such a molecular assay should also permit the analysis of a broad spectrum of less common mutations associated with isoniazid resistance.

Current diagnostic tools and algorithms mainly prioritize rifampicin resistance detection which allows for Hr-TB to remain undetected in many cases [2]. Evidence indicates increased rates of poor treatment outcomes such as treatment failure, relapse and progression to MDR-TB when patients with Hr-TB are treated with first-line TB drugs [3]. These less favourable treatment outcomes emphasize the importance of expanding DST of isoniazid to ensure patients with Hr-TB are quickly switched to adequate regimens.

The standard reference for DST of isoniazid is phenotypic culture-based DST (pDST). Molecular DST may be used instead of or in addition to pDST, with same-day results instead of weeks to months with pDST. Additionally, it can better distinguish between low- or high-level isoniazid resistance based on the mutation(s) detected, which may inform decisions on whether to include high-dose isoniazid in treatment regimens [4]. Line probe assays (LPA) have often been used for relatively rapid identification of isoniazid resistance but are labour intensive and rely on procedures that are often only possible in well-equipped laboratories. Other molecular assays targeting isoniazid resistance include the moderate complexity automated nucleic acid amplification tests (NAATs) Abbott RealTime MTB RIF/INH (Abbott Park, IL, USA), BD MAX MDR-TB (Franklin Lakes, NJ, USA), FluoroType MTBDR (Hain Lifescience, Nehren, Germany), cobas MTB-RIF/INH (Roche Diagnostics, Rotkreuz, Switzerland), or low complexity NAATS such as Xpert MTB/

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XDR (Cepheid, Sunnyvale, CA, USA) [5]. Whole genome sequencing has already become the norm in some high-income low TB burden countries and screens large proportions of the genome of *M. tuberculosis* complex isolates. In low- and middle-income countries, technologies like the MinIon or Illumina iSeq, increasingly affordable sequencing platforms, may improve differentiation of TB, and DST and in the future [6].

Cepheid's Xpert MTB/XDR has demonstrated high accuracy in the detection of isoniazid resistance in a recent clinical evaluation on direct sputum samples, with a sensitivity of 94% and specificity of 100% using a composite reference standard of pDST and whole genome sequencing [1]. The assay utilizes probes for four genetic regions associated with isoniazid resistance: the *inhA* promoter, the *katG* and *fabG1* genes, and the *oxyR-ahpC* intergenic region [1]. It thereby surpasses the LPAs and the moderate complexity NAATs in scope, which only target the *katG* gene and *inhA* promoter [1]. Although lacking head-to-head comparisons, Xpert MTB/XDR may exhibit slightly higher sensitivity than and similar specificity to these two alternatives [5]. Other benefits include its reliance on Gene Xpert equipment which is already used extensively for the Xpert MTB/RIF (Ultra) assays and that it can be implemented as a point-of-care test.

One of the main shortcomings of Xpert MTB/XDR and most molecular DST methods is that they are only able to detect mutations in a limited number of regions, a product of the number and size of the probes included when designing the assay. While the Xpert MTB/XDR leads by testing for isoniazid resistance in four genetic regions, other uncommon or unidentified mutations may very well still escape detection [7]. A systematic review of the mutations associated with resistance to isoniazid described how the relative prevalence of *katG* mutations in the 315 codon varies from as much as 88.4% in Africa to 67.7% in Asia [7]. Furthermore, approximately 18.4% of phenotypically isoniazid-resistant isolates from Asia did not contain mutations in the regions currently screened by molecular DST [7]. This illustrates another danger that has already been identified by other authors—that of artificially selecting *M. tuberculosis* complex strains with mutations that are not interrogated by current molecular DST methods [7,8]. In TB high-prevalence countries, the rate at which new mutations arise may be higher such that the landscape of isoniazid resistance could differ from that of lower burden nations. For instance, the evaluation of the Xpert MTB/XDR that reported an overall sensitivity of 94% for detection of isoniazid resistance also reported a reduction in sensitivity to 80% in samples originating from a centre in New Delhi, India [1]. Limitations aside, the assay shows immense potential for improving the early diagnosis of Hr-TB and other drug resistance in peripheral settings. However, this potential will not be fully realised if it is implemented as a reflex test for patients with rifampicin resistance confirmed by the Xpert MTB/RIF (Ultra), as has been previously argued [8]. In fact, this approach

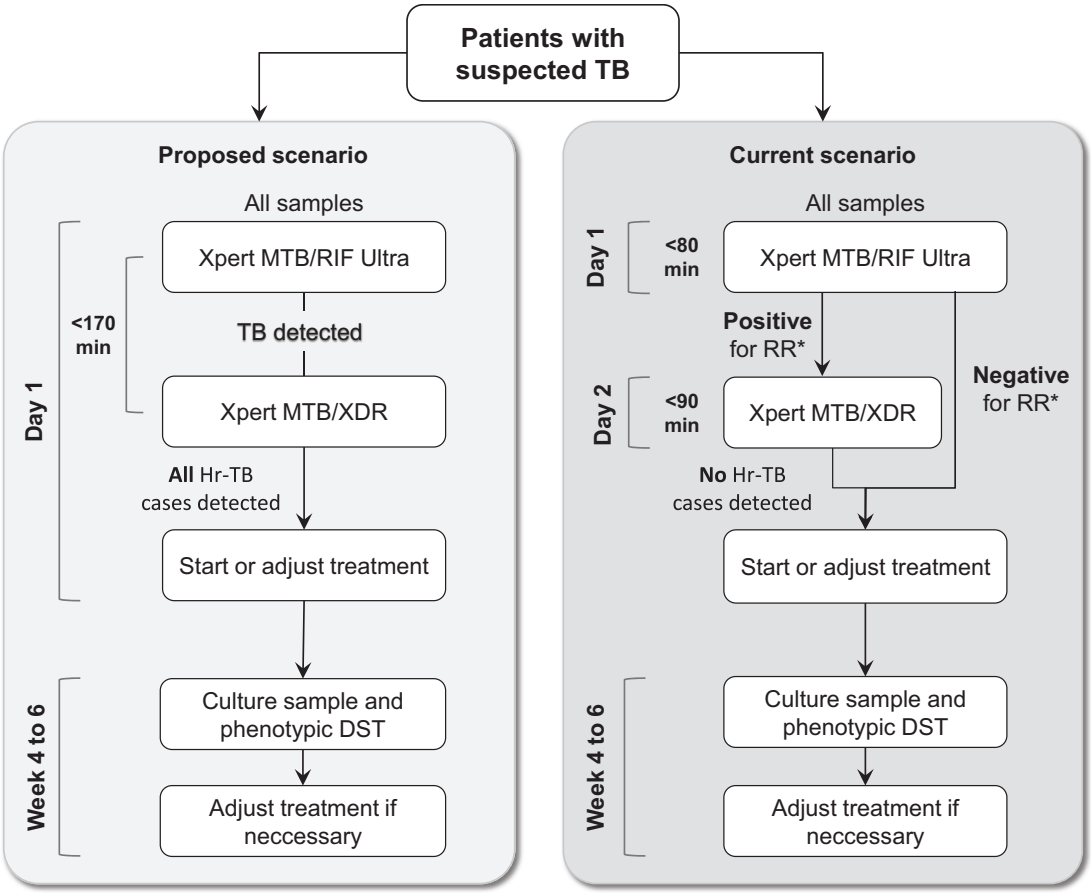

Fig. 1. Comparison between Xpert MTB/RIF use in all cases positive for MTB versus use only in cases positive for MTB with confirmed *rpoB* resistance. DST, drug susceptibility testing; Hr-TB, isoniazid-resistant rifampicin-susceptible TB; RR, rifampicin resistance; TB, tuberculosis. *Assuming samples are positive for *Mycobacterium tuberculosis* (MTB). Samples negative for MTB not included in flowchart.

will continue to miss the largest group of patients infected with a resistant *M. tuberculosis* complex strain, those with Hr-TB. Information from the manufacturer Cepheid is currently conflicting, with one publication describing the assay should be used for all samples positive for *M. tuberculosis* complex, whereas information on their website describes that in rifampicin susceptible cases, Xpert MTB/XDR should be the next step in cases of suspected high risk of Hr-TB or MDR-TB [1,9]. However, how this risk is ascertained is not specified.

It remains to be seen whether the Xpert MTB/XDR or any other emerging technology can outperform the diagnostic accuracy of the LPAs and deliver on the promise of rapid, affordable, and extended molecular DST with minimal infrastructure and personnel requirements. To ensure excellent sensitivity for isoniazid resistance detection, research on the mutations conferring isoniazid resistance across world regions and especially in countries with a high TB/MDR-TB burden is crucial. Artificial intelligence and targeted next generation sequencing are a few innovative approaches that could help address this gap.

Our view is that we should focus on the comprehensive use of assays such as the Xpert MTB/XDR. We propose that the XDR-TB cartridge be used sequentially for all samples declared positive for MTB by Xpert MTB/RIF Ultra even when *rpoB* gene resistance is not detected. This approach would allow for a significant increase in Hr-TB case detection and would thereby help boost appropriate treatment. Treatment would be further guided by the Xpert MTB/XDR as it allows exclusion of fluoroquinolone resistance, which is central to the management of Hr-TB [4]. Fig. 1 displays this proposed scenario and compares it to a scenario in which only samples with confirmed *rpoB* resistance undergo further testing with the Xpert MTB/XDR. An important but currently unquantifiable risk of increasing detection of Hr-TB is that of undetected baseline rifampicin resistance. Treating these patients as Hr-TB could increase the risk of pre-XDR-TB due to acquired levofloxacin resistance.

Additional testing will undoubtedly also have cost implications, with current pricing of Xpert MTB/XDR at $20 USD per test [10]. Cost effectiveness will vary depending on the local burden of Hr-TB. However, were regimens such as the novel 4-month rifapentine-based regimen for DS-TB containing moxifloxacin to be widely adopted, fluoroquinolones would then become first-line drugs [10]. Therefore, DST that addresses fluoroquinolone resistance would become crucial to prevent amplifying resistance. Consequently, pressure on companies to reduce prices of point-of-care DST targeting fluoroquinolones could greatly increase [10].

In conclusion, we suggest a different positioning of this new assay given the larger discussion that is Hr-TB. Detection of Hr-TB should not be dictated by the cartridge but rather by the presence of TB in clinical samples, which on its own should lead to testing for Hr-TB and other resistance patterns. Moreover, there is a need to further our understanding of the distribution of global isoniazid resistance and its genetic causes. With this knowledge, we can envision a future where no patient with Hr-TB slips under the radar, and all patients with Hr-TB receive the correct regimen and as such prevent the development of MDR-TB.

Author's contributions

Conceptualization: VBV, OWA, RMA, and HAMK. Writing, original draft: All authors. Writing, review & editing: All authors.

Transparency declaration

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